Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 606–611
Food Chemistry
www.elsevier.com/locate/foodchem

A rapid microwave-assisted derivatization process for the determination of phenolic acids in brewer's spent grains

Mallouchos Athanasios, Lagos Georgios, Komaitis Michael *

Agricultural University of Athens, Food Science and Technology Department, Iera Odos 75, Athens 11855, Greece

Received 3 February 2006; received in revised form 8 May 2006; accepted 21 May 2006

Abstract

A simple and fast method for the TMS derivatization of phenolic acids in a closed vial using microwave irradiation followed by GC/MS analysis is proposed. A full factorial design was used to investigate the effects of two independent variables, namely, time and power of microwave irradiation, on the yield of silylation reaction. The optimal conditions were tested against the classical heating derivatization procedure. No significant differences were found between the classical heating and microwave-assisted derivatization. Chromatographic separation of the nine phenolic acids examined was achieved in 16 min. The mass spectral fragmentation patterns of the derivatives obtained by the proposed method were identical to those from the classical heating. Four different batches of brewer's spent grain were extracted and analyzed for the total phenolic acid content. Significant differences between the batches of spent grains were found for all analytes. The total phenolic acid content varied between 2688 and 4884 μg/g.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Phenolic acids; GC/MS; Microwave; TMS derivatives; Spent grain

1. Introduction

Phenolic acids are a group of aromatic secondary plant metabolites widely spread throughout the plant kingdom. There is a great interest in the food industry because they improve the quality, the nutritional value and the antioxidative properties of foods (Adom & Liu, 2002; Herrmann, 1989; Maga, 1978; Parr & Bolwell, 2000). Brewer's spent grain (BSG) is the major by-product of the brewing industry. It is available in large quantities, but its main application has been limited to animal feeding. Recently, attempts have been made to use BSG as a source of phenolic acids (Bartolomé, Santos, Jiménez, Del Nozal, & Gómez-Cordovés, 2002; Mussatto, Dragone, & Roberto, 2006).

The most common technique used for the qualitative and quantitative analysis of phenolic acids in plants, including grains, is high performance liquid chromatography with photodiode array or electrochemical detection (Chen, Zuo, & Deng, 2001; Robbins, 2003). However, HPLC often does not provide sufficient separating performance and the UV–Vis spectrum does not supply sufficient identifying power. To overcome these problems many researchers have employed LC/MS or GC/MS technique (Ayaz, Hayirlioglou-Ayaz, Gruz, Novak, & Strnad, 2005; Naczk & Shahidi, 2004; Robbins, 2003). Phenolic acids have low volatility and decompose when heated above their melting point (158–251 °C) (Stadler, Welti, Stampfli, & Fay, 1996). To tackle this problem in GC analysis, silylation derivatization technique was employed. Sosulski, Krygier, and Hogge (1982) and Krygier, Sosulski, and Hogge (1982a) developed a procedure for the determination of free, esterified and insoluble-bound phenolic acids

Abbreviations: BSG, brewer's spent grain; MSTFA, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide; BSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; BSA, *N*,*O*-bis(trimethylsilyl)acetamide; TMS, trimethylsilyl; PTFE, polytetrafluoroethylene; TMCS, trimethylchlorosilane; IS, internal standard; TPAR, ratio of the total peak area by microwave heating relative to the total peak area by classical procedure; RRT, relative retention time.

* Corresponding author. Tel./fax: +30 210 5294681.
E-mail address: achem@aua.gr (K. Michael).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.05.040

in various cereals. Zuo, Wang, and Zhan (2002) reported a GC/MS method for the determination of 15 phenolic acids as trimethylsilyl derivatives in cranberry juice. Wu, Haig, Prately, Lemerle, and An (1999) investigated phenolic acids in wheat using an excess of MSTFA as derivatizing agent followed by gas chromatography–tandem mass spectrometry. However, these derivatization procedures require typically half to one hour. Recently, Chu, Chang, Liao, and Chen (2001) employed microwave energy in conjunction with BSA to promote the derivatization of phenolic acids in wine.

The aim of this work was to improve the microwave-accelerated derivatization procedure for phenolic acids by optimizing the parameters through experimental design as well as by employing the same glass apparatus as that used in classical derivatization methods. BSTFA was the derivatizing agent instead of BSA. The proposed protocol was used in the analysis of phenolic compounds in BSG samples.

