LC–SSI-MS Techniques as Efficient Tools for Characterization of Nonvolatile Phenolic Compounds of a Special Hungarian Wine

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Abstract

The utility of high-performance liquid chromatography–mass spectrometry using electrospray (ESI) and sonic spray (SSI) ionization for the characterization of nonvolatile phenolic compounds is tested using the special Hungarian wine Tokaj aszu of 1983 vintage. Besides caffeic-, ferrulic-, chlorogenic-, and 3,4-dimethoxycinnamic acids; 3,4-dimethoxycinnamoyl-, ferruloyl-, and galloyl-glucose; gentisic acid-β-Δ-glucoside; theogallin; and resveratrol-3-*O*-β-Δ-glucoside, 26 flavonoids can be identified. It is shown that because of its higher sensitivity, SSI is a more powerful tool for characterization and monitoring of nonvolatile phenolic compounds than ESI.

Introduction

Today, modern food analysis entails much more than the analysis of characteristic and active constituents of the products. There is a need for quality assurance of food, nutrition, and dietary supplement products during their production, storage, and shelflife, respectively. The food industry is under increased scrutiny from the government and public interest groups to contain costs and yet consistently deliver safe products to the market. Thus, the analytical control of products for human consumption, with special regard to the environmental and health connections, is a very important everyday problem.

It has been demonstrated (1,2) that in contrast to most countries where a high saturated fat intake was correlated to coronary heart disease (CHD) mortality, in certain parts of France mortality was significantly low despite a relatively high fat intake. Consumption of wine was one dietary factor that could partly explain this low mortality from CHD. This epidemiological evidence thus suggested that the intake of wine may counteract the effect of a high fat diet in reducing the incidence of CHD and was referred to as the "French Paradox". The in vivo antioxidant properties of polyphenols such as flavonoids and related compounds in wine in retarding atherogenesis were proposed as an explanation for the French Paradox (2–4). In addition, natural antioxidants from plant foods such as polyphenols, among them flavonoids, may also be effective in reducing thrombosis (cerebrovascular stroke), a fatal event in a large proportion of death from CHD. In vitro and in vivo data effectively demonstrate the antioxidant efficacy of structurally diverse flavonoids under many circumstances of oxidative stress (1,3–6).

There are a large number of studies on flavonoids and other phenolic compounds and nonphenolic constituents of different red wines (7–21); however, data for white wines are rare in the literature. Therefore, we set our sights on the study of the nonvolatile phenolic constituents of a world-wide famous wine produced in Tokaj, Hungary.

The wine-growing area of Tokaj is found in the frontier zone of the northeastern part of Hungary in the agro-ecological area of the mountains of Tokaj-Zemplen at a medium elevation of 500 m. Its climate shows continental character with mild, sunny, and slightly rainy autumns. This area has considerably diverse soils; in the Tokaj region it is chiefly loess mixed with rubbles of rocks of volcanic origin. Exclusively white wines are produced in this wine-growing area, particularly from varieties of grapes suitable for wine made from hand-selected noble rot grapes (aszu). In order to characterize the main nonvolatile phenolic components of "Tokaj aszu", liquid chromatography–mass spectrometry (LC–MS) techniques applying different ionization modes were used. Although some publications deal with the application of LC–MS techniques, mainly LC–electrospray ionization (ESI)-MS and LC–thermospray (TSP)-MS, to characterize organic compounds like flavonoids in fruit juices and wines (14,22–29),

sonic-spray ionization (SSI) (30,31) has never been applied to the analysis of Hungarian or other wines.

SSI, as part of the Hitachi family of atmospheric pressure LC–MS interfaces, was developed by Hirabayashi et al. (30). This is a new type of atmospheric-pressure ionization (API) interface. In SSI, the effluent from the high-performance liquid chromatography (HPLC) column (100–300 µL/min) flows into a stream of nitrogen gas with a linear velocity between Mach 1 (333 m/s) and Mach 3 (1000 m/s). Under these conditions, the mobile phase is evaporated and the molecular ions are formed in an atmospheric pressure ion source, without any application of heat or high voltage. The sampling orifice was heated to approximately 100–120ºC. This was to suppress the charged droplets produced by adiabatic expansion when ions are introduced into the first intermediate pressure region. Gaseous ions were analyzed with a very flexible ion-trap mass spectrometer. SSI produces protonated molecules and ionizes with lower fragmentation, thus higher sensitivity and much more simple mass spectra could be achieved. The fragment ions were probably produced by collisional dissociation in the intermediate pressure regions of the mass spectrometer.

