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Antioxidative and prooxidative effects of extracts made from cherry liqueur pomace

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Abstract

The effect of phenolics extracted from cherry liqueur pomace on the Fenton reaction was tested in a model system, where hydroxyl radicals from iron (II) reduction of H_2O_2 reacted with ethanol, forming 1-hydroxyl radicals. The extent of the radical formation was monitored by electron spin resonance (ESR) detection of spin adducts formed from 1-hydroxyethyl radicals and 4-pyridyl-1-oxide-*N*-*tert*-butylnitrone (POBN). Small phenol–iron ratios gave low amounts of detected radicals, indicating an antioxidative effect of the extracts, whereas high phenol–iron ratios gave high levels of radicals, indicating decreasing antioxidant or dominating prooxidant effects. Extracts made with pure solvents (water, methanol, ethanol, acetone or 2-propanol) gave higher antioxidant effects than extracts made with 70% aqueous solvent mixtures. The prooxidant effect of the extracts increased with the polarity of the extraction solvent. Gallic acid gave a prooxidative effect, *p*-coumaric acid only an antioxidative effect, while epicatechin gave an antioxidative effect at low concentrations and a prooxidative effect at high concentrations.

Keywords: Cherry liqueur pomace extract; Antioxidants; Prooxidants; ESR; 1-Hydroxyethyl radical; Fenton reaction

1. Introduction

Cherries contain phenolic compounds that have significant antioxidant effects (Burkhardt, Tan, Manchester, Hardeland, & Reiter, 2001; Chaovanalikit & Wrolstad, 2004a, 2004b; Wang, Nair, Strasburg, Booren, & Gray, 1999). Adding cherry tissue to beef patties has been demonstrated to inhibit lipid oxidation (Britt, Gomaa, Gray, & Booren, 1998). Pomace from the production of cherry liqueur is currently a wasteproduct with no value. However, it is a potential source of interesting cherry antioxidative phenolic compounds and isolation of the phenolic compounds on a large scale could very likely be achieved by extraction of the pomace.

Phenolic compounds, extracted from plant materials, have been demonstrated to have antioxidative properties in various model systems and in several foods, where they are finding increasing use (Andersen, Lauridsen, & Skibsted, 2003; Moure et al., 2001; Schwartz et al., 2001). However, often the extracted phenolic compounds have also been found to possess prooxidative effects, in addition to the antioxidative effects, but the conditions that favour one of the two effects over the other (for various foods) are generally poorly described and understood.

The prooxidative activity of plant phenolics is primarily associated with the ability to reduce Fe(III) and Cu(II). Iron initiates or increases oxidative stress in food or other biological systems through the Fenton reaction, where Fe(II) reacts with H_2O_2, resulting in the formation of the short-lived highly reactive hydroxyl radical, $^{\cdot}OH$, Eq. (1). Similar reactions are also possible with Cu(I) instead of Fe(II).

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$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (1)$$

Phenolics with a lower reduction potential for the phenoxyl radicals than the metal ion in its higher oxidation number, are able to reduce Fe(III) or Cu(II), thus generating the prooxidative lower oxidation state of the metal ion (Simic, Jovanovic, & Niki, 1992). The most powerful reducing agents are in general the most efficient radical scavengers, and the balance between antioxidative effects, based on radical scavenging, and prooxidative effects, based on iron activation, is thus very delicate (Andersen et al., 2003).

In the present study, extracts made from cherry liqueur pomace have been tested in a model system that has been designed to simulate the oxidative behaviour of foods, where oxidation that is initiated by the Fenton reaction produces hydroxyl radicals that are predominantly trapped by various organic solutes (Morelli, Russo-Volpe, Bruno, & Scalzo, 2003). The model system is also relevant in relation to food systems such as precooked meat and meat products, which contain free iron ions, and where the oxidative deterioration of unsaturated lipids and proteins is initiated by iron-catalyzed reactions, especially cleavage of preformed lipid hydroperoxides, or Fenton reactions involving hydroperoxides from bacterial sources. The phenolics extracted by solvents of different polarities from cherry liqueur pomace, have been tested in the model system in order to assess their potential as food antioxidants. The overall antioxidative or prooxidative activities of the extracts have been examined as a function of the concentration of phenolics and metal ions. Furthermore, the pure phenolic compounds coumaric acid, gallic acid, and epicatechin, have also been tested, with the purpose of illustrating the different behaviours of phenols in the model system.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu phenol reagent, and analytical grade iron (II) sulphate heptahydrate were obtained from Merck (Darmstadt, Germany). Sodium carbonate heptahydrate was from Riedel-de Haën (Seelze, Germany). Fremy's salt (potassium nitrosodisulfonate), gallic acid monohydrate, naringenin and (−)-epicatechin were from Aldrich Chemical Co. (Milwaukee, WI, USA). *p*-Coumaric acid, (+)-catechin, caffeic acid and quercetin were from Sigma–Aldrich Chemie (Steinheim, Germany). Vanillic acid was from Fluka Chemie AG (Buchs, Switzerland). Hydrogen peroxide (35%) was from Bie & Berntsen A/S (Rødovre, Denmark). α-(4-Pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) was from Sigma Chemical Co. (St. Louis, MO, USA). Water was purified through a Millipore Q-plus purification train (Millipore, Bedford, MA, USA).

2.2. Sample preparation

Pomace, from the production of a cherry liqueur, was obtained from Danish Distillers (Copenhagen, Denmark). The production was based on *Prunus cerasus* cv. Stevns cherry. The frozen pomace, without stones, was milled for 90 s under nitrogen in a water-cooled IKA-Universal mill model A10 (Jahnke & Kunkel, Staufen, Germany). Pomace samples of uniform particle size (250–500 μm) were obtained by using a sieving tower (J. Engelsmann, Ludvigshafen, Germany) with aperture sizes of 250 and 500 μm.

