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Raspberry seed extract effect on the ferroxidase activity of ceruloplasmin isolated from plasma

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ABSTRACT

In this study, the effect of the raspberry seed extract (RSE) on the ferroxidase activity of ceruloplasmin (Cp) isolated from healthy male subject serum was analyzed. The ferroxidase activity of Cp was determined by spectrophotometry using Fe(II) – histidine complex and ferrozine as a chromogenic reagent. The ferroxidase activity of ceruloplasmin was demonstrated in dose-dependent way within the range 22–66 μg/ml. The effect of RSE on Fe(II) concentration, measured as the decrease of Fe(II) concentration in samples and expressed as ΔFe(II), was found to be a dose-dependent within the range 1.20–51.56 μg dm/ml. The ferroxidase activity of Cp was influenced by the RSE within its studied range. However, the addition of the highest concentration of RSE (51.56 μg dm/ml) to the sample containing the highest level of Cp (66 μg/ml) did not affect its ferroxidase activity. It may be suggested that the competition for Fe(II) as the substrate limits the effect of RSE on Cp activity and causes no further changes in Fe(II) elimination.

1. Introduction

Ceruloplasmin (Cp), the main copper-binding plasma protein, plays the principal role in the oxidation and reduction processes in blood. The ability to oxidize Fe(II) to Fe(III) with reduction of molecular oxygen to water prevents releasing of partially reduced species of oxygen, i.e. superoxide anion, hydrogen peroxide, hydroxyl radical. This ferroxidase activity of Cp results in the uptake of Fe(III) by transferrin and the inhibition of the oxidation of various biomolecules, mainly lipids and proteins. The oxidase activity of Cp in plasma, measured with diamines as substrates, was found to be elevated during infection and inflammation (Louro, Cocho, Mera, & Tutor, 2000), in critical lower limb ischemia (Iskra & Majewski, 1999), colorectoral cancer (Zowczak et al., 2001), and suggested to be a biomarker of the acute phase reaction(see Table 1).

Cp and the other endogenic (albumin, bilirubin, uric acid, enzymes: superoxide dismutase and glutathione peroxidase) or exogenic (vitamins (A, E, C, β-carotene) biological substances demonstrate antioxidant activity. The same role is performed by some components of dietary beverages and products, such as red wine, green tea, fruit and vegetables, rich in polyphenolic compounds. There is still increasing interest in health benefits of polyphenol-rich food. Many of them may reduce the risk of coronary heart disease, cancer, allergy and inflammation (Brown, 1999; Visioli, Borsani, & Galli, 2000). Results of many studies have suggested an association between consumption of polyphenol-rich food and beverages and their profilactive role, and connected their beneficial effects with the antioxidant activity, namely their ability to scavenge hydroxyl radicals (Hanasaki, Ogawa, & Fukui, 1994) and superoxide anions (Magnani, Gaydou, & Hubaud, 2000).

Polyphenols represent the chemical structure with the hydroxy groups of the catechol-like moiety that donate hydrogen and is oxidized itself to semiquinone radical. Thus, the dihydroxy group is required for the participation of a polyphenol in the redox system and its antioxidant activity. On the other side, the semiquinone radical may accept hydrogen and participate in the mechanism of prooxidation, acting as an oxidant agent. The dual behavior of the polyphenolic compounds is still less explored than their antioxidant function. The prooxidant activity appears to be responsible for the harmful biological effects of polyphenols. In the presence of some metal ions polyphenols may play an important role in the mutagenesis or promotion of cancer (Sahu & Gray, 1996), and the oxidative damage of DNA and lipids *in vitro* (Yamashita, Tanemura, & Kawanishi, 1999).

The antioxidant or the prooxidant activity of phenolic compounds depends on many factors such as pH of the solution, their chelating properties and bioavailability. The presence of compounds of both reducing and oxidative properties in blood and other biological fluids may affect their antioxidant/prooxidant activity while competing for the substrate, i.e. free radicals, reactive oxygen species and transition metal ions. The nature of their contacts may depend on some chemical factors such as concentration, polarity and the oxidation state of antioxidants, or

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physiological factors such as their localization, the activity of, the rate of pathological changes.