2. Materials and methods

2.1. Materials

trans-Ferulic, *trans*-*p*-coumaric, *p*-hydroxybenzoic, vanillic, syringic, protocatechuic, salicylic, *trans*-caffeic and gallic acid were purchased from Sigma–Aldrich (Greece). Stock solutions (1000 mg/l) of these acids were prepared in methanol. All solvents used were of analytical grade. Spent grains were provided from a local brewery in wet form. They were dried at 60 °C overnight, ground in an electrical grinder to obtain a fine powder and stored in air-tight bags for further analyses. A domestic microwave oven (KOG-3767, DAEWOO) used in this study had a total capacity of 850 W.

2.2. TMS derivatization of phenolic acids

To obtain the TMS derivatives of phenolic acids, 0.5 ml of a working solution (10 mg/l) of phenolic acids was added in a conical vial (10 ml) with PTFE-lined screw-cap, and dried under nitrogen. Then, 0.3 ml pyridine, 0.2 ml BSTFA plus 1% TMCS (Sigma–Aldrich) and 0.05 ml internal standard [tetradecane (100 mg/ml) was used in the case of optimization experiments/salicylic acid (10 mg/ml) was used in the case of the construction of calibration curves and in the samples] were added. The resulting mixture was irradiated in a microwave oven. Two parameters, time and microwave power were examined using a full factorial design (Table 1) in order to find the optimum conditions for derivatization. For comparison reasons, TMS derivatives of phenolic acids were prepared in a similar manner, using the conventional heating method in a water bath at 70 °C for 30 min. All statistical analyses were carried out using the Statistica 6.0 software (Stat Soft Inc.).

Table 1
Analytical conditions as input factors and total peak area ratio (TPAR)[a] as the output factor in a full factorial experiment[b]

Standard order[c]	Run order[d]	Time (s)	Power (W)	TPAR
1	3	30	850	1.01
2	4	90	220	1.02
3	5	90	620	0.990
4	8	180	620	1.00
5	9	180	850	1.08
6	1	30	220	1.05
7	6	90	850	0.965
8	7	180	220	0.986
9	2	30	620	1.05

[a] TPAR: ratio of the total peak area by microwave heating relative to the total peak area by classical procedure.
[b] The design was replicated twice (total number of runs 27).
[c] No randomized.
[d] Randomized.

2.3. Isolation of phenolic acids

The total phenolic acid content in brewer's spent grains was determined by adding 1 N NaOH (10 ml) to the sample (0.5 g oven dried BSG) followed by incubation for 20 h at 20 °C under N_2 atmosphere and stirring. After centrifugation (7000 rpm for 20 min), the supernatant was collected, acidified with concentrated HCl to pH 2 and extracted six times with diethyl ether/ethyl acetate (1:1 v/v) at a solvent to water phase ratio of 1:1. The extracts were combined and evaporated to dryness in a rotary evaporator (30 °C). The dry residue was transferred with 4 ml of solvent into vials containing anhydrous sodium sulfate and kept refrigerated until further use.

2.4. GC/MS instrumentation and conditions

The GC analyses were performed using a FISONS 8000 Series GC system (Model 8060) interfaced to an MD-800 (FISONS) quadrupole mass-selective detector. The mass spectra for the TMS analytes were obtained via electron impact ionization at 70 eV. The transfer line was maintained at 300 °C. Detection was performed in the full scan mode (mass range 50–500 m/z, rate 1.5 scans/s). The GC was equipped with a split/splitless injector and a CP-Sil 8CB capillary column (Chrompack) (30 m × 0.32 mm, 0.25 μm film thickness). Oven temperature was programmed from 80 to 250 °C at 10 °C/min and then raised to 280 °C with a rate of 20 °C/min. It remained there for 2 min. Injector temperature was set at 260 °C and the injection volume was 1 μl in the splitless mode for 30 s. Helium (99.999% purity) was used as a carrier gas with a flow rate of 1 ml/min.

