

Content of Individual Phenolic Acids in Worts and Beers and their Possible Contribution to the Antiradical Activity of Beer

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ABSTRACT

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Phenolic acids are widely distributed in foods and raw materials. They are easily absorbed by humans due to their simplicity. Once they enter the blood plasma, they act as antioxidants. Beer can be a rich source of phenolic acids in the diet. The aim of this study was to determine the concentrations of phenolic acids in two experimental worts and beers as well as in nine market beers (using HPLC-UV). An examination of the total antiradical activities of phenolic acids with *in vitro* model systems (using ABTS and DPPH free radicals), at the concentrations comparable to those detected in beers, was performed. Only low fractions of the main phenolic acids present in barley malt (ferulic, vanillic and p-coumaric acid) were detected in the experimental worts. Moreover, the concentrations of phenolic acids significantly decreased until the last steps of beer production. The main beer phenolic acids (vanillic and ferulic acid) exerted a lower share of total antiradical activity against both free radicals (calculated as the sum of the individual activities of all acids detected in beer) than the minor phenolic acids (caffeic, chlorogenic, o-coumaric, sinapic or syringic acid). The synergies, between individual phenolic acids in pairs, were also studied with *in vitro* model solutions using free radicals. The total antiradical activity of the compounds studied in pairs, was at the most as high as the sum of the antiradical activity of the individual phenolic acids, but in most cases it was considerably lower (i.e. no synergy was detected).

Key words: antioxidant, beer, ferulic acid, phenolic acids, synergy, wort.

Abbreviations: ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl radical, TAA: total antiradical activity.

INTRODUCTION

Barley and beer are rich sources of phenolic compounds that can be divided into three groups: flavan-3-ols derivatives, flavon glycosides and phenolic acids^{23,33,48}. These phenolics are described as powerful antioxidants *in vitro*^{16,20,34,35,42} and as very strong anticarcinogenic compounds *in vivo*⁶⁰. A growing number of papers address the

absorption of phenolic compounds from the gastrointestinal tract and their transformations in blood plasma^{8,21,29,41,44,47,50,52,55,58}. Many of the existing papers in this subject area prove undoubtedly that phenolic acids (Fig. 1), among all classes of phenolic compounds in foodstuffs (including barley and beer), are the most easily absorbed from the intestinal tract^{41,45}. Phenolic acids from foods have been detected in urine⁹ and are present in blood in considerable concentrations¹³. Even when the consumption of more complex phenolic compounds is high, they are degraded and simple phenolic acids are excreted^{14,26,53,54}. The relative simplicity of phenolic acids is the reason why they are degraded to a lesser extent than stronger antioxidants *in vitro*, such as proanthocyanidins, and as a consequence, they can exert antioxidant activity *in vivo*¹⁸. The distribution of phenolic acids in foods and raw materials is wide, and the concentrations are considerable. For example, ferulic acid, the main phenolic acid in barley and beer, is present in the esterified form, but an esterase activity of bacterial origin is present in the human gastrointestinal tract^{1,15}. Once phenolic acids enter the human body, their role cannot be underestimated. For example, since the early 1980s, a great number of papers concerning the role of oxidative stress in the development of many diseases have appeared. Among others, the role of free radical formation in neuronal and glial cell death in the central nervous system has also been identified in the pathogenesis of serious neurodegenerative diseases, such as Alzheimer's disease^{2,7,57,59}. Alzheimer's disease and other non-Alzheimer's dementias can be taken as good examples of possible future studies involving free radicals and natural antioxidants from foods, because currently the only patient treatment is symptomatic, using acetylcholine esterase inhibitors, with an underestimated role of nutrition during the pre-Alzheimer phase^{3,22,27,28}.

A question that can be asked is "Which phenolic acids, present in the most popular typical lager beers consumed, exert substantial antiradical activity, taking into consideration the antiradical activity of the specific compound and its concentration in the particular beer?" The aim of this study was to attempt to evaluate the contribution of each of the identified phenolic acids, to the antiradical activity ascribed to all phenolic acids detected in the beer. Taking into consideration the concentrations of phenolic acids in the studied beers, it was also of interest to examine if there was synergy between different phenolic acids with *in vitro* studies using ABTS and DPPH free radicals.

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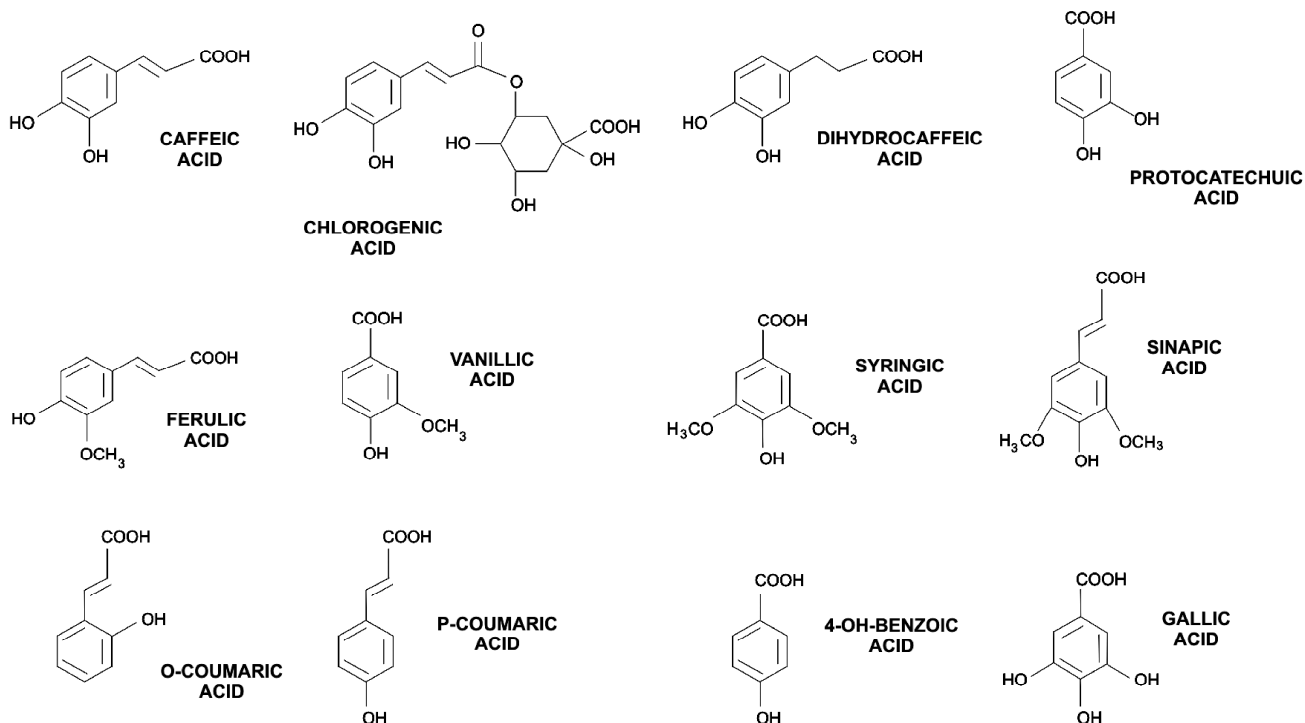


Fig. 1. Structure of phenolic acids.