2.3. Extraction of phenolics

Extracts were prepared by mixing 0.5 g of milled cherry liqueur pomace with 4 ml of the extraction mixture (water, or neat methanol, ethanol, acetone, or 2-propanol, or each of these solvents in 70% aqueous mixtures). The air in the tube was replaced with nitrogen and the phenolics were extracted at 20 °C by shaking (200 rpm) for 20 min. The mixture was subsequently centrifuged (2 min, 1100*g*), and the filtrate was evaporated to dryness using a rotary vacuum evaporator and a 35 °C hot water bath. The evaporation took between 5 and 20 min. The residue was dissolved in 6 ml 25% aqueous ethanol, that was adjusted to pH 2 with acetic acid in order to stabilize the phenolics. The extracts were covered with nitrogen and stored at −18 °C prior to use (maximum 21 days).

2.4. Quantification of the total amount of phenolic compounds

The amount of total phenolics in extracts was determined according to the Folin–Ciocalteu procedure (Singleton & Rossi, 1965). Samples (200 μl, two replicates) were mixed with 1.0 ml of Folin–Ciocalteu's reagent (diluted 1:10 with water) and 0.8 ml of a 7.5% solution of sodium carbonate was added. The absorption at 765 nm was measured after 30 min with a Cary 3 UV–Vis spectrophotometer (Varian Techtron Pty. Ltd, Mulgrave, Victoria, Australia). The total phenolic content was expressed as gallic acid equivalents (GAE) in mg per litre of extract.

2.5. HPLC analysis of phenolic compounds in cherry extracts

A modification of the method of Andersen, Outtrup, Riis, and Skibsted (1999) was used. Analysis was carried out with a Hewlett–Packard series 1050 HPLC system (Palo Alto, Ca, USA) equipped with a 25 × 4.6 mm

(i.d.) Macherey-Nagel ET Nucleosil® reversed phase 10 μm C_{18} stainless-steel column (Düren, Germany). The mobile phase consisted of 2.5% aqueous acetic acid (solvent A) and solvent B was a mixture of water, methanol and acetic acid (2.5:95:2.5). The gradient, applied at a flow rate of 1.0 ml min^{-1}, was: 0–5 min, 100% A–90% A; 5–30 min, 90% A–60% A; 30–80 min, 60% A–0% A. An ESA Analytical Coulochem II dual-channel electrochemical detector equipped with an ESA Model 5010 analytical cell (Chelmsford, MA, USA) was used for detection with channel 1: 0 mV and channel 2: 600 mV. Extracts were filtered and 10 μl aliquots were injected into the HPLC system. Peak identification was achieved by comparison with retention times of pure phenolic compounds.

2.6. ESR assay based on reduction of Fremy's salt radical

The extracts or solutions of the phenolic compounds (*p*-coumaric acid, gallic acid or (−)-epicatechin) were diluted with 25% ethanol, and 3 ml of the diluted solutions were mixed with 200 μl of Fremy's salt (820 μM) dissolved in a 25% saturated sodium carbonate solution. The concentration of the Fremy's salt solution was adjusted, based on spectrophotometric measurements. By correlating ESR spectra of Fremy's salt solutions with spectra of a stable radical (TEMPO), the exact concentration of Fremy's salt radical was used to calculate an extinction coefficient for the anion of Fremy's salt ($\varepsilon_{270} = 933\ M^{-1}\ cm^{-1}$). The ESR spectra were recorded with a Jeol JES-FR30 ESR spectrometer (JEOL Ltd., Tokyo, Japan) 5 min after the mixing. The measurements were carried out at room temperature with a microwave power of 4 mW, and a modulation width of 0.25 mT. The intensity of the ESR signal was measured as the height of the central line relative to the height of a Mn(II)-marker (JEOL Ltd., Tokyo, Japan) attached to the cavity of the spectrometer. The antioxidative activity was calculated on the basis of linear regression of results from experiments with four to five different concentrations of the sample or solution of the phenolic compound. The antioxidative activity was expressed as mmol Fremy's radicals reduced by 1 l of sample or as mol Fremy's radicals reduced per mole of the phenolic compound.

2.7. Fenton reaction model system with ESR detection of POBN spin adducts

Four ml of 3.2 mM POBN dissolved in 1.0 M aqueous ethanol were mixed with 20 μl of 1.1–44 mM $FeSO_4$ and 50 μl of either a sample or 25% aqueous ethanol (pH 2) as a reference. Finally, 80 μl of 24 mM H_2O_2 were added. An aliquot of the reaction mixture was withdrawn directly into an ESR quartz capillary tube with an interior diameter of 0.75 mm (Wilmad, Buena, NJ, USA), and the ESR spectrum was recorded 2 min after the addition of the H_2O_2 solution on a Jeol JES-FR30 ESR spectrometer (JEOL Ltd., Tokyo, Japan). The measurements were carried out at room temperature with a microwave power of 4 mW, and a modulation width of 0.1 mT. The intensity of all signals were recorded relative to the intensity of a Mn(II)-marker (JEOL Ltd., Tokyo, Japan) attached to the cavity of the spectrometer. Measurements were carried out with varying concentrations of cherry extract or pure phenolics (*p*-coumaric acid, gallic acid or (−)-epicatechin) and the degree of inhibition (I_{ESR}) was calculated from the height of the central peak of the ESR signal of the spin adduct by the following formula:

$$I_{ESR} = \left(1 - \frac{\text{Peak height}_{sample}}{\text{Peak height}_{reference}}\right) \times 100\%.$$