2.5. Identification and quantitation

The phenolic acids in the BSG extracts were identified by matching retention times and mass spectral data with those of the authentic compounds and from Wiley and Nist libraries. A series of five standard composite mixture solu-

Table 2
Characteristics ions and relative abundances[a] of the TMS derivatives of phenolic acids

Phenolic acid	MW of the derivative formed	$[M]^+$	$[M-H_3]^+$	$[M-CH_2O]^+$	$[M-CH_3-CO_2]^+$	$[M-TMSO]^+$	$[M-177]^+$
4-Hydroxy-benzoic (*p*-hydroxy-benzoic)	138 + 2TMS = 282	282 (17/17)	267 (89/86)[b]		223 (88/85)	193 (68/66)	105 (4/4)
2-Hydroxy-benzoic (salicylic)	138 + 2TMS = 282		267 (55/51)[b]			193 (6/6)	105 (1/1)
4-Hydroxy-3-methoxy-benzoic (vanillic)	168 + 2TMS = 312	312 (38/41)	297 (79/82)[b]	282 (26/28)	253 (47/51)	223 (55/64)	135 (8/10)
4-Hydroxy-cinnamic (*trans-p*-coumaric)	164 + 2TMS = 308	308 (30/30)[b]	293 (49/49)[b]		249 (32/32)	219 (62/60)	131 (2/2)
4-Hydroxy-3-methoxy-cinnamic (*trans*-ferulic)	194 + 2TMS = 338	338 (45/46)[b]	323 (34/34)[b]	308 (30/32)	279 (8/8)	249 (33/33)	161 (4/4)
3,4,5-Trihydroxy-benzoic (gallic)	170 + 3TMS = 458	458 (38/36)[b]	443 (15/14)		399 (5/5)	369 (2/3)	281 (84/88)
3,4-Dihydroxy-cinnamic (caffeic)	180 + 3TMS = 396	396 (28/26)[b]	381 (8/7)			307 (5/5)	219 (58/60)
4-Hydroxy-3,5-dimethoxy-benzoic (syringic)	198 + 2TMS = 342	342 (30/29)[b]	327 (50/52)	312 (36/39)	283 (12/14)	253 (26/29)	165 (4/4)
3,4-Dihydroxy-benzoic (protocatechuic)	154 + 3TMS = 370	370 (25/23)[b]	355 (15/14)		311 (14/14)	281 (7/7)	193 (100/100)

[a] The numbers in parentheses indicate relative abundances obtained by microwave irradiation and conventional heating, respectively.
[b] Target ions used for the quantitation.

tions of various concentrations were analyzed in triplicate and used for the construction of the calibration curves. The derivatives were quantified using the peak area ratios (analyte-to-IS) of the target ions as given in Table 2.

3. Results and discussion

3.1. Confirmation of the derivatives

The classical derivatization procedure using conventional heating requires a reaction time of half to one hour upon completion of the reaction because heat transfer occurs mainly by conduction. The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. Polar molecules (such as phenolic acids) and ionic solutions absorb microwave energy strongly because they have a permanent dipole moment. This results in rapid rise of the temperature and fast completion of the reaction (Eskilsson & Björklund, 2000). To exploit this advantage, a common microwave oven and PTFE-lined screw-capped vials commonly used in classical derivatization procedures were employed. There was no need for modification of the apparatus.

The silylation reaction is a nucleophilic substitution reaction. The strong electron withdrawing group (CF_3) of BSTFA stabilizes the negative charge of the leaving group and consequently facilitates the occurrence of the substitution reaction. Oxygen atom of the hydroxyl groups of phenolic acids acts as a nucleophilic site where the TMS group is covalently linked (Chu et al., 2001).

The efficiency of separation and detection of phenolic acids using the GC/MS technique was tested on a standard mixture consisting of the nine phenolic acid TMS derivatives and the internal standard. The instrument parameters were optimized to obtain a baseline separation of the analytes in a short elution time (16 min). The retention times of the derivatives obtained by microwave heating were similar to those obtained by classical heating. The linearity of each phenolic acid was examined by the construction of calibration curves. Satisfactory linearity was obtained as the correlation coefficients were very high (>0.997).