MATERIALS AND METHODS

Reagents

The following chemicals were purchased from Sigma-Aldrich: ferulic, p-coumaric, sinapic, o-coumaric, 4-OH-benzoic, 3-OH-benzoic, protocatechuic, chlorogenic, caffeic, syringic, vanillic acid standards, ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid, diammonium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%). DPPH (2,2'-diphenyl-1-picrylhydrazyl) was purchased from ICN Biomedicals, USA. Other reagents used were of analytical grade (P.O.Ch. Gliwice, Poland).

Malt and beers

Malt and beers used in the study were provided by "Perła" Browary Lubelskie S.A., Lublin, Poland. The moisture content of malt was evaluated after drying at 130°C for 3 h. Samples of Goolman (a full light beer, wort extract 11.5% (w/w), 5.5% (v/v) alc. max., bitterness 13–17 BU, colour 8 ± 2 EBC units) and Perła (a full light beer, wort extract 12.2% (w/w), 6.2% (v/v) alc. max., bitterness 15–19 BU, colour 9 ± 2 EBC units) were produced by the infusion method and samples were analyzed from the following production stages: worts after heating at 52°C, 63°C and 72°C, wort after filtration, wort after boiling with hops, "green" beer after the main fermentation, beer after lagering, beer after the stabilization with PVPP, and beer after pasteurization and packaging (canned or bottled). The malt to water ratio during wort production was 150 g:155 g/L and 170 g:180 g/L in the case of Goolman and Perła, respectively. Two series of samples were gathered for each beer analyzed.

The total antiradical activities of both beers (measurement described in Methods) were as follows: 2.1 mmol of

Trolox/dm³ (ABTS, Goolman), 0.9 mmol of Trolox/dm³ (DPPH, Goolman) and 2.3 mmol of Trolox/dm³ (ABTS, Perła), 1.4 mmol of Trolox/dm³ (DPPH, Perła).

In order to compare these beers with other commercial beers, some analyses were performed using the following beers: Heineken (pale, 11.4% (w/w), alc. max. 5.06% (v/v)), Żywiec (pale, 12.5% (w/w), alc. max. 5.6% (v/v)), Carlsberg (pale, 11.4% (w/w), alc. max. 5% (v/v)), Warka (pale, 12.1% (w/w), alc. max. 5.7% (v/v)), Tyskie (pale, 11.7% (w/w), alc. max. 5.6% (v/v)), Żubr (pale, 12.1% (w/w), alc. max. 6.0% (v/v)), Tatra (pale, 12.0% (w/w), alc. max. 6.0% (v/v)), Harnaś (pale, 12.1% (w/w), alc. max. 6.0% (v/v)), Zwierzyniec (pale, 11.4% (w/w), alc. max. 5% (v/v)).

Methanol extraction of free phenolic acids from malt

Phenolic acids were extracted from malt as described by Maillard and Berset³⁴. Malt (1 g) was milled in a laboratory mill (Müszergyar Rt. QC-109/2, Hungary) and extracted with methanol (5 mL) in a conical flask. The tightly closed flask was shaken in the dark for 1.5 h at ambient temperature (Certomat R, B. Braun Biotech International., 170/min). After centrifugation (7,000 g, 30 min, 6°C, centrifuge MPW-365, Mechanika Preczyzna, Warsaw, Poland) the supernatant was stored at minus 20°C until HPLC-UV analysis. Extractions were carried out in triplicate.

Mild alkaline hydrolysis and extractions of phenolic acids from malt, worts and beers

Hydrolysis was performed according to Nardini et al.⁴⁰ Ground malt (1 g) was transferred to a conical flask and sonicated on ice (Sonics Vibra Cell, at 70% amplitude/50 sec pulses and 5 sec off, total time 20 min) with NaOH

solution (0.8 mol/L, 20 mL). The sample was purged for 5 min using CO₂ and left in the dark for 24 h at ambient temperature. After hydrolysis, the pH was adjusted to 1.0 using HCL solution (2 mol/L) and the sample was extracted three times, each time using a fresh portion of ethyl acetate (50 mL). The organic part was evaporated to dryness (40°C, 0.9 bar vacuum, Büchi, Flawil, Switzerland, water bath TW-1 Cabrolab, Poland) and the residue dissolved in methanol (10 mL). The samples were filtered using Millipore filters (0.45 µm) and analyzed by HPLC-UV. Extractions were carried out in duplicate.

Wort or beer (30 mL) was mixed with 15 mL of NaOH solution (2 mol/L) containing EDTA (10 mmol/L) and 1% (w/v) of ascorbic acid (to prevent the loss of phenolic acids⁴⁰) and extracted for 30 min, followed by adjustment of the pH to 1.0 using HCl solution (2 mol/L) and addition of 0.5 g of KCl. The samples were extracted three times, each time using a fresh portion of ethyl acetate (10 mL). The organic part was evaporated to dryness (40°C, 0.9 bar vacuum, Büchi, Flawil, Switzerland, water bath TW-1 Cabrolab, Poland) and the residue dissolved in methanol (10 mL). The samples were filtered using Millipore filters (0.45 µm) and analyzed by HPLC-UV. Extractions were carried out in duplicate.

HPLC-UV separations and quantifications of phenolic acids in malt, worts and beers

The HPLC system consisted of two Gilson 306 separation module piston pumps, a Gilson PhotoDiode Array Detector 170, Gilson loop (20 µL), manometric module Gilson 805, and a dynamic mixer 811C. A Waters Symmetry C18 column (USA, 250 mm, 4.6 mm i.d., 5 µm) and a Waters Symmetry C18 precolumn (5 µm, 8 × 20 mm) were used for separations. Eluents used were as follows. Eluent A: 1% (w/v) acetic acid solution in distilled deionised water. Eluent B: 50% HPLC-grade acetonitrile in distilled deionised water. Signals were monitored at 320 nm, 280 nm, 260 nm and 360 nm according to Kim et al.³² with the program as follows: START 92% A, 8% B 0–10 min; 70% A, 30% B 10–40 min; 60% A, 40% B 40–55 min; 92% A, 8% B 55–70 min. The eluent flow was 0.8 mL/min (2,050 p.s.i.).

