The Determination of Ferulic Acid in Sugar Beet Pulp

PETRA JANKOVSKÁ, JANA ČOPÍKOVÁ and ANDRIY SINITSYA

Institute of Chemical Technology – Department of Carbohydrates Chemistry and Technology, Prague, Czech Republic

Abstract

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The content of ferulic acid in sugar beet pulp was determined by reverse phase high-pressure liquid chromatography (HPLC) and UV/VIS-spectroscopy. The acid extracts of pectin carrying feruloyl groups were prepared for analysis. To release ferulic acid from pectin the hydrolysis in alkaline medium (pH = 12.5) was performed. Both non-hydrolysed and hydrolysed extracts were measured by UV/VIS-spectroscopy after pH adjustment to the value of 10. The absorbance maximum was observed at 372 nm (ester of ferulic acid) for non-hydrolysed extracts and at 345 nm (sodium ferulate) for hydrolysed extracts. The HPLC estimation of ferulic acid was made in hydrolysed extracts only. The content of ferulic acid in sugar beet pulp was in the range of 0.3–0.9% (m/m). The data obtained by application of the particular methods to one set of samples were statistically compared. The results of all methods were in good agreement with each other.

Keywords: ferulic acid; sugar beet pulp; HPLC; UV/VIS-spectroscopy

Ferulic acid is a phenolic compound of plant origin with summary formula $C_{10}H_{10}O_4$. According to the systematic nomenclature, ferulic acid is called 3-(4-hydroxy-3-methoxyphenyl)propenoic acid (ANONYM 1996). The semisystematic name is 3-methoxy-4-hydroxycinnamic acid. The ferulic acid forms two geometric (*cis* and *trans*) isomers. The *trans* isomer is a white crystalline substance whereas the *cis*-isomer is a yellowish liquid substance (SCHOENROCK 1997). Ferulic acid occurs mainly as a *trans* isomer.

In the nature, ferulic acid is found as esters covalently bound to cell wall polysaccharides, i.e. particularly to cell wall pectins (FRY 1982), hemicelluloses and cereal pentosans. Sugar beet and spinach pectins are heteropolysaccharides that consist of rhamnogalacturonan backbone and neutral sugar side chains with linked feruloyl groups (VORAGEN *et al*. 1994). In citrus fruits, the ferulic acid occurs bound to oligosaccharides only. Citrus and apple pectins usually employed in confectionery do not contain any ferulic acid (MAGA *et al*. 1992).

Hemicelluloses from various plant sources also contain ferulic acid as a minor phenolic component. The chemical structure of cereal pentosans that belong to the total fibre is characterised by the linear chain of xylopyranose units and side chains of L-arabinofuranose residues (PUSSAYAWIN & WETZEL 1987). In the case of these polysaccharides, the ferulic acid is covalently bound to the ends of side chains.

The presence of feruloyl groups influences physical and chemical properties of cell wall polysaccharides. Ferulic acid can form dimers (SAULNIER *et al*. 1999) through oxidative coupling that may serve to cross-link cell-wall polymers and contribute to the mesh-like network of the cell wall (IIYAMA *et al*. 1994). This phenomenon participates in the formation of three-dimensional structure of plant cell wall (RALPH *et al*. 1994).

Oxidative gelation of cereal pentosans was reported to be of great importance in relation to the bread-making properties of wheat and rye flours (IZYDORCZYK & BILIADERIS 1995; VINKX & DELCOUR 1996). Oxidative gelatinization, which takes place during dough mixing, leads to a cross-linking of these polysaccharides as flour components by feruloyl coupling. This process contributes to the rheological properties of dough. The unsaturated carbon-carbon bonds of ferulic acid also react with the thiol groups of proteins and form an intermolecular cross-linking between polysaccharides and proteins (SMITH & HARTLEY 1983).

The sugar beet pectin has poor gelling properties in comparison with apple or citrus pectins. In the presence of oxidising agents the good quality gel from sugar beet pectin is formed (VORAGEN *et al*. 1986). Two ferulic acid

residues are coupled in the reaction to form a dimer, which is the third way to obtain gel from sugar beet pectins (VORAGEN *et al*. 1994).