Several reports have indicated interaction between antioxidants endogenously produced and derived from the diet (Chan, Chow, & Chiu, 1999; Machlin & Bendich, 1987). Gutteridge demonstrated that the ferroxidase activity of Cp was inhibited when the molar ratio of ascorbate to Cp was greater than 200 (Gutteridge, 1991). The inhibitory effect of ascorbate was observed at pH 6.0, but not at its physiological value (7.4) (Lovstad, 1997). The interaction between phenolic compounds of biological significance and ceruloplasmin in plasma or other antioxidants of blood plasma are poorly studied. It may be expected that the supplementation of humans with the exogenic antioxidants might affect the activity of the redox system and the total antioxidant status of a body. The competition for the limited amount of substrates might also take place and change the antioxidant barrier of a body.

Polyphenol-rich berries were shown to be effective antioxidants, and the extracts of raspberries, blackberries, blueberries, cranberries, and elderberries contain phenolic acids and flavonoids (Bagchi, Sen, Bagchi, & Atalay, 2004; Han, Shen, & Lou, 2007). Phenolic acids derived from hydroxybenzoic acid and hydroxycinnamic acid account for about one third of the total intake of dietary polyphenols (Han et al., 2007). The dietary sources of phenolic acids are fruit juices, and fruit or seed extracts where their concentration depends on a fruit cultivar and even the type of fruit tissue (localization). For example, ellagic acid was higher in the extract of raspberry seeds than of pulps (Juranic et al., 2005).

It is well known that polyphenols can act as antioxidants by radical scavenging, transient metal ion chelation, and inhibition of xanthine oxidase activity. Furthermore, curcumin, quercetin, and resveratrol increase the concentration of glutathione and the activities of antioxidant enzymes: glutathione peroxidase, superoxide dismutase, catalase *in vivo* and *in vitro* (Han et al., 2007). Some authors recognize that chelation is the main mechanism of polyphenols action (Rice-Evans, Miller, & Paganga, 1996) rather than free radical scavenging (Ferrali et al., 1997; Lopes, Schulman, & Hermes-Lima, 1999).

In plasma, Fe(II) ions are oxidized to Fe(III) by Cp, and chelated or oxidized by polyphenols, thus the possible relation and competition of Cp and polyphenols in the elimination of Fe(II) needs to be studied. The aim of the present study was to investigate the effect of complex mixture of polyphenolic compounds on the antioxidant activity of Cp. Therefore, the effect of raspberry seed extract on the ferroxidase activity of Cp isolated from a serum sample of a healthy subject was investigated.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

$(NH_4)_2$ $Fe(SO_4)_2$ $6H_2O$ (Mohr's salt), $(NH_4)_2$ SO_4, NaCl, KH_2PO_4, K_2HPO_4, CH_3COOH, CH_3COONa, chloroform, ethanol were purchased from POCh Spółka Akcyjna (Gliwice, Poland). 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine -4′,4″-disulfonic acid sodium salt (ferrozine), histidine, hexane, Folin & Ciocalteu's phenol reagent, DEAE-Sephadex A-25 chloride form, caffeic acid, ellagic acid, quercetine were purchased from Sigma–Aldrich (St. Louis, MO, USA). All reagents and solvents were of analytical grade of purity.

2.1.2. Preparation of raspberry seed extract

Raspberry seeds, obtained as the waste from the food industry (Zakłady Przemysłu Owocowo-Warzywnego Kotlin Sp. z o.o.), were first dried, comminuted, defatted with hexane and finally extracted by using 80% aqueous ethanol. The solvent was evaporated at 45 °C and dry residue was dissolved in 96% ethanol. The ethanol solution of raspberry seed extract contained 17.8 mg of dry mass/ml. The total content of phenolic compounds determined by using the Folin–Ciocalteu method (Singleton & Rossi, 1965), and expressed in mg of caffeic acid was 2370.5 mg per 100 g of dry matter of seed.

For further studies, the raspberry seed extract was prepared as a solution diluted in PBS (0.05 M, pH 7.38) in a ratio 1:100 (v/v).