Preparation of samples for the evaluations of total antiradical activity

Turbid samples of wort were centrifuged (7,000 g, 30 min, 6°C, centrifuge MPW-365, Mechanika Preczyzna, Warsaw, Poland). Beers were degassed by sonication (Sonics Vibra Cell) on ice (20% amplitude/30 sec pulses and 30 sec off, total time 10 min).

Total antiradical activity measured using ABTS

The analysis was performed as described by Miller et al.³⁷ with modifications as proposed by Re et al.⁵¹. A solution of ABTS (7 mmol/L) with potassium persulphate (2.45 mmol/L) was prepared and left for 24 h at ambient temperature. Directly prior to analysis, the absorbance of this solution, measured at 743 nm, was adjusted to 0.70 ± 0.02. Measurements were conducted at 20°C. The volumes of reagents in the spectrophotometric cuvette were as follows: 1.5 mL of ABTS solution, 0.13 mL of deionised water and 0.02 mL of sample. Changes in absorbance

at 734 nm were measured after 60 sec (Spekol 11, Carl Zeiss Jena, Germany). For each sample, the measurements were repeated at least six times. Total antiradical activity was expressed using Trolox (an analog of vitamin E) with a calibration curve obtained using a series of Trolox solutions.

Total antiradical activity measured using DPPH

The antiradical activity was measured using the method developed by Brand-Williams et al.¹⁰ A volume of 0.05 mL of studied sample was added to 1.95 mL of a 0.06 mmol/L DPPH solution in methanol. The decrease in the absorbance was measured spectrophotometrically at 515 nm every 1 min until the absorbance reached a plateau. The exact DPPH concentration that remained in the solution was calculated from the calibration curve. For each sample, the measurements were repeated six times at minimum. Total antiradical activity was expressed using a calibration curve obtained with a series of Trolox solutions.

Evaluation of the total antiradical activity of model solutions of phenolic acids using DPPH or ABTS – examining synergy/antagonism between phenolic acids

Model solutions of phenolic acids were prepared by dissolving the HPLC standards in minimal volumes of 99.7% ethanol followed by dilution to the desired concentrations with distilled deionised water. The concentrations of the phenolic acids under investigation were similar to the content of the free acids estimated by HPLC-UV in the Goolman beer. Two series of solutions of phenolic acids were prepared. Total antiradical activity was measured with ABTS or DPPH as described earlier.

RESULTS AND DISCUSSION

Phenolic acid content in malt and beers

The concentrations of the phenolic acids in the malt used for the production of Goolman and Perla beers are presented in Table I. Tables II and III present free and total alkali extractable phenolic acids contents in Goolman beer and Tables IV and V in Perla beer. The key values have been bolded in the Tables to underline the most important results. The free phenolic acid content in both worts and beers was significantly lower than the corresponding free phenolic acid content in malt. The most abundant total alkali extractable phenolic acid in malt was ferulic acid (as previously described by others³⁴), followed by vanillic, p-coumaric and 4-OH-benzoic acid, in line with other authors that indicated that ferulic, p-coumaric and vanillic acids were the most abundant in barley malt^{24,30}. Only a portion of the total alkali extractable phenolic acids were extracted from the malt into the wort during mashing. For example, the total ferulic acid content in wort (52°C) reached less than 50% of the corresponding content in malt in the case of both experimental beers. The concentrations of the other major phenolic acids (vanillic, p-coumaric and 4-OH-benzoic acid) were also significantly lower in the case of both experimental worts in comparison to the content in malt. High concentrations of free as well as total alkali extractable phenolic

Table I. Content of phenolic acids in experimental malt.

Phenolic acid	Free acid concentration	Total alkali extractable phenolic acids
	($\mu\text{mol}/100\text{ g}$ of dry mass of malt) ^a	(free + bound phenolic acids) ($\mu\text{mol}/100\text{ g}$ of dry mass of malt) ^a
protocatechuic acid	n.d. ^b	0.7 ± 0.06
4-OH-benzoic acid	0.66 ± 0.07	12.47 ± 1.46
3-OH-benzoic acid	0.51 ± 0.07	5.18 ± 0.15
chlorogenic acid	1.16 ± 0.06	3.95 ± 0.25
vanillic acid	3.21 ± 0.18	44.78 ± 3.69
syringic acid	0.35 ± 0.05	1.31 ± 0.05
caffeic acid	0.44 ± 0.06	1.67 ± 0.06
p-coumaric acid	1.34 ± 0.18	15.65 ± 0.73
ferulic acid	13.90 ± 0.15	50.16 ± 2.63
sinapic acid	0.13 ± 0.04	0.49 ± 0.04
o-coumaric acid	0.18 ± 0.61	6.88 ± 0.49

^a Mean values ± standard deviation, n = 3.^b Not detected.**Table II.** Concentration of free phenolic acids in Goolman beer.

Phenolic acid	Concentration of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Wort, 52°C	Wort, 63°C	Wort, 72°C	Wort after filtration	Wort after boiling with hops	Beer after main fermentation	Beer after maturation	Beer after filtration and stabilisation (PVPP)	Canned beer after pasteurisation
chlorogenic	n.d. ^b	n.d.	n.d.	0.19 ± 0.01	0.61 ± 0.03	0.58 ± 0.02	0.57 ± 0.05	0.21 ± 0.01	0.22 ± 0.02
vanillic	2.30 ± 0.11	2.60 ± 0.13	1.72 ± 0.21	1.77 ± 0.24	1.32 ± 0.07	1.50 ± 0.11	1.26 ± 0.11	1.18 ± 0.14	0.62 ± 0.07
caffeic	n.d.	0.20 ± 0.01	0.18 ± 0.02	0.24 ± 0.02	0.51 ± 0.06	0.30 ± 0.04	0.20 ± 0.02	0.18 ± 0.01	0.16 ± 0.02
syringic	n.d.	0.22 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.13 ± 0.01
p-coumaric	0.25 ± 0.02	0.79 ± 0.04	1.25 ± 0.03	0.94 ± 0.07	1.64 ± 0.12	1.27 ± 0.22	1.03 ± 0.07	1.06 ± 0.09	0.98 ± 0.10
ferulic	3.44 ± 0.17	11.39 ± 0.32	9.99 ± 0.15	10.24 ± 0.10	10.27 ± 0.09	8.57 ± 0.09	7.85 ± 0.45	3.96 ± 0.21	3.74 ± 0.12
sinapic	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
o-coumaric	0.25 ± 0.02	0.15 ± 0.01	0.23 ± 0.01	0.31 ± 0.01	0.39 ± 0.02	0.55 ± 0.04	0.54 ± 0.02	0.33 ± 0.03	0.39 ± 0.01
total	6.26 ± 0.33	15.37 ± 0.54	13.52 ± 0.44	14.82 ± 0.47	14.88 ± 0.41	12.94 ± 0.54	12.10 ± 0.74	7.07 ± 0.52	6.28 ± 0.36

^a Mean values ± standard deviation, n = 3.^b Not detected.**Table III.** Concentration of free phenolic acids (after mild alkaline hydrolysis) in Goolman beer.