From the characteristic main fragments and their relative abundances shown in Table 2, it is evident that the fragmentation patterns of all of the derivatives obtained by microwave heating are similar to those obtained by classical heating. The molecular ion $[M]^+$ for all of the TMS derivatives is a prominent peak in the mass spectrum. Generation of the [M−15] fragment (loss of a methyl group via α-cleavage) and the [M−59] fragment (subsequent loss of CO_2 after rearrangement) are well established cleavage patterns for TMS esters. Loss of TMSO, [M−89], is also a fragmentation pathway common for derivatized carboxylic acids, because the acylium cation formed is a stable species. Derivatives possessing a methoxy group on the phenyl ring, such as vanillic, ferulic and syringic acid, produce the [M−30] fragment which represents the loss of a molecule of formaldehyde. For gallic, caffeic and protocatechuic acids, the major fragmentation route generates a predominant [M−177] peak. However, there are no reports describing the origin of this fragment for phenolic acids (Robbins, 2003). The base peak in all mass spectra, except from that of protocatechuic, was the fragment with *m*/*z* 73, representing the TMS group.

3.2. Optimization of the derivatization process

After the confirmation of the derivatives of the phenolic acids, it was necessary to examine the effect of microwave power and time of applying this power to the reaction

mixture on the yield of the silylation reaction. A full factorial experimental design with two factors, three levels and two replications, with a total number of 27 runs, was employed (Table 1). The results were analyzed by using multiple regression analysis and visualized by response surface plots. The output factor for the trials was the ratio of the total peak area by microwave heating relative to the total peak area by classical procedure (TPAR). The target was to obtain a value equal or greater to 1. A value of 1 means that the yield of the silylation reaction using microwave heating is equal to that using conventional heating. The values of TPAR ranged from 0.965 to 1.08 and in most cases were greater to 1 (Table 1). Analysis of variance (ANOVA) of the independent variables showed that both quadratic and interaction effects were highly significant ($p < 0.001$ or $p < 0.05$). However, the linear effects were insignificant. The coefficient of determination indicating the model's ability to explain the relationships between the factors was 64.6%. Fig. 1 depicts response surface of the effects of the two variables, namely time and microwave power, on TPAR. It is evident that the highest settings of time and power (180 s, 850 W) increased significantly the total peak area ratio, i.e. the yield of silylation reaction. Thus, these settings were chosen for further experimentation as the most appropriate. However, one can choose a lower setting of time (30 s) in order to speed up the procedure and get similar results with those of conventional heating. Higher settings of time, more than 180 s are not recommended for safety reasons (high pressure is formed inside the glass vial and there is risk of cracking).

To compare the efficiency of microwave irradiation under the chosen settings (180 s, 850 W) a separate experiment was conducted. The data were compared with the results obtained by the classical heating method and presented in Fig. 2. Unpaired *t*-tests showed that there were no statistical differences at the 95% significance level between the peak areas of each phenolic acid under the different reaction conditions. However, microwave heating gave slightly higher peak areas, in most cases, than the classical method. Five replicate analyses were performed utilizing each method with the RSDs ranging from 1.5% to 10%. Chu et al. (2001) investigated microwave-accelerated derivatization of phenolic acids in a vessel with a cooling trap and employed BSA as the silylating agent. Although the experimental conditions were quite different (reaction vial, derivatizing agent, microwave power and time), their findings were similar to ours.

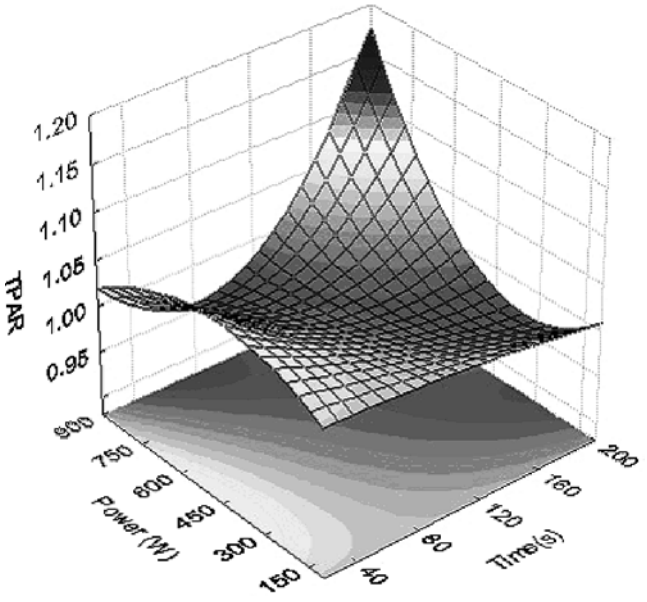

Fig. 1. Response surface plot for the effects of microwave power and time on total peak area ratio (TPAR) of phenolic acids.