2.1.3. Isolation and purification of ceruloplasmin from human serum samples

Ceruloplasmin was isolated from healthy male subject serum according to the procedure based on the protein precipitation with ammonium sulfate followed by adsorption and elution from DEAE-Sephadex A-50 (Hilewicz-Grabska, Zgirski, Krajewski, & Płonka, 1988). The proteolytic degradation of Cp was inhibited by the addition of aprotinin and 6-aminocaproic acid (EACA) to serum. Aprotinin, the inhibitor of several serine proteases, and 6-aminocaproic acid (plasminogen inhibitor, binds and inactivates carboxypeptidase B) prevent Cp degradation, and as a result the ferroxidase activity of isolated Cp was stable within the period of the study. The structural integrity of Cp and the presence of copper ions are required for the enzyme to effectively catalyze iron loading into transferrin and ferritin (Van Eden & Aust, 2000). Thus, metal-chelating agent as EDTA was not used since it. An efficient metal-chelating agent may cause the loss of copper ions, and the irreversible decrease in Cp activity (Sokolov, Zacharova, Shavlovskii, & Vasil'ev, 2005). Methylparaben (a bacteriostatic agent and preservative) and gentamicin (a bactericidal antibiotic) were added to preserve and conserve the sample.

In brief, DEAE-Sephadex A-25 chromatography, eluted with 0.2 M acetate buffer (pH 5.5 contained 0.125 M NaCl) were applied sequentially. To the pooled fractions from the previous step ammonium sulfate was added to remove globulins and precipitated with ammonium sulphate to reach 58% saturation. The sediment was dissolved in H_2O, precipitated with ethanol–chloroform (9:1, v/v). The final product was dialysed overnight against 0.05 M phosphate buffer, pH 6.82. Purified Cp sample was conserved by chloroform. The purification procedure yielded essentially pure preparation of Cp, and the absorbance ratio A_{610}/A_{280} ratio reached the value of 0.044. Although Sokolov, Zacharova, Shavlovskii, and Vasil'ev (2005) obtained higher value of the ratio (0.050 and 0.052) for Cp preparation isolated on PR-Sepharose column (PR – protamine, i.e. salmon protamine used to form a complex Cp–PR) but for Cp isolated from large volumes of blood plasma (higher than 200 ml). In the present study smaller volume of plasma was used (100 ml). The purity of Cp was expressed as the ratio of protein concentration measured at 610 nm and 280 nm, and calculated as 95.5%. Cp concentration in a phosphate buffer (0.05 M, pH 7.38) with 0.15 M NaCl was 1.17 mg/ml. The choice of the environment for studying the interaction between Cp and RSE was one of the basic questions in this study. The PBS was chosen because the phosphate buffer represents one of the main components of physiological buffering system. The acetate buffer (Sokolov et al., 2005) or Hepes (Welch, Davis, & Aust, 2002) may be more convenient for the measurement of the ferroxidase activity of Cp isolated from serum of different species and used for studies performed in at lower pH value (5.5) than physiological range (slightly above 7.0). Moreover, the mixture of Cp and RSE solutions contain 50 mM of NaCl added to reach equal volume of each sample.

2.2. Methods

2.2.1. The ferroxidase activity

The measurement of the ferroxidase activity is based on the reaction between Fe(II)-histidine complex and ferrozine [disodium

salt of 3-(2-pyridyl)-5,6-bis (4-phenylosulfonic acid)-1,2,4-triazine)]. Fe(II) ions are oxidized in the presence of Cp to Fe(III) and the remaining amount of Fe(II) forms more stable complex with ferrozine in the oxidation state specific reaction yielding a product measured spectrophotometrically (Juan & Aust, 1998).

It is well known that phosphate and bicarbonate ions provide oxygen ligands for the iron and promote the oxidation of Fe(II) (Welch et al., 2002). According to Welch and his co-authors for iron to safely exist in buffers such *in vivo*, it must be chelated and form iron species that is relatively redox inactive. In our experiment Fe(II) ions are complexed with histidine. The choice of histidine as a ligand for Fe(II) was based on the method for the measurement of the ferroxidase activity of Cp. Histidine, as nitrogen containing chelator stabilizing the Fe(II) ions, was chosen to bind Fe(II) in a stable and low-molecular complex in order to avoid iron autooxidation *in vitro* (Van Eden & Aust, 2000). Welch and co. observed also that in the presence of NaCl (50 mM) the autooxidation of Fe(II) was very slow, probably because the pH sufficiently dropped to inhibit the reaction.