Phenolic acid	Concentration of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Wort, 52°C	Wort, 63°C	Wort, 72°C	Wort after filtration	Wort after boiling with hops	Beer after main fermentation	Beer after maturation	Beer after filtration and stabilisation (PVPP)	Canned beer after pasteurisation
protocatechuic	0.23 ± 0.02	0.30 ± 0.02	0.42 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.03	0.24 ± 0.02
4-OH-benzoic	2.85 ± 0.19	2.66 ± 0.22	2.61 ± 0.21	2.43 ± 0.17	1.78 ± 0.10	1.66 ± 0.08	1.62 ± 0.08	0.67 ± 0.05	0.17 ± 0.02
3-OH-benzoic	3.05 ± 0.13	4.13 ± 0.25	4.70 ± 0.22	3.18 ± 0.21	3.49 ± 0.24	1.88 ± 0.11	1.14 ± 0.06	0.56 ± 0.06	0.11 ± 0.01
chlorogenic	1.01 ± 0.05	1.21 ± 0.15	1.02 ± 0.05	1.03 ± 0.04	2.69 ± 0.15	2.05 ± 0.07	2.26 ± 0.10	1.20 ± 0.07	0.91 ± 0.10
vanillic	2.33 ± 0.18	4.28 ± 0.17	2.24 ± 0.11	1.88 ± 0.09	1.50 ± 0.14	1.60 ± 0.10	1.63 ± 0.12	1.47 ± 0.06	0.74 ± 0.03
caffeic	0.23 ± 0.01	0.40 ± 0.03	0.48 ± 0.03	0.41 ± 0.02	0.48 ± 0.06	0.41 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.23 ± 0.01
syringic	0.22 ± 0.02	0.51 ± 0.04	0.86 ± 0.06	0.74 ± 0.05	0.24 ± 0.03	0.18 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
p-coumaric	0.40 ± 0.02	1.27 ± 0.09	1.86 ± 0.10	2.10 ± 0.09	2.23 ± 0.16	1.32 ± 0.07	1.28 ± 0.08	1.51 ± 0.15	1.01 ± 0.06
ferulic	22.88 ± 1.83	47.11 ± 1.83	50.84 ± 3.51	51.36 ± 2.93	46.98 ± 2.28	41.56 ± 1.23	41.22 ± 1.18	28.02 ± 0.68	26.38 ± 1.01
sinapic	0.19 ± 0.03	0.12 ± 0.01	0.20 ± 0.17	0.10 ± 0.01	0.24 ± 0.20	0.18 ± 0.02	0.25 ± 0.02	0.22 ± 0.01	0.17 ± 0.02
o-coumaric	2.39 ± 0.15	2.08 ± 0.10	1.76 ± 0.08	1.38 ± 0.09	1.33 ± 0.15	1.60 ± 0.20	1.25 ± 0.07	1.17 ± 0.10	0.77 ± 0.09
total	35.78 ± 2.63	64.07 ± 2.91	66.99 ± 4.57	63.83 ± 3.72	61.17 ± 3.53	52.68 ± 1.92	51.28 ± 1.75	35.45 ± 1.23	30.87 ± 1.38

^a Mean values ± standard deviation, n = 2.

acids were detected at different stages of wort production in both experimental beers (Tables II–V). Of note is that the highest content of free and total alkali extractable p-coumaric acid in Perła beer (but not in Goolman beer) was observed in the beer after filtration and stabilisation with PVPP and maturation, respectively. This result should be studied further in the future, since both beers were produced using the same equipment. A most important difference was the grist to water ratio. In Goolman beer, only vanillic and p-coumaric acid were present in the worts and beers, and predominantly in the free form

(Table II) and caffeic, sinapic, chlorogenic, ferulic or syringic acids were present in the total alkali extractable form (Table III). In Perła worts and beers, 4-OH-benzoic, 3-OH-benzoic, vanillic, syringic, ferulic sinapic and o-coumaric acids were present mainly in the total alkali extractable form. In both beers, the changes in the free phenolic acid content were not uniform. In the case of some phenolic acids, a decrease in content was observed after boiling the worts with hops or after the main fermentation until pasteurisation and packaging, but with some phenolic acids, the concentrations were constant until the

Table IV. Content of free phenolic acids in Perla beer.

Phenolic acid	Concentrations of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Wort, 52°C	Wort, 63°C	Wort, 72°C	Wort after filtration	Wort after boiling with hops	Beer after main fermentation	Beer after maturation	Beer after stabilisation and (PVPP)	Bottled beer after pasteurisation
4-OH-benzoic	n.d. ^b	n.d.	0.11 ± 0.01	0.18 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.12 ± 0.03
3-OH-benzoic	0.17 ± 0.01	n.d.	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.11 ± 0.01	n.d.
chlorogenic	0.06 ± 0.01	0.05 ± 0.01	1.16 ± 0.07	1.18 ± 0.05	0.92 ± 0.08	0.98 ± 0.05	0.72 ± 0.05	0.39 ± 0.06	0.39 ± 0.05
vanillic	0.93 ± 0.05	2.52 ± 0.10	4.50 ± 0.27	4.42 ± 0.65	1.72 ± 0.12	1.59 ± 0.09	1.44 ± 0.13	1.05 ± 0.06	1.18 ± 0.04
caffeic	0.18 ± 0.01	0.24 ± 0.01	0.12 ± 0.06	0.21 ± 0.02	0.63 ± 0.10	0.37 ± 0.01	0.42 ± 0.05	0.37 ± 0.01	0.38 ± 0.01
syringic	0.18 ± 0.01	0.27 ± 0.01	0.23 ± 0.07	0.17 ± 0.01	0.13 ± 0.01	0.12 ± 0.03	0.17 ± 0.01	0.05 ± 0.01	0.09 ± 0.01
p-coumaric	0.09 ± 0.01	0.11 ± 0.01	0.17 ± 0.03	0.41 ± 0.02	0.68 ± 0.06	0.51 ± 0.03	1.37 ± 0.10	1.73 ± 0.12	1.21 ± 0.09
ferulic	2.26 ± 0.07	4.53 ± 0.12	11.03 ± 0.51	15.53 ± 0.63	15.45 ± 0.28	0.83 ± 0.13	0.98 ± 0.03	0.77 ± 0.07	0.65 ± 0.12
sinapic	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	n.d.	n.d.
o-coumaric	0.24 ± 0.01	0.18 ± 0.01	0.16 ± 0.02	0.20 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.20 ± 0.02	0.16 ± 0.01
total	4.14 ± 0.19	7.92 ± 0.28	17.62 ± 1.06	22.43 ± 1.42	19.91 ± 0.70	4.76 ± 0.40	5.51 ± 0.43	4.80 ± 0.37	4.18 ± 0.36

^a Mean values ± standard deviation, n = 2.^b Not detected.**Table V.** Total content of phenolic acids (after mild alkaline hydrolysis) in Perla beer.