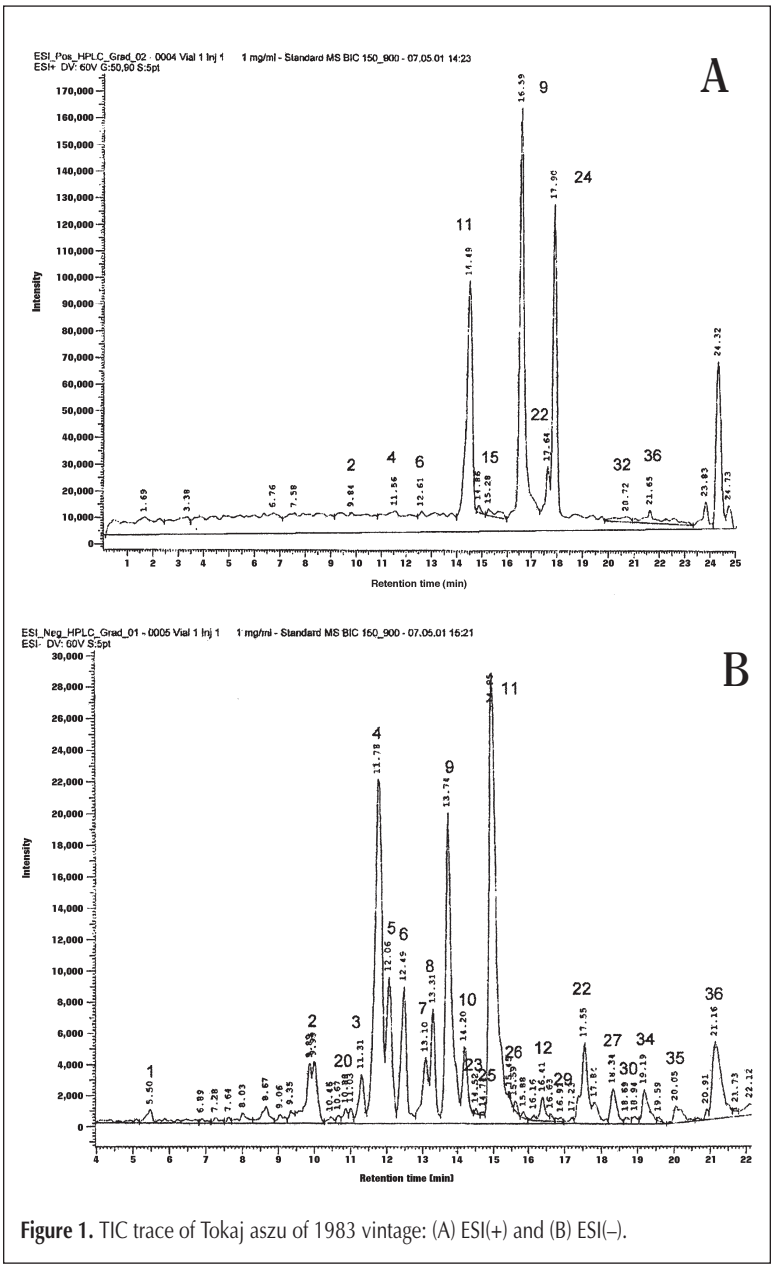

Figure 1. TIC trace of Tokaj aszu of 1983 vintage: (A) ESI(+) and (B) ESI(–).

Experimental

Chemicals and sample treatment

All organic solvents were HPLC grade and were purchased from Merck KGaA (Darmstadt, Germany).

The study was carried out with Tokaj aszu essence of 1983 vintage, which had been obtained from a local wine maker of Tokaj, Hungary. No extraction or any special purification was performed before HPLC separation. Alcohol was removed from the wine by evaporation at 30ºC and reduced pressure in a rotary evaporator. After the evaporation, the extract was freeze dried at –70ºC. The high-density oil-like residue was dissolved in methanol and injected into the HPLC.