3.3. Sample analysis

The proposed method was employed in the analysis of brewer's spent grains extracts. Four batches of different time periods were analyzed in triplicate. The major phenolic acids found after alkaline hydrolysis of the spent grains were *trans*-ferulic and *trans*-*p*-coumaric acid (Table 3), as expected (Nordkvist, Salomonsson, & Aman, 1984). GC/MS data showed that two peaks with RRT 1.65 and 1.84 had nearly identical mass spectra with those of authentic *trans*-*p*-coumaric and *trans*-ferulic acid TMS derivatives, respectively. These peaks were tentatively identified as the *cis*-isomers of the corresponding acids. Accordingly to Jung, Jeon, and Bock (2002) and Krygier, Sosulski, and Hogge (1982b), authentic *trans*-isomers were treated with 4 N NaOH for 4 h at room temperature to produce the *cis*-forms. The mixture, after TMS derivatization, exhibited peaks with the same RRT as the ferulic and *p*-coumaric acid isomers present in the spent grains samples. Based on these results, the two peaks were confirmed as the *cis*-isomers of *p*-coumaric and ferulic acids. They were quantified using the calibration curves of the *trans*-isomers, as their mass spectra were similar.

The ferulic acid content (sum of isomers) of the spent grain samples varied between 1805 and 3099 μg/g, while that of *p*-coumaric acid (sum of isomers) fluctuated

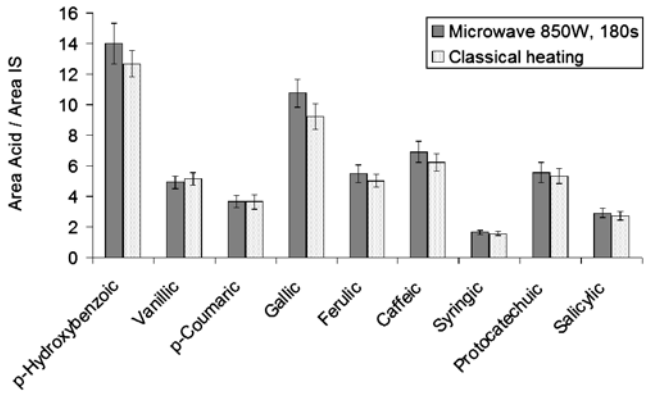

Fig. 2. Comparison of phenolic acids derivatives produced by microwave irradiation and classical heating (mean value of five replicates and standard deviation).

Table 3
Phenolic acid content in spent grains (μg/g)

Analyte	RRT	Spent grains			
		Batch 1	Batch 2	Batch 3	Batch 4
m-Hydroxy-benzoic acid	1.38	n.q.	n.q.	n.q.	n.q.
p-Hydroxy-benzoic acid	1.39	10.6 ± 1.7^{a}	16.3 ± 0.0^{b}	11.1 ± 0.6^{a}	11.5 ± 0.2^{a}
Vanillic acid	1.61	23.2 ± 1.8^{a}	20.4 ± 3.3^{a}	32.1 ± 1.1^{b}	34.9 ± 0.1^{b}
cis-*p*-Coumaric acid	1.65	252 ± 21^{a}	188 ± 3^{b}	308 ± 10^{c}	350 ± 7^{d}
Protocatechuic acid	1.70	n.q.	n.q.	n.q.	n.q.
Syringic acid	1.81	12.7 ± 0.7^{a}	13.1 ± 0.6^{a}	17.4 ± 0.7^{b}	18.4 ± 0.7^{b}
cis-Ferulic acid	1.84	180 ± 7^{a}	147 ± 11^{b}	317 ± 5^{c}	383 ± 18^{d}
trans-*p*-Coumaric acid	1.86	998 ± 159^{a}	645 ± 78^{b}	1064 ± 105^{a}	1373 ± 115^{c}
Gallic acid	1.91	n.q.	n.q.	n.q.	n.q.
cis-Caffeic acid	1.94	n.q.	n.q.	n.q.	n.q.
trans-Ferulic acid	2.07	1737 ± 136^{a}	1658 ± 180^{a}	2134 ± 168^{b}	2716 ± 202^{c}
trans-Caffeic acid	2.14	n.q.	n.q.	n.q.	n.q.
Sinapic acid	2.27	n.q.	n.q.	n.q.	n.q.
Sum of isomers of ferulic acid		1917 ± 143^{a}	1805 ± 191^{a}	2451 ± 174^{b}	3099 ± 220^{c}
Sum of isomers of coumaric acid		1251 ± 180^{a}	833 ± 81^{b}	1372 ± 115^{a}	1720 ± 122^{c}
Total phenolic acid content		3214 ± 327^{a}	2688 ± 275^{b}	3883 ± 292^{c}	4884 ± 343^{d}