3. Results and discussion

3.1. Cherry pomace extracts

Pomace from the production of cherry liqueur was milled and extracted with the aim of extracting phenolic compounds. Methanol, ethanol and acetone in solvent–water mixtures of 70% and the pure solvents (including water) were chosen for the extraction, based on previous use in different studies to extract phenolics from berries, berry seeds and berry press residues (Alonso, Bourzeix, & Revilla, 1991; Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1998; Heinonen, Meyer, & Frankel, 1998; Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995; Landbo & Meyer, 2001). 2-Propanol was included as extraction solvent in order to examine the capacity of a less polar protic solvent for extraction of phenolic compounds. After extraction and evaporation of the solvents the extracted solids were redissolved in a 25% ethanol–water mixture.

Quantification of the total amount of phenolics in the extracts by the Folin–Ciocalteu method showed that 70% solvent–water mixtures extracted the phenolics more efficiently than the pure solvents, and the 70% acetone–water mixture gave the highest yield of extracted phenolics (Table 1).

HPLC analysis with electrochemical detection demonstrated the presence of several phenolic compounds in the extracts, as illustrated with by the chromatograms of the ethanol extracts in Fig. 1. Generally, the extracts made with 70% solvent–water mixtures contained more complex mixtures of phenolic compounds than did the pure solvent extracts. (+)-Catechin, caffeic acid, (−)-epicatechin, *p*-coumaric acid, and naringenin were some of the most abundant phenolic compounds that were detected in the extracts.

The antioxidative capacity of the extracts toward radicals was examined by the quenching of the stable

Table 1
Total phenolics and antioxidative capacities of extracts from cherry pomace

Extraction mixture	Phenolic compounds *mg GAE/l*	Antioxidative capacity *mmol Fremy's salt radical quenched per litre extract*
Water	13.7 ± 2.0	≈0
70% methanol	36.5 ± 2.3	1.00 ± 0.28
70% ethanol	37.1 ± 9.6	0.96 ± 0.13
70% acetone	92.8 ± 15.5	3.47 ± 0.39
70% 2-propanol	39.9 ± 0.5	0.73 ± 0.03
100% methanol	21.3 ± 4.5	≈0
100% ethanol	17.4 ± 1.2	≈0
100% acetone	16.3 ± 3.3	≈0
100% 2-propanol	9.8 ± 1.2	≈0

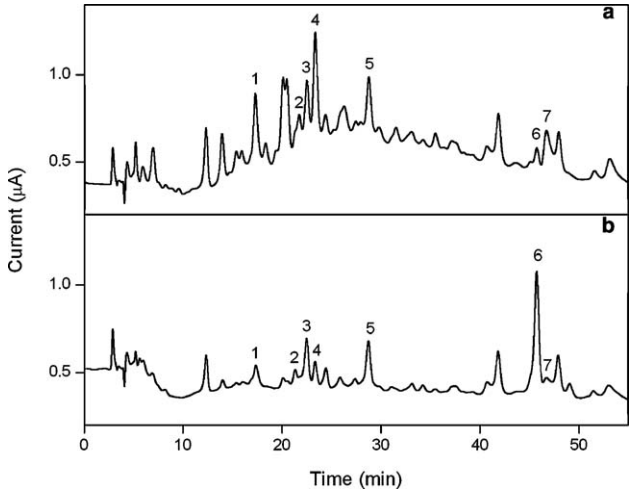

Fig. 1. HPLC chromatograms of phenolic compounds extracted from cherry pomace with: (a) 70% aqueous ethanol and (b) 100% neat ethanol. The identified peaks are: (1) (+)-catechin; (2) vanillic acid; (3) caffeic acid; (4) (−)-epicatechin; (5) *p*-coumaric acid; (6) naringenin; and (7) quercetin.

Fremy's salt radical. The extent of the reaction was monitored by the decay of the ESR signal from Fremy's salt, and the antioxidative capacity was calculated from the amount of Fremy's salt that was quenched 5 min after mixing (Table 1). Only extracts containing relatively high amounts of phenolic compounds gave an antioxidative activity towards Fremy's salt radical, and the extracts made with pure solvents did not react with Fremy's salt.

3.2. The Fenton reaction model system

The antioxidant and prooxidant activities of the phenolic extracts were tested in a model system, where organic radicals (1-hydroxyethyl radicals) were generated according to reactions (1) and (2) ("Fenton chemistry"). The quantification of the extent of the reactions, i.e. the amount of produced radicals, was based on trapping 1-hydroxyethyl radicals with the spin trap 4-pyridyl-1-oxide-*N*-*tert*-butylnitrone (POBN) and detection of the generated spin adducts by ESR. The high concentration of ethanol (1 M) in the reaction mixture assures that hydroxyl radicals formed by the Fenton reaction, reaction (1), are trapped by ethanol forming mainly 1-hydroxyethyl radicals, reaction (2) (Andersen & Skibsted, 1998; Asmus, Möckel, & Henglein, 1973; Pou et al., 1994).

$$CH_3CH_2OH + {}^{\bullet}OH \rightarrow CH_3^{\bullet}CHOH + H_2O \quad (2)$$

The 1-hydroxyethyl radicals are either trapped, reaction (3), or react with antioxidants, reaction (4), or with oxygen, reaction (5), generating alkylperoxyl radicals that decay to hydroxyperoxyl radicals and acetaldehyde, reaction (6) (von Sonntag & Schuchmann, 1997).