HPLC–MS system and operating conditions

LC–MS was carried out using a Merck-Hitachi (Merck KGaA) model M-8000 LC/3DQMS ion trap mass spectrometer equipped with a LaCrom HPLC system (Merck KGaA) consisting of 7100 pump, automatic solvent degasser, L-7250 autosampler, L-7455 diode-array detector, and a LiChropher 100-RP-18 (5-µ × 4.6-mm i.d. × 250-mm length) reversed-phase column. The column temperature was 20–25ºC. The elution conditions were: flowrate, 0.8 mL/min; solvent A, water–formic acid (98:2, v/v); and solvent B, acetonitrile–water–formic acid (80:18:2, v/v/v). Elution began with isocratic A (100%) for 5 min, followed by linear gradients from 10% to 30% B in 25 min and from 30% to 100% B in 5 min and isocratic elution with 100% B for 5 min, followed by washing and re-equilibrating the column. UV-vis detection was monitored at 280–310 and 520 nm, respectively. The column was connected to the mass spectrometer ESI or SSI interfaces via a fused-silica capillary (length, 100 cm; 75-µm i.d.). The flow was split after UV-vis detection so that 250 µL/min went to the electrospray or sonic-spray source. Positive and negative ionization ESI- and SSI-MS were used for the detection of the nonvolatile phenolic compounds. The optimum SSI conditions were: nitrogen pressure, 1 bar (100 KPa); spray gas flowrate, 3 L/min; temperature of the nitrogen spray gas, 250ºC; temperature of first aperture, 120ºC; attenuation time, 500 ms; scan range, *m/z* 150–900; scan time, 1 s; and a drift voltage of 60 V. ESI mass spectra were recorded with the same instrument.

The experimental conditions were: desolvator temperature, 200ºC; temperature of first aperture,

140°C; nitrogen nebulizer pressure, 1.5 bar; nitrogen drying gas temperature, 200°C at 6 L/min; capillary voltage, 3500 V; and drift voltage, 60 V.

Results and Discussion

Positive and negative ionization ESI and SSI total ion current (TIC) traces obtained from the Tokaj aszu essence of 1983 vintage sample are shown in Figures 1A and B, as well as Figures 2A and B, respectively.

On the basis of simple comparison of these figures' characteristics, differences could be recognized between the TIC traces. In the case of the ESI, the negative ionization mode is more advantageous than the positive one, but there are significant differences between the abundances.

Although these differences could not be observed so markedly in the case of SSI, the number of peaks in the mass spectra have been found to be substantially higher compared with the ESI counterparts. Characteristic examples are shown in Figures 3 and 4A and 4B. The Merck-Hitachi M-8000 LC/3DQMS has two special features: ASC (automatic sensitivity control) and FNF (filtered noise fields). ASC and FNF combined offer a unique solution to avoid space charging causing mass shifts and loss of sensitivity. By using FNF, we received noise-filtered TIC trace because unwanted background compounds could be simply excluded from the MS measurement. Under the optimized ESI and SSI conditions, depending on the ionization mode, all phenolic compounds generated protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ions without significant fragmentation.

In the ESI(+) and SSI(+) spectra (Figures 3 and 4A), a relatively large number and more abundant background ion can be observed.

Figure 4B clearly shows that the SSI(–) mass spectrum is very simple, consisting predominantly of deprotonated molecular ion, and, under these conditions, no adducts, dimers, or other ions were produced.

According to earlier results (32), in case of thermally labile molecules, SSI can offer an advantage over ESI because no thermal energy is transferred to the molecule producing an unclear ionization. With a lack of characteristic fragment ions in an SSI spectrum, the authenticity of the identification can be increased by MS–MS or collision-induced decomposition (CID) techniques (especially at the unknown compounds). In addition, the overall stability of the SSI-MS–MS