2.2.2. The effect of raspberry seed extract on the ferroxidase activity of Cp

The investigation of the effect of raspberry seed extract on the ferroxidase activity of Cp was performed for the following reaction mixtures:

- Cp isolated from serum in the concentration range of 22–66 μg/ml;
- the raspberry seed extract in the concentration range: 1.20–51.56 μg dm/ml;
- a mixture of chosen concentration of Cp (22; 33; 44; 66 μg/ml) and variable concentrations of the raspberry seed extract (1.20–51.56 μg dm/ml);
- a mixture of chosen concentration of the raspberry seed extract and various concentrations of Cp.

The concentrations of Cp and RSE used in the experimental model were chosen based on previous measurements of Cp ferroxidase activity level, and the results of preliminary study on the capacity of different RSE amounts to eliminate Fe(II) from the sample.

2.3. Procedure

The appropriate amounts of the Cp and/or RSE solution were added to the test tubes and the total volume of each sample (525 μl) was reached by the addition of 50 mM NaCl solution. The blank sample contained only 525 μl of 50 mM NaCl. Tubes were tightly closed and incubated for 1 min at 37 °C. After incubation, 1.0 ml of the solution of $(NH_4)_2\ Fe(SO_4)_2 \cdot 6H_2O$ (0.22 mM) complexed with histidine (1.1 mM) in a ratio 1:1 (v:v), prepared freshly, was added to each of the sample and mixed. The concentration of Fe(II) in the final reaction mixture was 71.4 μM. The incubation was stopped after 1 min, and 100 μl of the reaction mixture was added to the next tube with 900 μl of ferrozine solution (4.0 mM). The excess of Fe(II) remaining as the complex with ferrozine was quantitated at 564 nm (E_{564} = 27.9 $mM^{-1}\ cm^{-1}$). The effect of spontaneous oxidation of Fe(II) to Fe(III) was diminished by simultaneous incubation of the samples and the blank. The period of incubation of the mixture of Cp and RSE with Fe(II)-histidine complex, i.e. 1 min, limited the autooxidation of Fe(II) and the oxidative effect of the phosphate ions. The time of incubation (1 min) was chosen based on our previous investigation. The results obtained after 30 s period of incubation were non reproducible and thus unacceptable, and after longer time of incubation (2, 3, 5, 10 min) further changes in Fe(II) concentration were not observed.

The ferroxidase activity of the samples was calculated from the equation: $[(A_{blank}–A_{sample})/27.9]$ and expressed as the change in Fe(II) [ΔFe(II)] concentration in μmol/l of the sample.

2.4. Statistical analysis

All determinations were carried out 6 times and the results were expressed as means ± standard deviation. Statistical differences were estimated by using Student's *t*-test. The significance level was accepted at $p < 0.001$.

3. Results

In the first part of the study, the effect of RSE on the ferroxidase activity of Cp was studied in the mixtures containing both of these antioxidants. The studied range of RSE concentration was 1.20 to 51.56 μg dm/ml, and that of Cp was 22–66 μg/ml. The addition of various amounts of RSE to the sample with a constant amount of Cp caused significant decrease in Fe(II), shown as ΔFe(II) μmol/l in Fig. 1. The effect of RSE on the ferroxidase activity of Cp was dose-dependent for each of Cp concentration. The ferroxidase activity of RSE was dose-dependent within the range of 1.2–35.7 μg dm/ml (Fig. 1), and that of Cp within the range of 22–66 μg/ml (Fig. 2).

The decrease in Fe(II) observed in the samples containing constant RSE level but increasing Cp (Fig. 2) was found to be significant and dose-dependent for lower levels of RSE (1.2–11.9 μg dm/ml). The results presented in Fig. 2 shows that for higher concentrations of RSE (23.80–51.56 μg dm/ml) the increase in concentration of Cp did not significantly affect the ferroxidase activity.