In addition, ferulic acid and their derivatives have some non-food applications. For example, tocopheryl ferulate and C1-C30 esters of ferulic acid are used in cosmetics and pharmacology due to their antioxidative, antibacterial, anticancerous and antihepatotoxic effects (CROTTY *et al*. 1998; SCHOENROCK *et al*. 1997). These compounds are usually prepared by chemical synthesis.

The sugar beet pulp is a by-product of sugar industry and raw material for isolation of feruloyl contained sugar beet pectin. The determination of ferulic acid could be important for characterisation of sugar beet pulp as a potential source of this phenolic compound.

The aim of this study was the determination of ferulic acid in a set of 24 samples of sugar beet pulp by HPLC and UV/VIS-spectroscopy.

METHODS AND MATERIALS

Materials: The samples of sugar beet pulp were obtained from various sugar-factories at various harvesting times. The specification of samples is shown in Table 1. The ferulic acid standard (purum 98%) was purchased from Fluka AG, Germany. The common chemicals were bought from Lachema Ltd., Czech Republic.

Sample Preparation: Dry sugar beet pulp was ground on a laboratory grinder for 1 minute. 1 g of sample was placed into a flat-bottom flask and 100 ml of 0.2 mol/l HCl were added and kept for 30 min at laboratory temperature. Then the flask was put into a water bath at 85°C for 60 min. The warm suspension was filtrated and the final volume was measured. The pH of some extracts assigned as non-hydrolysed was adjusted by 0.2 mol/l NaOH to the value of 10. The extracts assigned as hydrolysed were prepared by one hour alkali hydrolysis (pH = 12.5) at 25ºC. The hydrolysis was finished by adding 0.2 mol/l HCl to obtain pH = 10.

Table 1. Specification of the samples of sugar beet pulp and the content of ferulic acid (% m/m) obtained by methods I–III

Sample No.	Sugar beet factory	Extractor type	Harvesting year	Ferulic acid content, % (m/m)		
				I	II	III
1	Opava-Vávrovice, Slezská a.s.	DdS	1995	0.543	0.521	0.560
2	Opava-Vávrovice, Slezská a.s.	DdS	1994	0.511	0.493	0.445
3	Opava-Vávrovice, Slezská a.s.	DdS	1994	0.523	0.538	0.396
4	Opava-Vávrovice, Slezská a.s.	DdS	1994	0.609	0.550	0.580
5	Opava-Vávrovice, Slezská a.s.	DdS	1995	0.782	0.764	0.772
6	Cukrovary Žatec, s.p.	KDP	1991	0.556	0.580	0.496
7	Cukrovary Žatec, s.p.	KDP	1991	0.389	0.434	0.304
8	Cukrovary Žatec, s.p.	KDP	1991	0.335	0.367	0.302
9	Cukrovary Žatec, s.p.	KDP	1991	0.938	0.925	0.925
10	Cukrovary Žatec, s.p.	KDP	1991	0.363	0.368	0.305
11	Severofrukt, Trávčice, s.p.	KDP	1991	0.483	0.447	0.401
12	Severofrukt, Trávčice, s.p.	KDP	1991	0.498	0.448	0.380
13	Severofrukt, Trávčice, s.p.	KDP	1991	0.469	0.414	0.508
14	Severofrukt, Trávčice, s.p.	KDP	1991	0.510	0.449	0.498
15	Severofrukt, Trávčice, s.p.	KDP	1991	0.394	0.401	0.328
16	Cukrospol. Praha-Modřany	KDP	1994	0.570	0.540	0.580
17	Cukrospol. Praha-Modřany	KDP	1994	0.446	0.409	0.398
18	Cukrospol. Praha-Modřany	KDP	1994	0.401	0.398	0.456
19	Severofrukt, Trávčice, s.p.	KDP	1992	0.456	0.489	0.412
20	Keblice, ZD	KDP	1991	0.510	0.470	0.456
21	Keblice, ZD	KDP	1991	0.421	0.453	0.420
22	Keblice, ZD	KDP	1991	0.407	0.491	0.395
23	Keblice, ZD	KDP	1991	0.362	0.351	0.402
24	Keblice, ZD	KDP	1991	0.600	0.564	0.586

UV/VIS Spectroscopy: UV/VIS absorption spectra of non-hydrolysed and hydrolysed extracts were measured on double-beam UV 4 Unicam UV/VIS-spectrometer, band width 2 nm. The wavelength range was 190–500 nm and the resolution was 0.2 nm. The ferulic acid standard was used for calibration. The spectra were evaluated by Vision 3.31 (Unicam) software.