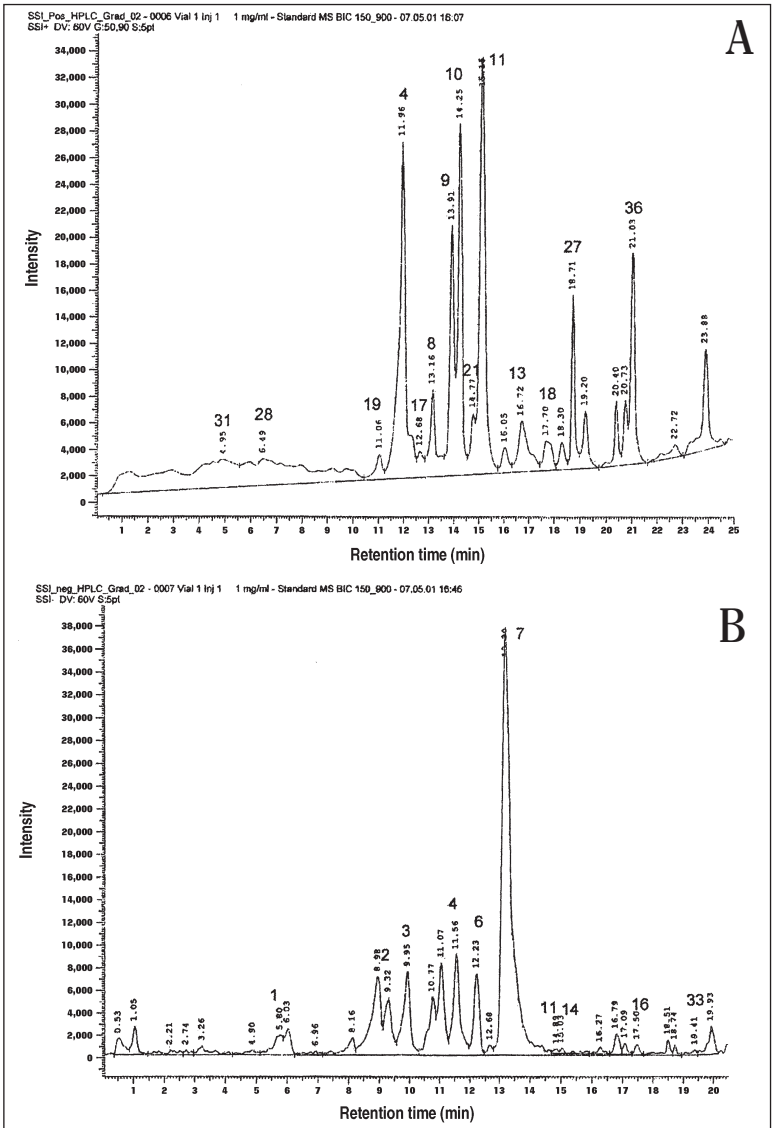

Figure 2. TIC trace of Tokaj aszu of 1983 vintage: (A) SSI(+) and (B) SSI(–).

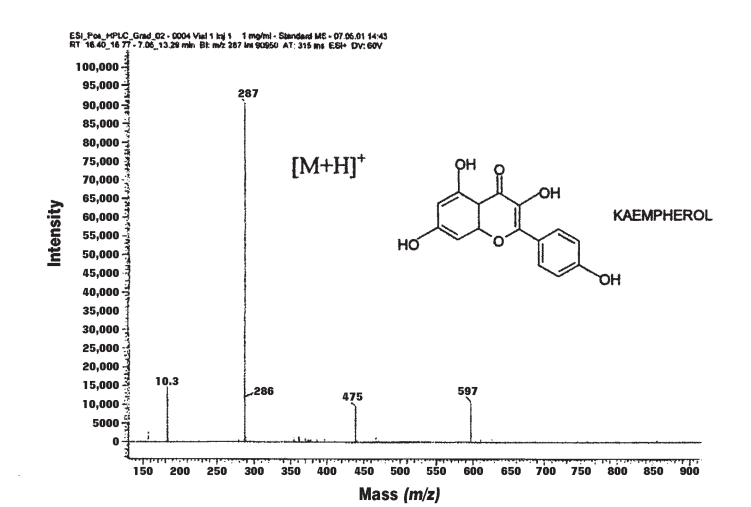

Figure 3. ESI(+) mass spectrum of kaempherol.