$$CH_3^{\bullet}CHOH + POBN \rightarrow POBN/CH_3^{\bullet}CHOH \quad (3)$$

$$CH_3^{\bullet}CHOH + ArOH \rightarrow CH_3CH_2OH + ArO^{\bullet} \quad (4)$$

$$CH_3^{\bullet}CHOH + O_2 \rightarrow CH_3CH(OO^{\bullet})OH \quad (5)$$

$$CH_3CH(OO^{\bullet})OH \rightarrow CH_3CHO + HO_2^{\bullet} \quad (6)$$

Phenolics added to the reaction mixture compete with the spin trap in the scavenging of 1-hydroxyethyl radicals. This will lower the amount of spin adducts and accordingly, the decrease in the intensity of the ESR signal indicates antioxidative activity. Conversely, an increase in the signal intensity will reflect prooxidant activity of the added phenolics, due to increased formation of spin adducts.

The role of iron in the model system was examined in experiments where the concentration of iron was varied while the concentration of hydrogen peroxide was kept constant (Fig. 2). Below 20 μM Fe^{2+} the formation of

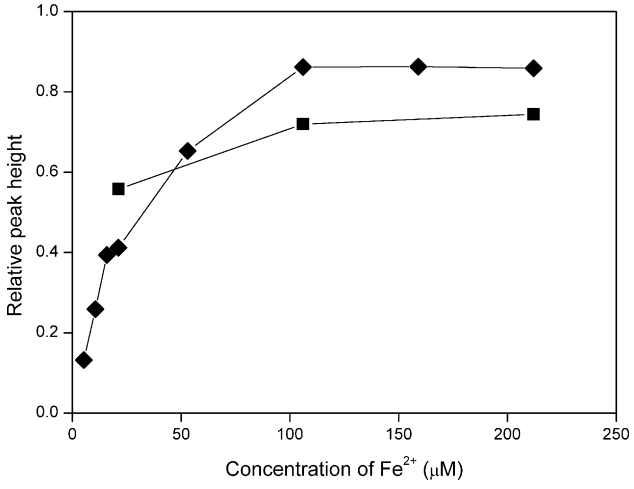

Fig. 2. The effect of varying the concentration of Fe^{2+} on the intensity of ESR-spectra of spin adducts formed in the Fenton/ethanol model system. Relative peak height of reference samples (◆) and of samples with added liquid cherry extract made with 70% aqueous acetone (■). The spin trap POBN (3 mM) and $FeSO_4$ dissolved in 1 M aqueous ethanol were mixed with either 25% aqueous ethanol (reference) or the liquid extract (1.1 ± 0.2 mg GAE/l) and H_2O_2 (463 μM).

spin adducts was proportional to the Fe^{2+} concentration, and above 100 μM Fe^{2+} the amount of spin adducts became constant. The observed ESR spectra of the POBN spin adducts were six-line spectra (triplets of doublets) with hyperfine coupling constants [$a_N = 15.57 \pm 0.02$ G; $a_H = 2.58 \pm 0.04$ G] which are identical to the values expected for spin adducts formed from POBN and carbon-centred alkyl radicals, such as the 1-hydroxyethyl radical, that are derived from ethanol (Andersen & Skibsted, 1998; Buettner, 1987).

A cherry liqueur extract prepared with 70% acetone was added to the reaction mixture at three different concentrations of Fe^{2+} (Fig. 2). The extract gave an antioxidative effect at high concentrations of Fe^{2+} (106 and 212 μM), which was observed as an inhibition of the ESR signal as compared to the experiments without added extract. On the other hand, the addition of extract increased the ESR signal at a low concentration of Fe^{2+} (21.2 μM), which corresponds to an increased amount of spin adducts, and thus a prooxidative effect.

The effect of the extract concentration was tested at two Fe^{2+} concentrations (Fig. 3). At 15.9 μM Fe^{2+} (a high phenol–iron ratio) the amount of spin adducts increased with the concentration of extract, indicating a prooxidative effect, which was linear above 0.2 mg GAE/l. At 106 μM of Fe^{2+} (a low phenol–iron ratio), a decrease in the amount of spin adducts was observed for all tested concentrations of extract, thus demonstrating an antioxidative effect. However, the antioxidative effect decreased with increasing extract concentration at concentrations above 0.2 mg GAE/l, and the decrease was linear above 0.3 mg GAE/l.

3.3. Antioxidative and prooxidative effects of different cherry liqueur pomace extracts

The antioxidative and prooxidative behaviour of all cherry liqueur pomace extracts were characterized in a model system with 106 μM Fe^{2+}. The antioxidative effect of the extracts was quantified as the degree of inhibition, I_{ESR}, calculated as the percentage decrease of the intensity of the ESR signals compared to a control solution without extract. The same general behaviour in the Fenton/ethanol model system was observed for all pomace extracts (Fig. 4). All extracts gave increasing antioxidative effects at low concentrations of extract in the model system, and decreasing antioxidative effects at higher concentrations. The linear increase of I_{ESR} that was observed at low concentrations of extracts indicates increasing antioxidative effects, which were quantified by the slopes, α^{anti}, i.e. the antioxidative effect per phenol unit (Fig. 5). Extrapolation of the decreasing linear part of the curve at high concentrations of extract in the model system was used to quantify the onset of a dominating prooxidative activity of the extracts, by determining the concentration, C^{pro}, where the extrapolation was equal to zero (Fig. 5).