The actual contribution of RSE to the ferroxidase activity of Cp was estimated for two chosen Cp concentrations. Fig. 3 presents the decrease in Fe(II) concentration in samples with varied amounts of RSE and constant amount of Cp (33 or 66 μg/ml). Those data were compared with values of ΔFe(II) calculated as a sum of the values obtained in experiments done for Cp and RSE separately (theoretical). For Cp concentration 66 μg/ml the theoretical decrease in Fe(II) was higher than the experimental value for various amounts of RSE. The experimental and theoretical values of ΔFe(II) differ significantly especially for higher concentration of RSE (23.8, 35.7 and 51.56 μg dm/ml).

In the second part of the study, the maximal concentration of Fe(II) eliminated by solutions of RSE alone and in the presence of Cp of varied concentrations was evaluated. The parameters of the regression between the reciprocal of concentration of RSE and the reciprocal of ΔFe(II) were calculated (Fig. 4). The maximal decrease in Fe(II) found for RSE alone was 14.06 μmol/l, and that for samples with RSE together with Cp were lower and within the range from 6.24 to 7.87 μmol/l (Table 1). The effect of Cp on the

Table 1
Data for $\Delta Fe(II)_{max}$ calculation in the mixture of Cp and RSE

Cp (μg/ml)	Double reciprocal curve $y = ax + b$*	$b = 1/\Delta Fe(II)_{max}$ at $1/RSE \rightarrow 0$	$1/b = \Delta Fe(II)_{max}$
Without Cp	$y = 6.1725x + 0.0711$	0.0711	14.0647
22	$y = 2.1353x + 0.1270$	0.1270	7.8740
33	$y = 2.1550x + 0.1351$	0.1351	7.4019
44	$y = 1.2517x + 0.1527$	0.1527	6.5487
66	$y = 0.6622x + 0.1602$	0.1602	6.2421

When $x = 0 \rightarrow y = b$, thus $1/\Delta Fe(II) = b \rightarrow 1/b = \Delta Fe(II)$ is the maximum of possible decrease in Fe(II) concentration.
* The equation $y = ax + b$ is factored as follows: $1/\Delta Fe(II) = a\ (1/RSE) + b$ (see Fig. 4).

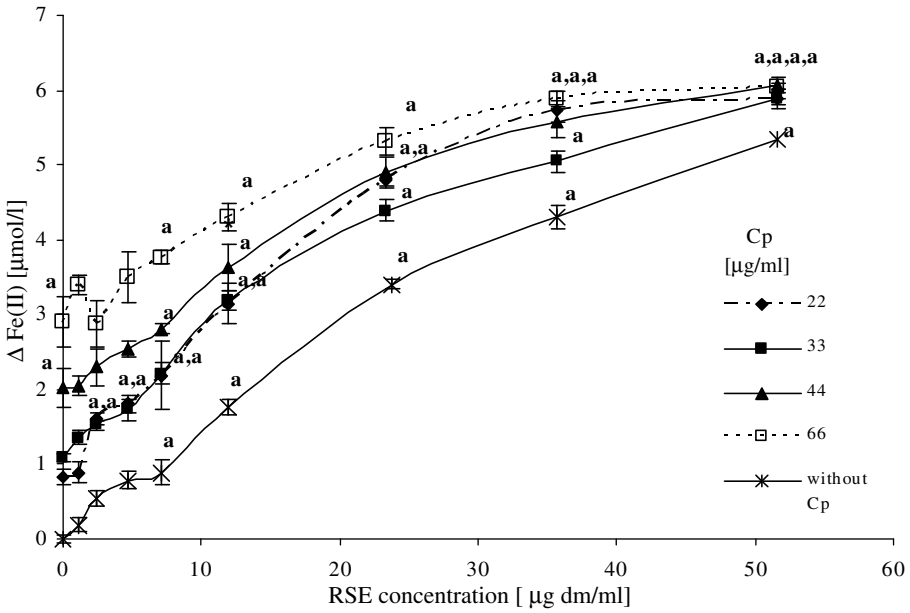

Fig. 1. Effect of raspberry seed extract (RSE) concentration on the ferroxidase activity (expressed as ΔFe(II)) of the mixture of ceruloplasmin (Cp) and RSE ($p < 0.001$, a – significant difference vs. samples without RSE).