Phenolic acid	Concentrations of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Wort, 52°C	Wort, 63°C	Wort, 72°C	Wort after filtration	Wort after boiling with hops	Beer after main fermentation	Beer after maturation	Beer after stabilisation and (PVPP)	Bottled beer after pasteurisation
protocatechuic	0.16 ± 0.02	0.38 ± 0.06	0.66 ± 0.07	0.27 ± 0.03	0.40 ± 0.02	0.45 ± 0.02	0.39 ± 0.03	0.33 ± 0.02	0.40 ± 0.01
4-OH-benzoic	11.76 ± 1.78	13.81 ± 1.59	13.10 ± 0.79	13.45 ± 0.33	5.29 ± 0.19	4.52 ± 0.17	1.23 ± 0.06	0.16 ± 0.02	0.19 ± 0.02
3-OH-benzoic	0.88 ± 0.18	2.75 ± 0.16	4.61 ± 0.19	4.37 ± 0.16	3.83 ± 0.20	5.79 ± 0.24	1.41 ± 0.09	1.24 ± 0.03	1.18 ± 0.08
chlorogenic	2.70 ± 0.22	2.48 ± 0.28	2.64 ± 0.20	2.65 ± 0.06	2.38 ± 0.09	2.35 ± 0.16	1.84 ± 0.09	1.14 ± 0.09	1.64 ± 0.10
vanillic	0.98 ± 0.06	75.42 ± 5.70	77.15 ± 5.17	62.63 ± 5.55	3.14 ± 0.25	2.90 ± 0.26	2.75 ± 0.19	2.90 ± 0.15	2.56 ± 0.19
caffeic	0.20 ± 0.02	0.23 ± 0.04	0.19 ± 0.01	0.26 ± 0.01	0.93 ± 0.03	0.53 ± 0.02	0.82 ± 0.10	0.74 ± 0.03	0.75 ± 0.09
syringic	0.22 ± 0.01	0.50 ± 0.02	1.11 ± 0.11	1.39 ± 0.06	0.22 ± 0.03	0.19 ± 0.01	0.24 ± 0.02	0.21 ± 0.03	0.22 ± 0.02
p-coumaric	0.12 ± 0.01	0.91 ± 0.04	1.30 ± 0.26	0.81 ± 0.03	1.24 ± 0.10	1.62 ± 0.14	1.66 ± 0.09	1.39 ± 0.04	1.58 ± 0.06
ferulic	34.30 ± 2.80	62.94 ± 4.50	81.72 ± 4.60	74.20 ± 4.96	73.57 ± 5.62	42.34 ± 2.59	45.58 ± 2.68	29.94 ± 3.20	24.56 ± 2.06
sinapic	0.09 ± 0.01	0.15 ± 0.01	0.19 ± 0.03	0.15 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	n.d. ^b
o-coumaric	17.47 ± 1.89	31.22 ± 3.37	29.49 ± 2.16	25.30 ± 1.08	11.12 ± 0.60	0.87 ± 0.08	0.80 ± 0.05	0.81 ± 0.06	0.88 ± 0.12
total	68.88 ± 7.00	190.79 ± 15.77	212.16 ± 13.59	185.48 ± 12.28	102.27 ± 7.14	61.66 ± 3.70	56.84 ± 3.39	39.01 ± 3.68	33.96 ± 2.75

^a Mean values ± standard deviation, n = 2.^b Not detected.

end of the process or even increased (e.g., syringic and vanillic acid in Perla, Table IV). It should be noted that there was no common mashing stage at which phenolic acids were simultaneously at the highest concentrations. The large amount of data gathered in the Tables II–V indicates that there was a considerable loss of some phenolic acids after wort boiling with hops and later stages of beer production, but in numerous cases, the decrease of the phenolic acid content was seen from the very beginning of the mashing process.

The content of the phenolic acids in the experimental beers were compared to the concentrations of the phenolic acids in nine market beers (Tables VI and VII). Both Goolman and Perla contained free phenolic acids at concentrations that generally were similar to those detected in other beers. The only exceptions in the case of Perla beer were ferulic acid (in a significantly lower concentration) and caffeic acid (in a significantly higher concentration) when compared to the other beers. On the other hand, the total alkali extractable caffeic acid content in Perla was the highest among all the beers studied, and the total alkali extractable 3-OH-benzoic and vanillic acid content in Goolman was lower than the corresponding content of these acids in Perla and in the other beers studied. Other

authors have also reported a wide range of phenolic acids in worts and beers. Floridi et al.¹⁹, using HPLC with coulometric array detection, described a wide range of free phenolic acids in worts. The results presented by these authors also indicated a substantial decrease of phenolic acid contents in beers in comparison to the corresponding worts. Nardini and Ghiselli⁴³ determined free and total alkali extractable phenolic acids in three beers of Italian, Austrian and German origin. Ferulic acid was the main phenolic acid in both forms, followed by other phenolic acids, present in the three beers always in considerably lower levels than ferulic acid. Phenolic acids were present in these beers mainly in the bound form. Vanbeneden et al.⁶¹, using HPLC-ECD, determined the content of the three main phenolic acids: ferulic (main phenolic acid) followed by p-coumaric and sinapic acid, but their analysis technique was created primarily for the simultaneous detection of volatile phenols and not phenolic acids in worts or beers. Bartolome et al.⁵ compared, among others, the phenolic acid content in normal and low-alcohol beers. Again, ferulic acid was the main phenolic acid, followed by p-coumaric, vanillic, p-OH-benzoic, caffeic and sinapic acids. The content of the phenolic acids in the low-alcohol beers was lower than in the standard beers

Table VI. Content of free phenolic acids in market beers.