All the cherry pomace extracts gave (with the high Fe^{2+} concentration) antioxidative effects that increased with the concentration of extract in the reaction mixture. The antioxidative effects levelled off and began to decrease at very high concentrations of extracts indicating the onset of prooxidative effects. A similar change from antioxidative effects at low phenol concentrations to prooxidative effect at high phenol concentrations

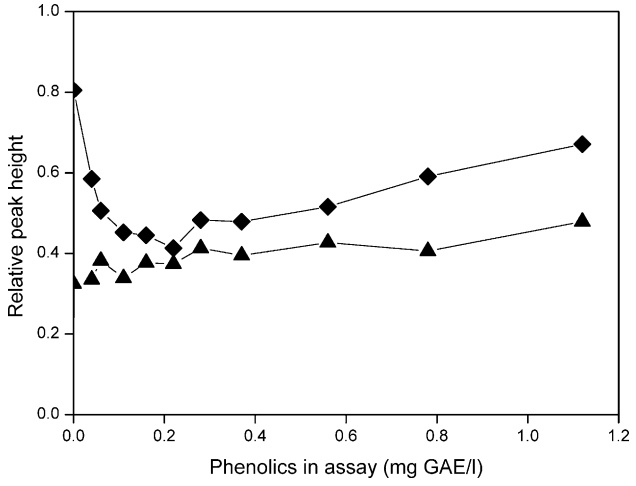

Fig. 3. The effect of varying the concentration of cherry pomace extract (70% aqueous acetone) on the intensity of ESR-spectra of spin adducts formed in the Fenton/ethanol model system. The reaction mixture contained either 106 μM Fe^{2+} (◆) or 15.9 μM Fe^{2+} (▲). The spin trap POBN (3 mM) and $FeSO_4$ dissolved in 1 M aqueous ethanol were mixed with the liquid extract and H_2O_2 (463 μM).

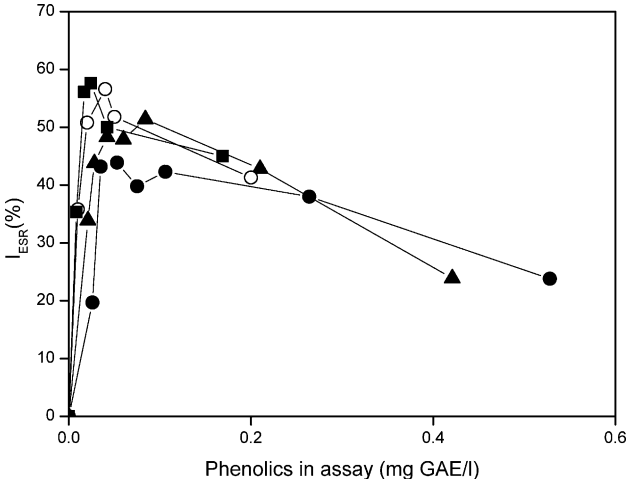

Fig. 4. The effect of varying amounts of different cherry liqueur pomace extracts on the inhibition of the ESR-signal in the Fenton/ethanol model system. The extracts were made with either 70% aqueous methanol (▲), 70% aqueous ethanol (●), 100% ethanol (○), or 100% acetone (■). The spin trap POBN (3 mM) and $FeSO_4$ (106 μM) dissolved in 1 M aqueous ethanol were mixed with a liquid extract and H_2O_2 (463 μM).

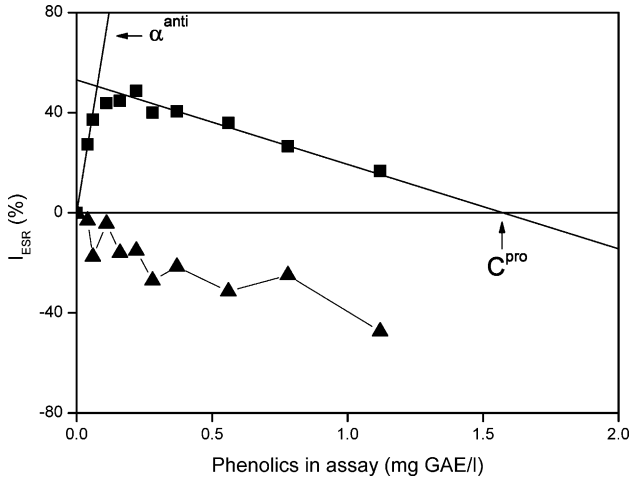

Fig. 5. Inhibition of the ESR-signal in the Fenton/ethanol model system at varying concentrations of pomace extract made with 70% aqueous acetone for two Fe^{2+} concentrations: 106 μM Fe^{2+} (■) and 15.9 μM Fe^{2+} (▲). The curve shape with 106 μM Fe^{2+} is described by two regression lines which are characterized by the following parameters: α^{anti} is the slope of the increasing line and C^{pro} is the concentration of extract where the extrapolated linear decreasing part of the curve is equal to zero. The spin trap POBN (3 mM) and $FeSO_4$ dissolved in 1 M aqueous ethanol were mixed with the liquid extract and H_2O_2 (463 μM).