Reverse-Phase HPLC: The hydrolysed extracts were used for HPLC analysis. The HPLC chromatography station was equipped with pump LCP 4000 (Ecom, Czech Republic), glass guard column 30 × 3.3 mm CGC 3.3-30 SZ (Tessek, Czech Republic), 250 × 4 mm Separon SGX C_{18} column (Tessek, Czech Republic) and variable wavelength UV/VIS-detector SpectroMonitor 3200 (Thermo Separation products, USA). The column was eluated isocratically at a rate of 1 ml/min. Citrate buffer (c = 0.01 mol per l, pH = 5.4) and methanol (88:12, V/V) were used as eluents. The measurement ran at laboratory temperature. Detection wavelength was 310 nm. This optimal value was obtained by evaluation of UV/VIS absorption spectra of 8 samples. The method of external standard was used for quantification. The chromatograms were evaluated by CSW 1.7 software (DataApex, Czech Republic).

RESULTS AND DISCUSSION

The UV/VIS absorption spectra of non-hydrolysed and hydrolysed extracts of sugar beet pulp are shown in Fig. 1. The absorbance maximum was observed at 372 nm for non-hydrolysed extracts and at 345 nm for hydrolysed extracts. These bands correspond to esters of ferulic acid and potassium ferulate, respectively (FRY 1982). The blue shift of the maximum indicated the hydrolysis of feruloyl groups and releasing of free ferulate. The HPLC chromatograms of hydrolysed extract and standard are shown in Fig. 2. Intense peaks eluted at T_r = 8 min indicated ferulic acid as a major phenolic component of hydrolysed extracts.

The samples of sugar beet pulp were analysed by the three described methods, i.e. UV/VIS absorption spec-

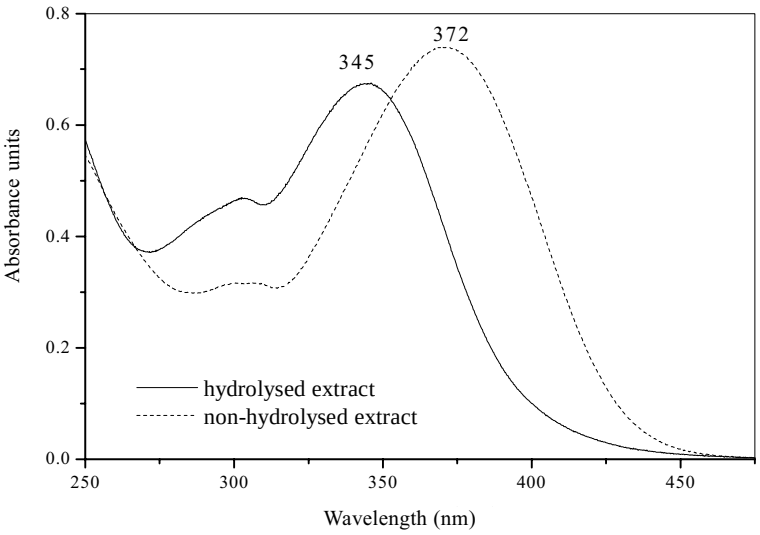

Fig. 1. UV/VIS absorption spectra of hydrolysed and non-hydrolysed extracts of the sample of sugar beet pulp