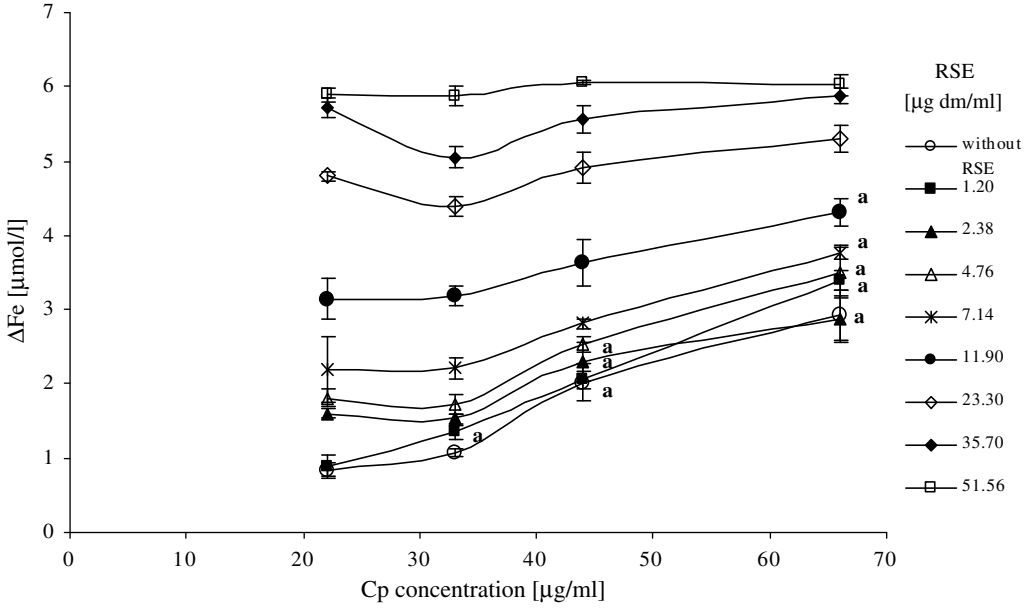

Fig. 2. Effect of ceruloplasmin (Cp) concentration on the ferroxidase activity of the mixture of Cp and raspberry seed extract (RSE) ($p < 0.001$, a – significant difference vs. the lowest Cp concentration 22 mg/ml).

maximal decrease in Fe(II) depends on its concentration, and is higher at the lowest Cp concentration (22 µg/ml) in comparison with its highest level (66 µg/ml).

4. Discussion

The increasing interest in the study of the activity of polyphenolic compounds, found in food of plant origin is still considered with respect to their antioxidant properties. Dietary polyphenols have been shown to protect against cardiovascular diseases by the inhibitory effect on LDL peroxidation, modification of hepatic cholesterol and lipoprotein metabolism, and anti-inflammatory effects by reduction of cytokines production during the oxidative stress (Biesalski, 2007; Rahman, Biswas, & Kirkham, 2006; Zern & Fernandez, 2005). It was suggested that some antioxidants in human diet may change the properties and play the prooxidant role depending on their concentration, production of reactive oxygen species and/or free radicals, and the presence of the other antioxidants (Sergedienė et al., 1999).

Many studies have indicated an interaction among endogenous and exogenous antioxidants. The presence of different types of antioxidants in biological fluids commonly acting in the redox reactions may cause unexpected relationships and the competition in the processes of scavenging of free radicals and reactive oxygen species. It may lead to the reversal of the antioxidant into