Mean values of three replicates ±SD (μg/g), n.q.: not quantified, RRT: retention time relative to tetradecane, a–d: different letters indicate significant differences ($p < 0.05$).

between 833 and 1720 μg/g. These values are in agreement with data reported by other authors (Bartolomé et al., 2002; Hernanz et al., 2001). The ferulic/*p*-coumaric acid ratio varied between 1.53 and 2.17. This was in accordance with findings of other researchers (Bartolomé et al., 2002). Furthermore, spent grains exhibited 5–10-fold higher levels of phenolic acids than the unprocessed barley (Hernanz et al., 2001; Maillard & Berset, 1995). *p*-Hydroxy-benzoic, vanillic and syringic acids were also found, but in lesser amounts (Table 3). These acids were not quantitatively determined in BSG previously by HPLC. *m*-Hydroxy-benzoic and sinapic acid were found in trace amounts according to peak heights. They were not quantified due to lack of authentic compounds. ANOVA showed significant differences ($p < 0.05$) in phenolic acid content among the different spent grain batches, which is in agreement with other investigators (Bartolomé et al., 2002; Hernanz et al., 2001). Phenolic acids are minor compounds in cereals. Their amount depends on the variety, time of harvest and the characteristics of the growing region. This explains the observed differences between the different batches.

To evaluate the whole analytical method (extraction, derivatization, GC/MS analysis), a recovery test was performed. Different known amounts of standard compounds were subjected to the entire analytical procedure with the samples in triplicate. The results are given in Table 4. Only 50% of the phenolic acids tested showed excellent recovery rates (over 90%). Although the protocatechuic, gallic, caffeic, *cis*-*p*-coumaric and *cis*-ferulic acids were identified from mass fragments, only the last two were quantitatively determined. The losses of the first three acids can be attributed to the reactive nature of the *o*-dihydroxy-phenols, which are more susceptible to oxidation to *o*-quinone than partially methylated phenolics, like ferulic acid (Krygier et al., 1982a; Sosulski et al., 1982). Reproducibility of the whole analytical procedure was satisfactory, with relative standards deviations ranging from 3.12% to 14.9%. The recovery test also proved that the *cis*-isomers of ferulic and *p*-coumaric acids occurred naturally in the spent grains samples, and were not produced as artifacts of the extraction. This was concluded from the fact, that their amounts in the unspiked samples of spent grains were the same with those of the spiked samples with the *trans*-isomers.

Table 4
Recovery (%) and RSD (%) of the whole analytical procedure

Analyte	Recovery (%)	RSD (%)
p-Hydroxy-benzoic acid	93.8	8.29
Vanillic acid	98.5	7.92
cis-*p*-Coumaric acid	n.d.	8.12
Protocatechuic acid	n.d.	n.d.
Syringic acid	94.4	5.62
cis-Ferulic acid	n.d.	3.95
trans-*p*-Coumaric acid	106	14.9
Gallic acid	n.d.	n.d.
trans-Ferulic acid	93.1	7.85
trans-Caffeic acid	n.d.	n.d.

Mean values from three replicates; n.d.: not determined.

4. Conclusion

The above results suggest that the preparation of TMS derivatives of phenolic acids using microwave irradiation in a closed vial is a very efficient method. It gives derivatives identical with those produced by the conventional heating method. Yields were better in most cases. Although the same glass apparatus was used, the derivatization time was reduced to a few seconds. Thirteen phenolic acids were identified in BSG samples, whereas seven of them were quantified. The method described in this report may also be used for the identification and quantification of phenolic acids in other agricultural by-products and plant-derived

foods. However, a potential problem should be mentioned. The localized extremely high temperature developing during the microwave process can decompose some heat sensitive phenolic compounds. If such decomposition is observed, the classical derivatization technique shall be the method of choice.