Phenolic acid	Concentrations of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Heineken	Żywiec	Carlsberg	Warka	Tyskie	Żubr	Tatra	Harnaś	Zwierzyniec
4-OH-benzoic	0.08 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-OH-benzoic	0.11 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.01	1.15 ± 0.08
chlorogenic	0.20 ± 0.02	0.50 ± 0.03	0.72 ± 0.09	0.16 ± 0.02	0.47 ± 0.04	0.22 ± 0.01	0.13 ± 0.02	0.16 ± 0.01	0.28 ± 0.01
vanillic	2.30 ± 0.09	0.46 ± 0.03	0.74 ± 0.09	0.61 ± 0.02	0.91 ± 0.05	0.65 ± 0.06	0.73 ± 0.05	2.13 ± 0.15	0.62 ± 0.05
caffeic	n.d. ^b	0.16 ± 0.01	0.15 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	n.d.	0.18 ± 0.01
syringic	n.d.	0.07 ± 0.01	0.11 ± 0.01	n.d.	0.07 ± 0.01	0.13 ± 0.01	n.d.	n.d.	0.11 ± 0.01
p-coumaric	0.02 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.64 ± 0.57	1.44 ± 0.13	1.36 ± 0.10	0.31 ± 0.06	0.12 ± 0.01
ferulic	3.78 ± 0.22	3.27 ± 0.10	4.22 ± 0.20	4.90 ± 0.23	4.00 ± 0.25	4.12 ± 0.20	2.30 ± 0.06	2.92 ± 0.12	6.20 ± 0.48
sinapic	0.03 ± 0.01	0.04 ± 0.01	n.d.	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	n.d.	0.02 ± 0.01	0.03 ± 0.01
o-coumaric	0.26 ± 0.02	0.44 ± 0.06	0.14 ± 0.02	0.25 ± 0.02	0.14 ± 0.02	0.19 ± 0.01	0.34 ± 0.02	0.18 ± 0.01	0.43 ± 0.03
total	6.78 ± 0.39	5.00 ± 0.26	6.13 ± 0.43	6.18 ± 0.32	6.44 ± 0.96	6.95 ± 0.44	5.02 ± 0.26	5.81 ± 0.37	9.12 ± 0.69

^a Mean values ± standard deviation, n = 2.^b Not detected.**Table VII.** Total content of phenolic acids (after mild alkaline hydrolysis) in market beers.

Phenolic acid	Concentrations of phenolic acids ($\mu\text{mol}/\text{dm}^3$) ^a								
	Heineken	Żywiec	Carlsberg	Warka	Tyskie	Żubr	Tatra	Harnaś	Zwierzyniec
protocatechuic acid	0.22 ± 0.03	n.d.	0.50 ± 0.02	0.07 ± 0.01	0.37 ± 0.02	0.44 ± 0.02	0.08 ± 0.01	n.d.	0.30 ± 0.02
4-OH-benzoic acid	n.d. ^b	0.17 ± 0.01	0.13 ± 0.01	n.d.	n.d.	0.21 ± 0.01	0.14 ± 0.01	n.d.	0.30 ± 0.01
3-OH-benzoic acid	1.79 ± 0.10	1.00 ± 0.06	3.61 ± 0.23	0.89 ± 0.04	3.08 ± 0.18	2.76 ± 0.09	n.d.	n.d.	2.53 ± 0.15
chlorogenic acid	0.72 ± 0.03	1.23 ± 0.04	0.93 ± 0.03	0.31 ± 0.01	2.02 ± 0.08	0.88 ± 0.02	1.84 ± 0.13	2.57 ± 0.18	1.67 ± 0.31
vanillic	6.44 ± 0.45	3.51 ± 0.15	1.06 ± 0.06	3.84 ± 0.16	3.92 ± 0.13	7.74 ± 0.27	11.23 ± 0.41	2.25 ± 0.04	0.70 ± 0.14
caffeic	0.31 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.19 ± 0.45	0.25 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.14 ± 0.01	0.25 ± 0.01
syringic	0.12 ± 0.01	0.05 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	n.d.	0.17 ± 0.01
p-coumaric	0.94 ± 0.11	0.96 ± 0.03	2.46 ± 0.09	2.78 ± 0.07	1.54 ± 0.05	3.10 ± 0.15	1.40 ± 0.04	0.49 ± 0.01	0.30 ± 0.01
ferulic	3.87 ± 0.07	15.92 ± 0.67	51.59 ± 16.96	52.38 ± 1.88	22.25 ± 1.03	38.03 ± 0.97	53.46 ± 1.95	5.80 ± 0.79	40.70 ± 2.41
sinapic	0.12 ± 0.01	0.25 ± 0.05	0.04 ± 0.01	0.04 ± 0.01	0.37 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.41 ± 0.02	0.16 ± 0.01
o-coumaric	1.42 ± 0.09	0.80 ± 0.13	3.00 ± 0.19	3.56 ± 0.16	0.48 ± 0.01	1.59 ± 0.14	3.55 ± 0.14	1.94 ± 0.07	3.67 ± 0.07
total	15.95 ± 0.91	24.02 ± 1.16	63.58 ± 17.62	64.19 ± 2.80	34.45 ± 1.55	55.08 ± 1.69	72.03 ± 2.72	13.60 ± 1.12	50.75 ± 3.15

^a Mean values ± standard deviation, n = 2.^b Not detected.

due to differences in the production process, yeast strains used, dealcoholization of beer etc. Montanari et al.³⁸ detected a number of free phenolic acids in four beers using HPLC-ECD and HPLC-DAD (two standard and two dealcoholized beers). Generally, the most abundant phenolic acids in all four beers were o-coumaric, ferulic, m-coumaric and 3-OH-benzoic acid, but the results were strongly influenced by the detection system applied. Qualitative comparison of the results presented in this study (Tables II–VII) and in the cited papers allows for the conclusion that the phenolic acids detected in this work are abundant in beers worldwide. Consequently, their role as low molecular (i.e. easy absorbed) antioxidants in beers can be important for human health⁴⁵. It is clear that comparison of the phenolic acid content in beers, reported in this paper and from other experiments, is extremely difficult due to the different malting and brewing technologies employed.

The contribution of phenolic acids to the total antiradical activity of beer

The percentage concentration of phenolic acids in the beer was compared to their percentage contribution to the total antiradical activity of the beer (which could be calculated in this study only with the assumption that the sum of the antiradical activity originating from all identified phenolic acids was 100%). Ferulic acid was present in the Goolman beer at the highest concentration (59.6% of all detected phenolic acids), but this was not reflected by its

contribution to the formation of antiradical activity measured using both methods with free radicals (38.0% and 28.6%, respectively, Table VIII). P-coumaric, vanillic and ferulic acids were the most abundant in Perła, but their percentage contribution to the total antiradical activity was significantly lower than what would have been expected (Table IX). On the other hand, in the case of the minor phenolic acids (i.e., caffeic, chlorogenic, o-coumaric, sinapic 4-OH-benzoic and syringic acids), the total antiradical activity (with both ABTS and DPPH) was higher than expected taking into consideration their relatively low content in beer (Tables VIII and IX). Indeed, there are many papers indicating that caffeic acid and chlorogenic acid are especially effective antioxidants in different oxidation systems^{11,36,39,46,62}. In this context, the significant role of minor phenolic acids in beers cannot be underestimated.

The total antiradical activity of phenolic acids in relation to their structure

When considering the antiradical activity of phenolic acids in beer, not only their molar concentrations, but also their molecular structures should be taken under consideration (the number of *hydroxy*- groups, ring substitution, presence of accepting/donating groups etc.). Natella et al.⁴⁶ analyzed the relationship of the structures of eight benzoic and cinnamic acids (4-OH-benzoic, protocatechuic, vanillic, syringic acids versus p-coumaric, caffeic, ferulic and sinapic acids, see Fig. 1) to their antiradical

Table VIII. Comparison of content of free phenolic acids in Goolman beer and the corresponding contribution to the total antiradical activity (TAA).