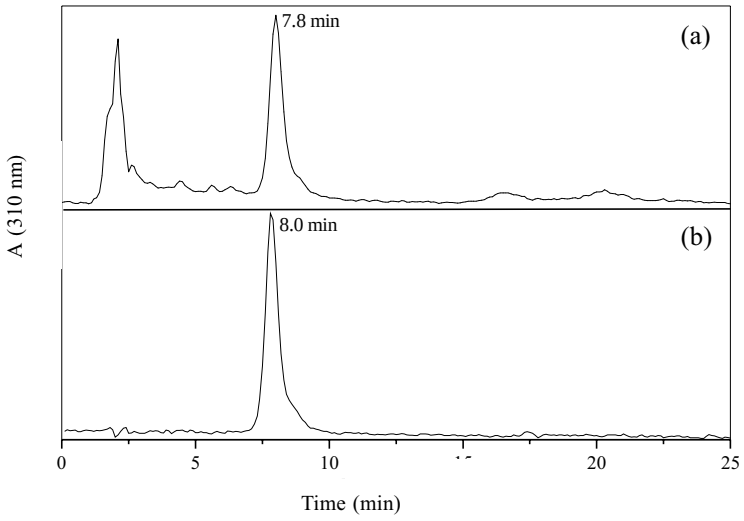

Fig. 2. HPLC chromatograms in C18 reverse-phase chromatography of non-hydrolysed extract of sugar beet pulp (a) and ferulic acid standard (b)

troscopy of non-hydrolysed and hydrolysed extracts (methods I and II, respectively) and HPLC of hydrolysed extracts (method III). The results are shown in Table 1. According to all these methods, the content of ferulic acid in sugar beet pulp was in the range of 0.3–0.9% (m/m). To evaluate the proposed methods one sample was analysed six times by each method and all extraction steps were repeated for each measurement. The obtained data, calculated averages and standard deviations are presented in Table 2.

Table 2. The values of six repeated measurements of the content of ferulic acid (%, m/m) in sample No. 9 determined by methods I–III and their statistical evaluation

Number of measurement	Ferulic acid content, % (m/m)		
	I	II	III
1	0.922	0.936	0.910
2	0.929	0.945	0.910
3	0.923	0.946	0.915
4	0.937	0.934	0.935
5	0.935	0.936	0.945
6	0.924	0.930	0.935
Mean	0.928 ± 0.006	0.938 ± 0.006	0.925 ± 0.015

The statistical evaluation of repeated measurements by *F*- and *t*-tests is shown in Table 3. The *F*-test proved that both methods I and II had the same variance as the value of *F*-test [$F_{0.05}$ (5.5) = 1.00] was lower than $F_{0.05}$(5.5) critical value [$F_{0.05}(5.5)_{critical}$ = 4.37]. On the contrary, method III had the different variance in comparison with both UV/VIS absorption methods. The results of t-test proved that the difference between averages of all three methods was insignificant as the statistical values of *t*-test $t_{0.05}(6)_{stat}$ (Table 2) were lower than or equal to the critical value $t_{0.05}(6)_{critical}$ = 2.57 for all comparisons.

Table 1 shows the content of ferulic acid in samples of sugar beet pulp from different regions of Bohemia, sugar factories, extractor types and harvesting years. The effect of Bohemian regions, harvesting years and the extractor type in a sugar factory on the content of ferulic acid in sugar beet pulp was not observed. The set of samples was not large enough to explain the differences between samples.

Table 3. Statistical evaluation of repeated measurements

Compared methods	$F_{0.05}$		$t_{0.05}$	
	$(5,5)_{stat}$	$(5,5)_{critical}$	$(5)_{stat}$	$(5)_{critical}$
I and II	1.00	4.46	0.50	2.57
I and III	5.56	4.46	1.92	2.57
II and III	5.55	4.46	2.57	2.57

The results of the particular methods were compared by linear regression analysis (Fig. 3). It can be concluded that there is a significant linear correlation (at the 0.05 level of significance). The slopes of regressions equations are near to 1 and the correlation coefficients are higher than 0.9. Therefore, the results obtained by the described methods correlate with each other.

The determination of ferulic acid by HPLC and UV/VIS absorption spectroscopy involves complex procedures of sample preparation that complicate the analysis. More-