Acknowledgments

The project is co-funded by European Social Fund and National Resources-EPEAEK II.

References

Adom, K. K., & Liu, H. R. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*, 6182–6187.

Ayaz, A. F., Hayirlioglou-Ayaz, S., Gruz, J., Novak, O., & Strnad, M. (2005). Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC–MS. *Journal of Agricultural and Food Chemistry, 53*, 8116–8122.

Bartolomé, B., Santos, M., Jiménez, J. J., Del Nozal, J. M., & Gómez-Cordovés, C. (2002). Pentoses and hydroxycinnamic acids in brewer's spent grain. *Journal of Cereal Science, 36*, 51–58.

Chen, H., Zuo, Y., & Deng, Y. (2001). Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. *Journal of Chromatography A, 913*, 387–395.

Chu, Y. T., Chang, H. C., Liao, C. Y., & Chen, C. Y. (2001). Microwave-accelerated derivatization process for the determination of phenolic acids by gas chromatography–mass spectrometry. *Talanta, 54*, 1163–1171.

Eskilsson, S. C., & Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A, 902*, 227–250.

Hernanz, D., Nunez, V., Sancho, I. A., Faulds, B. C., Williamson, G., Bartolomé, B., et al. (2001). Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. *Journal of Agricultural and Food Chemistry, 49*, 4884–4888.

Herrmann, K. (1989). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Critical Reviews in Food Science and Nutrition, 28*, 315–347.

Jung, Y. M., Jeon, S. B., & Bock, Y. J. (2002). Free, esterified and insoluble-bound phenolic acids in white and red Korean gingsengs (*Panax gingseng* C.A. Meyer). *Food Chemistry, 79*, 105–111.

Krygier, K., Sosulski, F., & Hogge, L. (1982a). Free, esterified and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry, 30*, 330–334.

Krygier, K., Sosulski, F., & Hogge, L. (1982b). Free, esterified and insoluble-bound phenolic acids. 2. Composition of phenolic acids in rapeseed flour and hulls. *Journal of Agricultural and Food Chemistry, 30*, 334–336.

Maga, J. A. (1978). Simple phenol and phenolic compounds in food flavor. *Critical Reviews in Food Science and Nutrition, 10*, 323–372.

Maillard, N. M., & Berset, C. (1995). Evolution of antioxidant activity during kilning: role of insoluble bound phenolic acids of barley and malt. *Journal of Agricultural and Food Chemistry, 43*, 1789–1793.

Mussatto, I. S., Dragone, G., & Roberto, C. I. (2006). Brewer's spent grain: generation, characteristics and potential applications. *Journal of Cereal Science, 43*, 1–14.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A, 1054*, 95–111.

Nordkvist, E., Salomonsson, A. C., & Aman, P. (1984). Distribution of insoluble bound phenolic acids in barley grain. *Journal of the Science of Food and Agriculture, 35*, 657–661.

Parr, A. J., & Bolwell, G. P. (2000). Phenols in plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture, 80*, 985–1012.

Robbins, J. R. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry, 51*, 2866–2887.

Sosulski, F., Krygier, K., & Hogge, L. (1982). Free, esterified and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flour. *Journal of Agricultural and Food Chemistry, 30*, 337–340.

Stadler, H. R., Welti, H. D., Stampfli, A. A., & Fay, B. L. (1996). Thermal decomposition of caffeic acid in model systems: identification of novel tetraoxygenated phenylindan isomers and their stability in aqueous solution. *Journal of Agricultural and Food Chemistry, 44*, 898.

Wu, H., Haig, T., Prately, J., Lemerle, D., & An, M. (1999). Simultaneous determination of phenolic acids and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in wheat (*Triticum aestivum* L.) by gas chromatography–tandem mass spectrometry. *Journal of Chromatography A, 864*, 315–321.

Zuo, Y., Wang, C., & Zhan, J. (2002). Separation, characterization and quantitation of benzoic and phenolic antioxidants in American Cranberry fruit by GC/MS. *Journal of Agricultural and Food Chemistry, 50*, 3789–3794.