Identification of phenolic and alcoholic compounds in wine spirits and their classification by use of multivariate analysis

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Headspace gas chromatography (HSS-GC)

Major volatile compounds were analysed by gas chromatography (GC).[1] Varian 3300 gas chromatograph GC system with a split/splitless injector and a flame ionisation detector (FID) was employed. For the headspace analyses, Hewlett Packard headspace sampler HP 5890 was used. The compounds of interest were separated on a RTX-624 (30 m × 0.32 mm, Agilent J&W, Santa Clara, CA, USA) capillary column with a film thickness of 1.8 μm with the following temperature programme: initial oven temperature was 38 °C for 4 min, then raised at 20 °C min to 170 °C, followed by 40 °C min to 190 °C and by 10 °C min to 220 °C. Operating conditions were kept constant: injector temperature: 220 °C, FID detector temperature: 250 °C, carrier gas: nitrogen, at a flow rate of 7.85 ml min^{-1}. The carrier gas pressure was 160 Psi, vial pressure 7 Psi and injection time 0.2 min. The samples were equilibrated by heating at 80°C for 10 minutes and injected by means of the headspace sampler in splitless mode (3 min). A headspace sampler was equipped with a standard 1-mL loop. The volatile compounds analysed were methanol and higher alcohols: *2*-butanol, *n*-propanol, isobutanol, *n*-butanol, isoamyl alcohol. Qualitative analysis was carried out by comparison of the retention times of the standards and the corresponding peaks obtained with the samples. A calibration curve for internal standardization employing pentan-3--ol as internal standard was built and used for quantification. Analyses were carried out in triplicate and their averages were calculated. Higher alcohol con-

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tents were estimated by the sum of *2*-butanol, *n*-propanol, isobutanol, *n*-butanol, isoamyl alcohol. Results were expressed as mg 100 mL^{-1} of absolute alcohol (AA).

HPLC ANALYSES OF POLYPHENOL COMPOSITION OF WINE SPIRITS

Chemicals

Gallic, vanillic and *trans*-cinnamic acids, syringaldehyde and vanillin were purchased from Sigma–Aldrich (Steinheim, Germany). Syringic and ellagic acid were obtained also from Fluka (Buchs, Switzerland). All of them were used as standards. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). The redistilled water was used in sample preparation, soutions and analyses.

Isolation of polyphenol fractions

The isolation of wine distillates polyphenol was performed by liquid–liquid extraction according to adapted method of Canas *et al.*[2] Extractions of samples were performed in duplicate. Samples (10 mL) were dealcoholized by means of rotary evaporator at 30 °C and diluted to the original concentration by distilled water. Then, the sample pH was adjusted to pH 2 with 2M HCl in order to form an aglycone and extracted three times with ethyl acetate (5 mL). The obtained three ethyl acetate extracts were combined and then evaporated to dryness in a rotary evaporator. The dry residue was dissolved in 1.5 mL of methanol and this solution was used for HPLC analyses. Extractions of samples were performed in duplicate.

Qualitative and quantitative HPLC analyses of polyphenol composition of wine spirits and brandies were performed. The analytical HPLC system was ProStar Varian equipped with a Varian Pro Star 330 photodiode array detector (Varian, Walnut Creek, CA, USA). The HPLC column was Nucleosil 5u C18 100A (Phenomenex, Torrance, CA, USA). The solvents for gradient elution were: A-0.2 % o-phosphoric acid, B-methanol, C-acetonitrile. The following gradient was used: 96 % A, 2 % B, 2 % C. The flow rate was 1.5 mL min^{-1}. Operating conditions were as follows: column temperature 30 °C and injection volume 20 μL. Chromatograms were recorded at 260, 280, 320 and 360 nm. The identification of the compounds was achieved by comparing UV spectra and the retention times of the separated peaks with the retention times of the standards. Quantification was made by the external standard method using calibration of standards as a reference and was based on peak area from HPLC analyses and mass concentration of compound. The polyphenols identified and quantified were: gallic acid (GA) and its derivate (dGA), ellagic acid (EA) and its derivate (dEA), vanillic acid (VA), syringic acid (Syr), vanillin (V), syringaldehyde (SYAL) and coniferaldehyde (CoAL). Results were expressed as mg 100 mL^{-1} of sample.
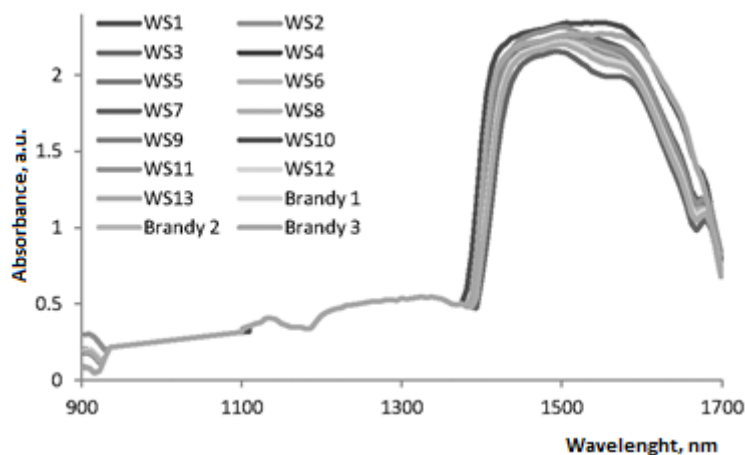
Fig. S-1. NIR spectra of wine spirit (WS) and brandies samples.

TABLE S-I. Partial least squares regression models obtained from NIR spectra (baseline, smoothing S-G, smoothing S-G & derivative) for identified alcohols in wine spirits used in spirit production – calibration and external validation set results, root mean square error of cross validation (*RMSEC*) and prediction (*RMSEV*), correlation coefficient for calibration (r_c^2) and validation (r_v^2) sets, residual predictive deviation (*RPD*) and ratio of error range (*RER*); GA-gallic acid; dGA-gallic acid derivate; VA-vanillic acid; Syr-syringic acid; V-vanillin; SYAL-syringaldehyde; CoAL-coniferaldehyde; EA-ellagic acid; dEA-ellagic acid derivate

Observed parameters in the partial least squares regression models		Calibration		Prediction		*RPD*	*RER*
		r_c^2	*RMSEC*	r_v^2	*RMSEV*		
Alcohols	2-Methylpropan-1-ol	0.97	4.28	0.94	2.00	3.57	13.42
	Propan-1-ol	0.94	2.62	0.89	4.02	3.98	11.73
	Butan-2-ol	0.93	3.95	0.87	0.50	3.25	9.86
	Butan-1ol	0.95	0.51	0.91	3.54	2.93	9.18
	Ethanol	0.99	0.52	0.94	0.53	7.83	20.80
Aging		0.99	0.13	0.98	0.14	6.78	14.93
Phenols	GA	0.92	0.01	0.89	0.12	2.20	6.20
	dGA	0.84	0.02	0.82	0.03	2.45	7.37
	VA	0.85	0.06	0.80	0.09	1.65	4.86
	Syr	0.86	0.07	0.82	0.11	1.78	5.42
	Syal	0.84	0.16	0.81	0.23	1.71	5.65
	V	0.85	0.07	0.79	0.11	1.65	4.86
	CoAL	0.85	0.08	0.82	0.09	2.38	6.07
	EA	0.91	0.69	0.87	1.12	2.04	5.89
	dEA	0.82	0.14	0.73	0.22	1.48	4.30

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