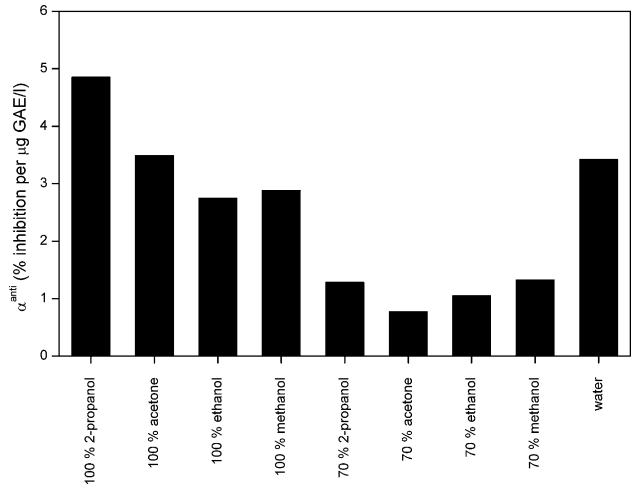

Fig. 6. The antioxidative activities, α^{anti}, in the Fenton/ethanol model system of different cherry liqueur pomace extracts prepared with solvents of different polarities.

has been observed in the ORAC assay for flavonoids (Cao, Sofic, & Prior, 1997), but the opposite trend has also been observed in antioxidant assays with copper or iron initiated oxidations (Tran, Cronin, Dearden, & Morris, 2000; Yen, Chen, & Peng, 1997).

The prooxidative effects of phenols have been reported to dominate at low phenol/iron ratios and antioxidative effects at high phenol/iron ratios (Strlic, Radovic, Kolar, & Pihlar, 2002), and Satué-Gracia, Heinonen, and Frankel (1997) observed, that the antioxidative activity decreased toward prooxidant activity when copper concentration was increased in a LDL/Cu system and in a lecithin–liposome system. An opposite trend was observed in the present model system where a low iron concentration (corresponding to a high phenol–iron ratio) only gave a prooxidative effect of the phenolic extract (Fig. 3), and also, at a higher concentration of iron, where the antioxidative effects decreased toward being prooxidative at increasing concentration of extracts, i.e. by increasing the phenol–iron ratio (Fig. 4). These effects are very likely caused by the excess of hydrogen peroxide compared to iron in the model systems with high phenol–iron ratios. Fe^{2+}, produced by the reduction of Fe^{3+} by phenols, reacts with excess hydrogen peroxide, leading to increased generation of radicals. On the other hand, at low phenol–iron ratios, iron is in excess compared to hydrogen peroxide, and the regeneration of Fe^{2+} by reduction of Fe^{3+} by the phenols does not give rise to formation of extra radicals due to the lack of hydrogen peroxide.

The antioxidative effects per phenol unit, α^{anti}, of all the extracts made with 70% solvent mixtures were markedly lower than the antioxidative effects of the extracts made with pure solvents (Fig. 6). The lowest value of α^{anti} was observed for the 70% acetone extract, which also had the highest concentration of phenolics (Table 1). This suggests that the extra phenolics that are extracted by the 70% solvent mixtures only contribute negligibly to the overall antioxidative effect of the extracts. Thus, the phenolics that are extracted with the pure solvents are on average the most effective as antioxidants. This observation suggests that the major contribution to the antioxidative effect of the extracts arises from a specific group of phenols that are extracted with the same efficiency by all the tested solvents and mixtures. Phenolic compounds that only give minor contributions to the overall antioxidative effects thus cause differences in the phenolic contents of the extracts.

The onsets of the prooxidative effect show a systematic distribution according to the polarity of extraction solvent (Fig. 7). The extracts prepared using pure solvents gave an overall prooxidant activity at a lower phenolic concentration than the corresponding aqueous 70% solvent mixture extracts. The difference between the pure solvent extracts and the water–solvent extracts was increased by lowering the polarity of the solvent.

3.4. Pure phenolic compounds

Apparently the extracts contain complex mixtures of phenols with very different antioxidant and prooxidant properties. (−)-Epicatechin and *p*-coumaric acid are two of the dominating phenolic compounds identified in cherries (Chaovanalikit & Wrolstad, 2004b). The antioxidant and prooxidant activities of three pure phenolic compounds, gallic acid, (−)-epicatechin, and

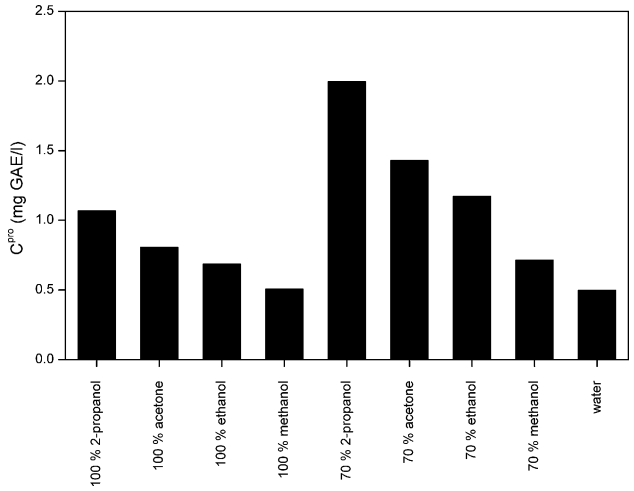

Fig. 7. The concentrations, C^{pro}, of phenolics in the liquid cherry extracts corresponding to the transition from an overall antioxidative activity to a prooxidative activity in the Fenton/ethanol modelsystem for different pomace extracts.