Phenolic acid	Phenolic acid concentration in beer ($\mu\text{mol}/\text{dm}^3$)	Content of the acid (%) ^a	TAA measured using ABTS (mmol of Trolox/ dm^3)	Contribution of the acid to the TAA measured using ABTS (%) ^b	TAA measured using DPPH (mmol of trolox/ dm^3)	Contribution of the acid to the TAA measured using DPPH (%) ^b
ferulic acid	3.74 ± 0.12	59.6	1.74 ± 0.01	38.0	0.65 ± 0.01	28.6
caffeic acid	0.16 ± 0.02	2.5	0.40 ± 0.01	8.7	0.39 ± 0.02	17.2
chlorogenic acid	0.22 ± 0.02	3.5	0.53 ± 0.02	11.6	0.37 ± 0.01	16.3
p-coumaric acid	0.98 ± 0.10	15.6	0.71 ± 0.01	15.5	0.22 ± 0.01	9.7
sinapic acid	0.04 ± 0.01	0.6	0.07 ± 0.01	1.5	0.04 ± 0.01	1.8
o-coumaric acid	0.39 ± 0.01	6.2	0.47 ± 0.02	10.3	0.28 ± 0.01	12.3
vanillic acid	0.62 ± 0.07	9.9	0.50 ± 0.01	10.9	0.21 ± 0.01	9.3
syringic acid	0.13 ± 0.01	2.1	0.16 ± 0.01	3.5	0.11 ± 0.01	4.8

^a 100% is taken as a sum of the acids detected in the beer.

^b Contribution of phenolic acid to the formation of total antioxidant activity when the sum of the antioxidant activities originating from all identified phenolic acids was taken as 100%.

Table IX. Comparison of content of free phenolic acids in Perla beer and the corresponding contributions to total antiradical activity (TAA).

Phenolic acid	Phenolic acid concentration in beer ($\mu\text{mol}/\text{dm}^3$)	Content of the acid (%) ^a	TAA measured using ABTS (mmol of Trolox/ dm^3)	Contribution of the acid to the TAA measured using ABTS (%) ^b	TAA measured using DPPH (mmol of trolox/ dm^3)	Contribution of the acid to the TAA measured using DPPH (%) ^b
ferulic acid	0.65 ± 0.12	15.6	0.38 ± 0.03	9.1	0.15 ± 0.01	5.2
caffeic acid	0.38 ± 0.01	9.1	0.87 ± 0.01	20.9	0.95 ± 0.01	33.0
chlorogenic acid	0.39 ± 0.05	9.3	1.00 ± 0.01	24.0	0.70 ± 0.01	24.3
p-coumaric acid	1.21 ± 0.09	28.9	0.60 ± 0.02	14.4	0.28 ± 0.01	9.7
o-coumaric acid	0.16 ± 0.01	3.8	0.17 ± 0.01	4.1	0.12 ± 0.01	4.2
4-OH-benzoic acid	0.12 ± 0.03	2.9	0.20 ± 0.02	4.8	0.10 ± 0.01	3.5
vanillic acid	1.18 ± 0.04	28.2	0.88 ± 0.01	21.2	0.49 ± 0.02	17.0
syringic acid	0.09 ± 0.01	2.2	0.06 ± 0.01	1.4	0.09 ± 0.01	3.1

^a 100% is taken as a sum of the acids present in beer.

^b Contribution of phenolic acid to the formation of total antioxidant activity when the sum of the antioxidant activities originating from all identified phenolic acids was taken as 100%.

activity in different oxidation systems. Some uniform conclusions could be drawn from this study. The higher antiradical activity of cinnamic acid derivatives in comparison to benzoic acid derivatives in the cited study was explained by the presence of the double bond in the propenoic chain, which stabilized the phenoxyl radical by resonance. Moreover, the electron withdrawing carboxylic group of cinnamic acids decreased the ability of the phenolic ring to donate the hydrogen. The authors also concluded that the *methoxy*- group substituted into the phenolic ring stabilized the phenoxyl radical, which caused the increase in the antiradical activity of the compound. The role of the double bond in the propenoic chain was also pointed out in another work³⁹. The authors proved that caffeic acid was a more potent antioxidant than dihydrocaffeic acid (lacking the 2,3-double bond) in a number of environments where oxidation occurred. The results obtained in the study presented herein are in accordance with the observations of cited authors because *dimethoxyhydroxy*- and *dihydroxy*- phenolic acids exhibited higher antiradical activity than would be expected taking under consideration only their concentrations (Tables VIII and IX). It is evident that a number of *methoxy*- groups substituted to phenolic ring at the *ortho*- position relative to the *hydroxy*- group is important in the formation of antiradical activity of the phenolic compound¹⁶. The substitution of the *methoxy*- group is far less effective than the addition of another *hydroxy*- group in the molecule. Indeed, it is a well known fact that the *hydroxy*- group is a far better electron donor than the *methoxy*- one. Also, the high antiradical action of caffeic and chlorogenic acid should be

mentioned is the formation of the intramolecular hydrogen bond between two *hydroxy*- groups and the ability to chelate the metal ions if these groups are placed at the *ortho*- position to each other. The presence of the second *hydroxy*- group in the *ortho*- or *para*- position is known to increase the antiradical activity of the compound due to the stabilization of phenoxyl radical as well as additional stabilization and formation of o-quinone or p-quinone^{11,16}. Gallic acid (although not quantitatively estimated in the beers in this study) was pointed out to be a more potent antioxidant than protocatechuic acid¹⁶. This can be used to explain the higher antiradical activity of caffeic and chlorogenic acid compared to ferulic acid. According to Cuvelier et al.¹⁶, the esterification of caffeic acid with quinic acid (chlorogenic acid) decreased its antioxidant activity, but the results presented in Tables VIII and IX suggest that chlorogenic acid is as potent as caffeic acid (at least in the conditions applied in this study). *Ab initio* and theory studies⁴ explain in detail the differences in the antiradical activity of phenolic acids on the basis of free phenoxyl radical formation from the parental phenolic acid. For example, in the case of caffeic acid, three different radical structures can be considered. Two structures correspond to the radical structures originating from the OH group at C(3) (one in the presence and one in the absence of intramolecular hydrogen bonds), and in the third structure the H• is eliminated from the OH group at C(4). The theory also explains the differences between the antiradical activities of different phenolic acids. When a *hydroxy*- or *methoxy*- group is added to the p-coumaric acid molecule at the *ortho*- position to the aroxyl group (yielding caffeic,

Table X. Total antiradical activity (mmol of Trolox/dm³) measured with the ABTS radical.