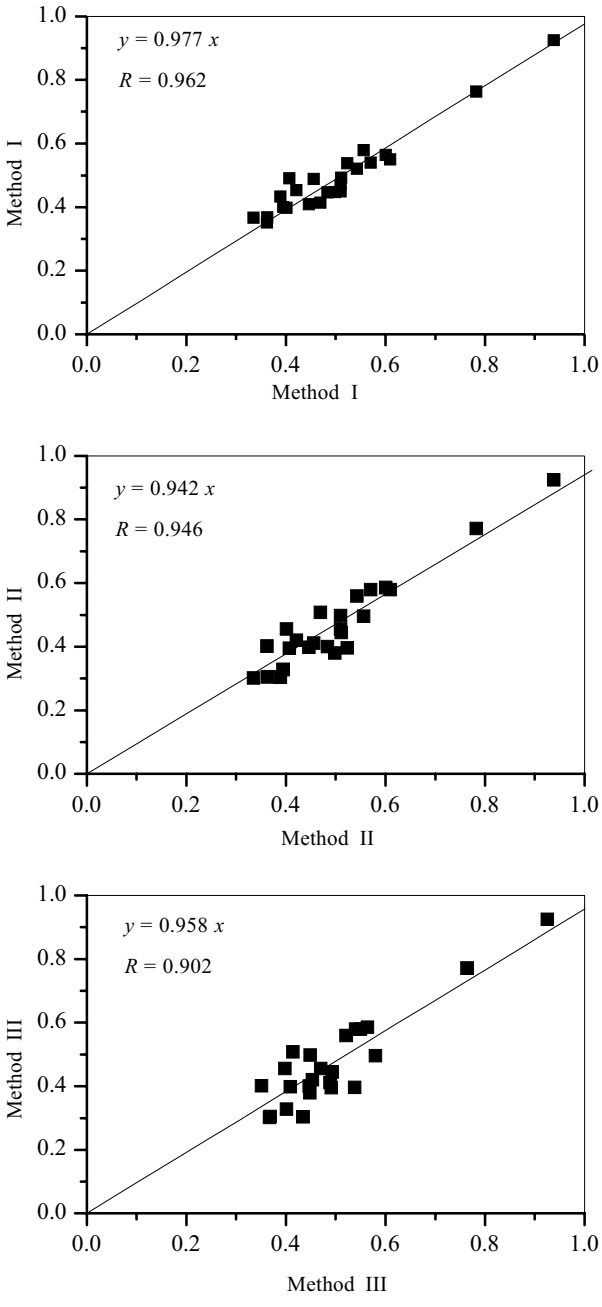

Fig. 3. Regression lines obtained for compared methods I–III of ferulic acid determination in sugar beet pulp

over, the contents of ferulic acid in the samples were relatively low, i.e. less than 1% (m/m) of dry matter. Nevertheless, all described methods are suitable and reliable for determination of ferulic acid in sugar beet pulp.

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Souhrn

JANKOVSKÁ P., ČOPÍKOVÁ J., SINITSYA A. (2001): **Stanovení kyseliny ferulové ve vzorcích vyslazených cukrových řízků**. Czech J. Food Sci., **19**: 143–147.

Ve vzorcích vyslazených řepných řízků byla pomocí UV/VIS-spektroskopie a vysokoúčinné kapalinové chromatografie (HPLC) stanovována kyselina ferulová. Pro stanovení byly připravovány kyselé extrakty. Pro uvolněni kyseliny ferulové vázané na řepný pektin pomocí esterové vazby byla prováděna alkalická hydrolýza (pH = 12,5). Pomocí UV/VIS-spektroskopie byly měřeny oba extrakty (nehydrolyzovaný a hydrolyzovaný) po úpravě pH na hodnotu 10. Maximum absorbance nehydrolyzovaného extraktu bylo 372 nm, což odpovídá esteru kyseliny ferulové. Maximum absorbance hydrolyzovaného extraktu bylo 345 nm, což odpovídá ferulátu sodnému. Pomocí HPLC byl zkoumán pouze obsah kyseliny ferulové v extraktech po alkalické hydrolýze. Obsah kyseliny ferulové v řepných řízcích se pohyboval od 0,3 do 0,9 %. Data získaná použitím jednotlivých metod při měření jednoho souboru vzorků byla statisticky porovnána. Výsledky všech použitých metod byly v dobré shodě.

Klíčová slova: kyselina ferulová; vyslazené řepné řízky; HPLC; UV/VIS-spektroskopie

Corresponding author:

Ing. PETRA JANKOVSKÁ, Vysoká škola chemicko-technologická, Ústav chemie a technologie sacharidů, Technická 5, 166 28 Praha 6, Česká republika

tel.:+420 2 24 35 31 13, fax.: +420 2 311 99 90, e-mail: petra.jankovska@vscht.cz