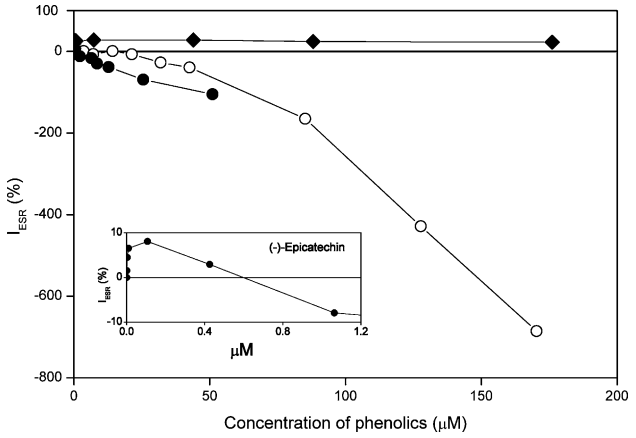

Fig. 8. The effect of varying the concentration of pure phenolic compounds on the inhibition of the ESR-signal in the Fenton/ethanol modelsystem. *p*-Coumaric acid (◆), (−)-epicatechin (●) and gallic acid (○). The inset shows the effect of (−)-epicatechin at low concentrations. The spin trap POBN (3 mM) and $FeSO_4$ (106 μM) dissolved in 1 M aqueous ethanol were mixed with the dissolved phenolics and H_2O_2 (463 μ M).

p-coumaric acid, were tested in order to examine the different likely properties of the extracted phenolic compounds. The three pure phenols were tested as antioxidants towards Fremy's salt (Table 2). Gallic acid was found to give the highest antioxidative effect, and (−)-epicatechin gave a lower antioxidative effect. *p*-Coumaric acid was not able to reduce Fremy's salt at any of the tested concentrations (⩽2.8 mM).

The three phenolic compounds were also tested as antioxidants in the Fenton/ethanol-model system. At low concentration, (−)-epicatechin and *p*-coumaric acid showed antioxidative effects by decreasing the intensity of the ESR signal by up to 10% and 27%, respectively (Fig. 8). At low concentration, the curve shape for (−)-epicatechin was similar to the curve shape for the extracts. The antioxidative effect of *p*-coumaric acid was constant at high concentrations and a prooxidant activity was not observed, in contrast to gallic acid that gave a prooxidative effect at all concentrations.

The three pure phenols, *p*-coumaric acid, (−)-epicatechin, and gallic acid, illustrate the very different properties that phenolic compounds can have in oxidation reactions. Gallic acid gave only a prooxidative effect in the Fenton/POBN model system, and *p*-coumaric acid only an antioxidative effect, whereas (−)-epicatechin gave both an antioxidative effect at low concentrations and a prooxidative effect at high concentrations. The prooxidant effects of phenolic compounds are likely caused by reduction of Fe^{3+} to Fe^{2+}, Eq. (7), which can generate new radicals through the Fenton reaction, Eq. (1).

$$ArOH + Fe^{3+} \rightarrow ArO^{\bullet} + H^{+} + Fe^{2+} \quad (7)$$

The oxidation potentials of the phenols are, therefore, expected to have a significant effect on the prooxidative properties. Prooxidative effects were not observed with *p*-coumaric acid that has the highest oxidation potential of the three pure phenols that were tested, whereas (−)-epicatechin and gallic acid both gave prooxidative effects (Table 2). (+)-Catechin, which is expected to have redox properties similar to (−)-epicatechin, and gallic acid have been shown to be able to reduce Fe^{3+}, whereas coumaric acid under the same conditions gave only a very low degree of conversion of Fe^{3+} to Fe^{2+} (Mellican, Li, Mehansho, & Nielsen, 2003). However, other effects, such as formation of phenol–metal complexes will also strongly influence antioxidant and prooxidative effects [Andersen et al., 2003; Miller, Castelluccio, Tijburg & Rice-Evans, 1996; Yoshino & Murakami, 1998].

The model system is concluded to be preferred over the simple Fenton assay previously described (Madsen, Nielsen, Bertelsen, & Skibsted, 1996), since it gives a more realistic evaluation of the plant extracts which also includes prooxidative effects.

The results from the test of the cherry pomace extracts in the model system suggest that water would be suitable as extraction solvent if only the antioxidative

Table 2
Antioxidative capacities of pure phenolic compounds

	Antioxidative capacity *mol Fremy's salt radical quenched per mol phenol*	Oxidation potential in aqueous solution *V vs. NHE*
Gallic acid	4.15 ± 0.20	0.56[a]
(−)-Epicatechin	1.32 ± 0.08	0.33[b]
p-Coumaric acid	<0.02	0.66[b]

[a] Furuno et al. (2002).
[b] Jørgensen and Skibsted (1998).

effects are considered. The use of organic solvents does not increase the extraction of the phenolics with antioxidative effects. A water extract could be used directly in food systems without further work up. The extract prepared with 70% 2-propanol gave the highest C^{pro} and this extract would therefore have antioxidant properties over a wider range of concentrations than the other extracts.

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References

Alonso, E., Bourzeix, M., & Revilla, E. (1991). Suitability of water/ethanol mixtures for the extraction of catechins and proanthocyanidins from *Vitis vinifera* seeds contained in a winery by-product. *Seed Science and Technology, 19*, 545–552.

Andersen, M. L., & Skibsted, L. H. (1998). Electron spin resonance spin trapping identification of radicals formed during aerobic forced aging of beer. *Journal of Agricultural and Food Chemistry, 46*(4), 1272–1275.

Andersen, M. L., Outtrup, H., Riis, P., & Skibsted, L. H. (1999). Do polyphenols influence the flavour stability of beer? *Proceedings of the 27th congress of the European brewery convention* (pp. 133–140). Zoeterwoude, The Netherlands: European Brewery Convention.

Andersen, M. L., Lauridsen, R. K., & Skibsted, L. H. (2003). Optimising the use of phenolic compounds in foods. In I. Johnson & G. Williamson (Eds.), *Phytochemical functional foods* (pp. 315–346). Cambridge: Woodhead Publishing Ltd.