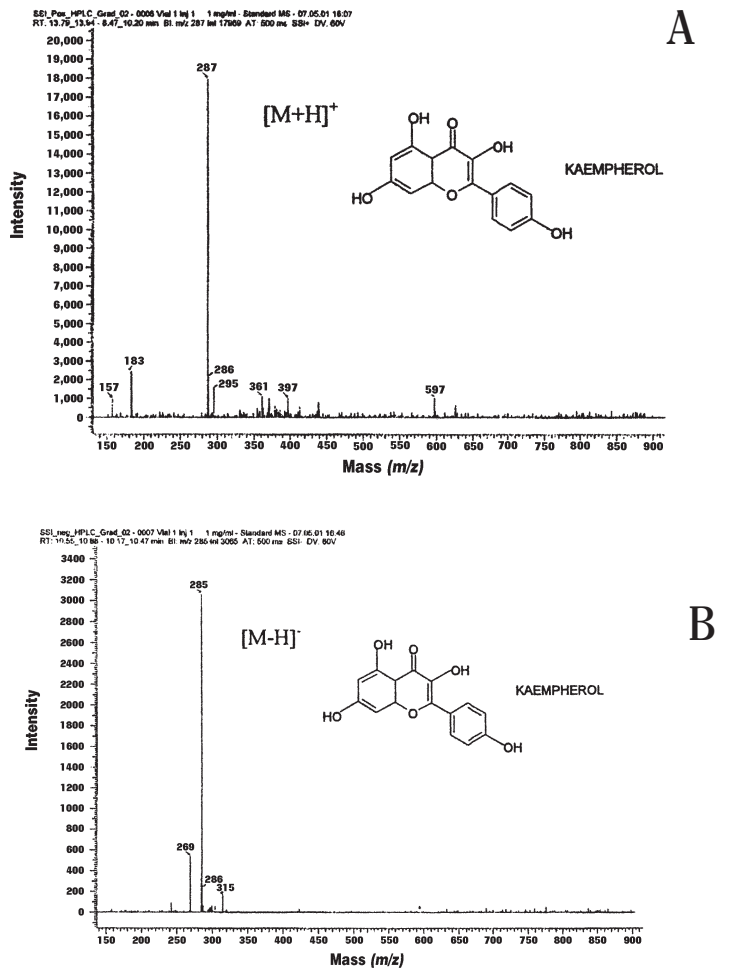

Figure 4. Mass spectrum of kaempherol: (A) SSI(+) and (B) SSI(–).

Table I. The Identified Compounds of Tokaj aszu of 1983 Vintage by Using SSI and ESI Techniques

Number	Name of compounds*	Number	Name of compounds
1	(+)-catechin	19	gentisic acid-Glu
2	caffeic acid	20	apigenin-7-*O*-Glu
3	apigenin	21	kaempherol-*O*-gallate
4	naringenin	22	catechin-3-*O*-Glu
5	(–)-epicatechin	23	kaempherol-3-*O*-Glu
6	(+)-gallocatechin	24	(–)-epicatechin-3-*O*-gallate
7	myricetin	25	quercetin-*O*-gallate
8	quercetin	26	epigallocatechin-*O*-gallate
9	kaempherol	27	*O*-ferruloyl-Glu
10	petunidin	28	pinocembrin-chalcone-*O*-Glu
11	chrysin	29	*p*-coumaroyl-kaempherol
12	theogallin	30	3,4-dimethoxycinnamoyl-*O*-Glu
13	ferrulic acid	31	pinocembrin-chalcone-*O*-di-Glu
14	clorogenic acid	32	apigenin-*O*-di-Glu
15	pinocembrin	33	(+)-gallocatechin-3-*O*-Ru
16	3,4-dimethoxycinnamic acid	34	quercetin-3-*O*-Ru
17	*O*-galloyl-Glu	35	epigallocatechin-3-*O*-Glu
18	resveratrol-3-*O*-Glu	36	kaempherol-3-*O*-Ru

* Glu: probably β-D-glucosyl, Ru: probably β-D-rutinosyl.

method has been proven to be extremely suitable for identification of the compounds, based on a set of standards and their HPLC relative retention times.

On the basis of the SSI-MS–MS method, several polyphenolic compounds have been identified in Tokaj aszu of 1983 vintage. Among the identified compounds, phenolic acid and their glycosides have been characterized.

The identified compounds are summarized in Table I. The exact anomeric configuration of the *O*-glucosides and the position of the galloyl-, ferruloyl-, and 3,4-dimethoxycinnamoyl residues at the glucose are yet not known.

Conclusion

Our results confirm that SSI overcomes the limitations of traditional ESI methods because it can ionize and analyze without using high voltage or high temperature. Thus, it is suitable mainly in negative ion mode for analyzing thermally unstable or highly polar compounds (or both) such as flavonoid-glycosides or carbohydrate derivatives of phenolic acids. SSI is also suitable for use with highly polar LC mobile phases.

Comprehensive information on the characteristic main nonvolatile organic constituents of a unique Hungarian wine speciality, Tokaj aszu, could be received by LC–SSI-MS techniques. By using the FNF technique, the undesired background compounds coming from the sample or from the mobile phase can be eliminated, resulting in easier ways for identification and for the application of the MS–MS mode.

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