Phenolic acid	p-coumaric acid (0.71 ± 0.01) ^a	o-coumaric acid (0.47 ± 0.02)	caffeic acid (0.40 ± 0.01)	sinapic acid (0.07 ± 0.01)	chlorogenic acid (0.53 ± 0.02)
p-coumaric acid (0.71 ± 0.01) ^a					
o-coumaric acid (0.47 ± 0.02)	0.68 ± 0.02				
caffeic acid (0.40 ± 0.01)	0.75 ± 0.01	0.43 ± 0.02			
sinapic acid (0.07 ± 0.01)	0.64 ± 0.01	0.38 ± 0.02	0.25 ± 0.02		
chlorogenic acid (0.53 ± 0.02)	0.74 ± 0.02	0.63 ± 0.02	0.60 ± 0.02	0.24 ± 0.01	
ferulic acid (1.74 ± 0.01)	1.85 ± 0.01	1.77 ± 0.02	1.73 ± 0.01	1.71 ± 0.01	1.76 ± 0.02

^a Number in the bracket is the antiradical activity of the solution containing one phenolic acid at the same concentration as in the studied mixture of two compounds. Intersections represent the total antiradical activity of the mixture of two compounds.

Table XI. Total antiradical activity (mmol of Trolox/dm³) measured with DPPH radical.

Phenolic acid	p-coumaric acid (0.22 ± 0.01) ^a	o-coumaric acid (0.28 ± 0.01)	caffeic acid (0.39 ± 0.02)	sinapic acid (0.04 ± 0.01)	chlorogenic acid (0.37 ± 0.01)
p-coumaric acid (0.22 ± 0.01) ^a					
o-coumaric acid (0.28 ± 0.01)	0.27 ± 0.01				
caffeic acid (0.39 ± 0.02)	0.41 ± 0.02	0.25 ± 0.02			
sinapic acid (0.04 ± 0.01)	0.26 ± 0.01	0.25 ± 0.02	0.60 ± 0.02		
chlorogenic acid (0.37 ± 0.01)	0.40 ± 0.02	0.41 ± 0.02	0.38 ± 0.02	0.40 ± 0.01	
ferulic acid (0.65 ± 0.01)	0.63 ± 0.01	0.79 ± 0.02	0.70 ± 0.01	0.64 ± 0.01	0.58 ± 0.02

^a Number in the bracket is the antiradical activity of the solution containing one phenolic acid at the same concentration as in the studied mixture of two compounds. Intersections represent the total antiradical activity of the mixture of two compounds.

ferulic or sinapic acids), changes in bond lengths and angles between functional groups appear. The already existing C(4)-O bond distance becomes longer when a second OH group is added (when caffeic acid is formed), or becomes shorter when a *methoxy*- group is added (as in the case of ferulic or sinapic acid formation). Thus, the already existing O-H bond distance at C(4) is shorter in caffeic acid and slightly longer in ferulic or sinapic acid as compared to this bond in p-coumaric acid. This could be an explanation of the differences in the antiradical activity of different phenolic acids, because a longer O-H bond means that the hydrogen can be more easily removed. Nevertheless, other molecular descriptors need to be established for a full explanation of the effectiveness of the antioxidant molecule (e.g. the heat of formation of the antioxidant and the free radical, the spin distribution in the free radical, the dissociation energy of the OH bond). The differences in the antiradical activity are also affected by the angle formed by the C(2)-C(3)-O bond when the *hydroxy*- or *methoxy*- group is substituted⁴.

Synergy/antagonism studies between phenolic acids

The knowledge of the average content of phenolic acids in beers was used for the examination of the synergy/antagonism between phenolic acids, since it is known that the antiradical activity depends on the concentration of the antioxidant. Because the synergy between different antioxidants in beer creates a very attractive perspective of the increment of the total antiradical activity of this beverage, it would be useful to know which particular phenolic acids could exert such an activity. The synergy (antagonism) between a number of the acids in pairs at the concentrations close to those detected in free form in the Goolman beer was studied (Table II). In most cases, the total antiradical activity of the mixture of two phenolic acids was considerably lower than the sum of the antiradical activities of individual phenolic acids (Tables X and XI). A higher total antiradical activity of the mixture of phe-

nolic acids was seen only in the case of caffeic acid with sinapic acid (with DPPH free radicals, Table XI). The literature is lacking the detailed information about the synergy/antagonism of phenolic acids in the presence of free radical species. In this paper, the study on synergy/antagonism between these compounds was undertaken because the papers of other authors bring contradictory results concerning the more complex polyphenols related to phenolic acids. In the past, the synergy as the possible mechanism of action between different phenolic compounds^{49,56}, non-phenolic antioxidants¹², phenolic and non-phenolic antioxidants^{6,17,25} and different antioxidant-rich foods³¹ was pointed out. Peyrat-Maillard et al.⁴⁹ evaluated the interactions between phenolic antioxidants in binary systems (each time with antioxidants at equal molar concentrations). The scientists used linoleic acid and 2,2'-azobis (2-amidinopropane) as the source of free radicals and observed the synergistic effects between rosmarinic acid and quercetin, or rosmarinic acid and caffeic acid, and antagonistic effects in the mixtures containing: α -tocopherol+caffeic acid, α -tocopherol+rosmarinic acid; (+)-catechin+caffeic acid; and caffeic acid+quercetin. Rossetto et al.⁵⁶ investigated the inhibitory effect of anthocyanins in the peroxidation of linoleic acid in the micelles in the presence of AAPH (2,2'-azobis (2-amidinopropane) dihydrochloride) and in the absence of (+)-catechin. The results of their work showed that catechin effectively regenerated malvidin 3-glucoside and peonidin 3-glucoside recycling the antioxidant efficiency of the two latter antioxidants. It seems reasonable to proceed with more detailed experiments using a broad range of phenolic acids and more complex food polyphenols.

SUMMARY

In summary, it can be concluded that barley malt is a rich source of phenolic acids both in the free and in the bound forms, and only limited parts of these acids are transferred into wort. More importantly, both free and

total alkali extractable phenolic acid concentrations decrease until the last steps of beer production. Finally, when considering the role of individual acids in the total antiradical activity of beer, not only the concentrations, but also the structures of these compounds should be taken into consideration. There is a need for further studies concerning the synergistic/antagonistic interactions between different phenolic compounds, especially phenolic acids in foods and beverages. Phenolic acids should be a group receiving special attention because of their natural origin, relatively high concentrations in foods and beverages, strong antioxidant actions, easy intestinal absorption and lack of negative side-effects for human health after their consumption from natural sources. Interactions (synergy or antagonism) between phenolic antioxidants and natural antioxidants from human plasma after beer consumption should be examined in detail in the near future.

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