Asmus, C. W., Möckel, H., & Henglein, H. (1973). A pulse radiolytic study of the site of OH· radical attack on aliphatic alcohols on aqueous solution. *Journal of Physical Chemistry, 77*, 1218–1221.

Britt, C., Gomaa, E. A., Gray, J. I., & Booren, A. M. (1998). Influence of cherry tissue on lipid oxidation and heterocyclic aromatic amine formation in ground beef patties. *Journal of Agricultural and Food Chemistry, 42*(12), 4891–4897.

Buettner, G. R. (1987). Spin trapping: ESR parameters of spin adducts. *Free Radical Biology and Medicine, 3*, 259–303.

Burkhardt, S., Tan, D. X., Manchester, L. C., Hardeland, R., & Reiter, R. J. (2001). Detection and quantification of the antioxidant melatonin in Montmorency and Balaton chart cherries (*Prunus cerasus*). *Journal of Agricultural and Food Chemistry, 49*(10), 4898–4902.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships. *Free Radical Biology and Medicine, 22*, 749–760.

Chaovanalikit, A., & Wrolstad, R. E. (2004a). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of Food Science, 69*(1), 67–72.

Chaovanalikit, A., & Wrolstad, R. E. (2004b). Anthocyanin and polyphenolic composition of fresh and processed cherries. *Journal of Food Science, 69*(1), 73–83.

Furuno, K., Akasako, T., & Sugihara, N. (2002). The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of flavonoids. *Biological and Pharmaceutical Bulletin, 25*(1), 19–23.

Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., & Törrönen, A. R. (1998). HPLC method for screening of flavonoids and phenolic acids in berries. *Journal of the Science of Food and Agriculture, 77*, 543–551.

Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry, 46*, 4107–4112.

Jørgensen, L. V., & Skibsted, L. H. (1998). Flavonoid deactivation of ferrylmyoglobin in relation to ease of oxidation as determined by cyclic voltammetry. *Free Radical Research, 28*, 335–351.

Kallithraka, S., Garcia-Viguera, C., Bridle, P., & Bakker, J. (1995). Survey of solvents for the extraction of grape seed phenolics. *Phytochemical Analysis, 6*, 265–267.

Landbo, A.-K., & Meyer, A. S. (2001). Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *Journal of Agricultural and Food Chemistry, 49*, 3169–3177.

Madsen, H. L., Nielsen, B. R., Bertelsen, G., & Skibsted, L. H. (1996). Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chemistry, 57*(2), 331–337.

Mellican, R. I., Li, J., Mehansho, H., & Nielsen, S. S. (2003). The role of iron and the factors affecting off-color development of polyphenols. *Journal of Agricultural and Food Chemistry, 51*(8), 2304–2316.

Miller, N. J., Castelluccio, C., Tijburg, L., & Rice-Evans, C. (1996). The antioxidant properties of theaflavin and their gallate esters – radical scavengers of metal chelators? *FEBS Letters, 392*, 40–44.

Morelli, R., Russo-Volpe, S., Bruno, N., & Scalzo, R. L. (2003). Fenton-dependent damage to carbohydrates: free radical scavenging activity of some simple sugars. *Journal of Agricultural and Food Chemistry, 51*(25), 7418–7425.

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., et al. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*, 145–171.

Pou, S., Ramos, C. L., Gladwell, T., Renks, E., Centra, M., Young, D., et al. (1994). A kinetic approach to the selection of a sensitive spin trapping system for the detection of hydroxyl radical. *Analytical Biochemistry, 217*, 76–83.

Satué-Gracia, M. T., Heinonen, M., & Frankel, E. N. (1997). Anthocyanins as antioxidants on human low-density lipoprotein and lecithin–liposome systems. *Journal of Agricultural and Food Chemistry, 45*(9), 3362–3367.

Schwartz, K., Bertelsen, G., Nissen, L. R., Gardner, P. T., Heinonen, M. I., Hopia, A., et al. (2001). Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *European Food Research and Technology, 212*, 319–328.

Simic, M. G., Jovanovic, S. V., & Niki, E. (1992). Mechanisms of lipid oxidative processes and their inhibition. In A. J. S. Angelo (Ed.), *Lipid oxidation in food. ACS symposium series* (Vol. 500, pp. 14–32). New York: American Chemical Society.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Strlic, M., Radovic, T., Kolar, J., & Pihlar, B. (2002). Anti- and prooxidative properties of gallic acid in Fenton-type systems. *Journal of Agricultural and Food Chemistry, 50*(22), 6313–6317.

Tran, T. T., Cronin, M. T. D., Dearden, J. C., & Morris, H. (2000). Determination of anti-oxidant activity by electron spin resonance spectroscopy. *Pest Managemant Science, 56*, 818–820.

von Sonntag, C., & Schuchmann, H.-P. (1997). Peroxyl radicals in aqueous solutions. In Z. Alfassi (Ed.), *Peroxyl radicals* (pp. 173–234). Chichester: Wiley.

Wang, H., Nair, M. G., Strasburg, G. M., Booren, A. M., & Gray, J. I. (1999). Antioxidant polyphenols from tart cherries (*Prunus cerasus*). *Journal of Agricultural and Food Chemistry, 47*(3), 840–844.

Yen, G. C., Chen, H. Y., & Peng, H. H. (1997). Antioxidant and pro-oxidant effects of various tea extracts. *Journal of Agricultural and Food Chemistry, 45*, 30–34.

Yoshino, M., & Murakami, K. (1998). Interaction of iron with polyphenolic compounds: application to antioxidant characterization. *Analytical Biochemistry, 257*, 40–44.