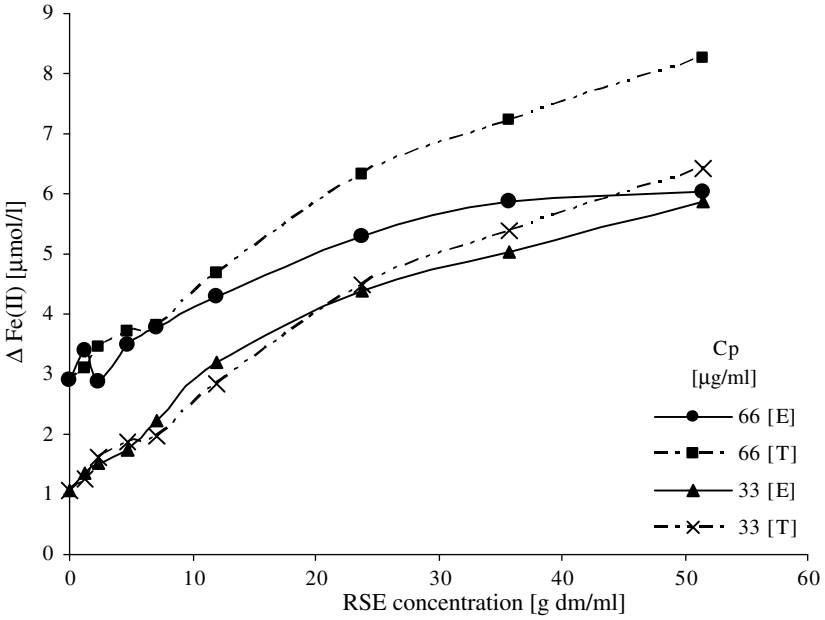

Fig. 3. The values of ΔFe(II) measured in samples containing both Cp and RSE (experimental, E) and the calculated values of ΔFe(II) for samples of Cp and RSE measured separately (theoretical, T).

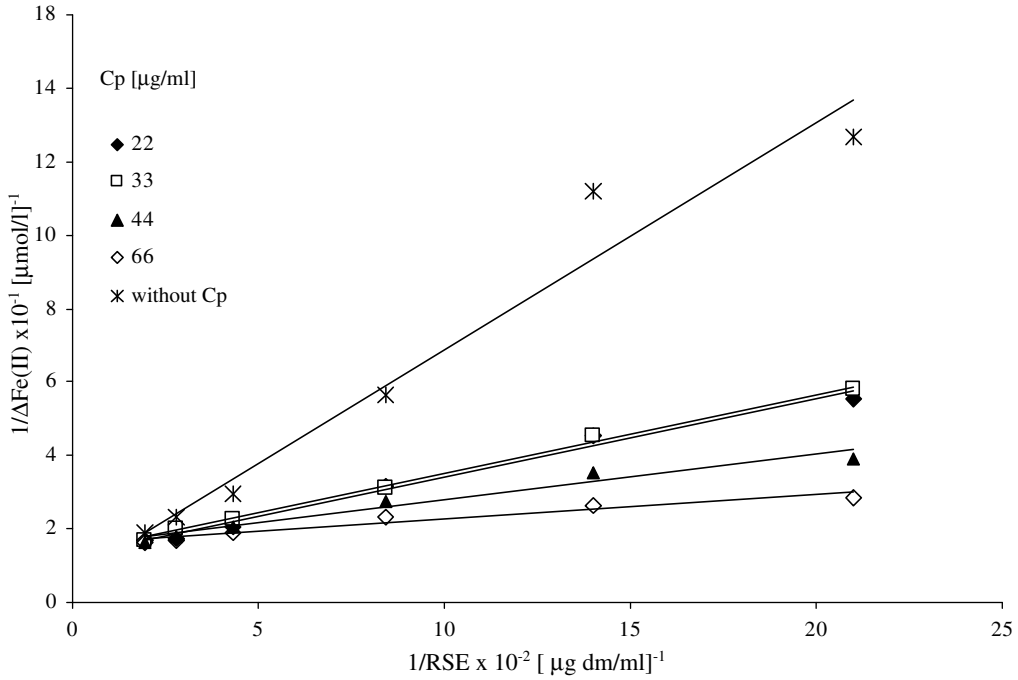

Fig. 4. Double reciprocal plot of [1/ΔFe(II)] vs. [1/RSE] in the presence of various concentration of Cp when RSE was in concentration range 4.76–51.56 (µg dm/ml)].

prooxidant activity at the high level or in specific conditions for one of them. The relationship between the low-molecular compounds, i.e. polyphenols, and the endogenic macromolecules with the enzymatic activity, such as Cp, remains under consideration. Gutteridge demonstrated the effect of ascorbate on the ferroxidase activity of Cp in dose-dependent way, and finally the loss of the activity at the molar ratio of ascorbate to Cp greater than 200 (Gutteridge, 1991). Finley and Cerklewski investigated the effect of ascorbic acid supplementation on the copper status in young adult men (Finley & Cerklewski, 1983). It was shown that copper status in serum, i.e. copper and ceruloplasmin concentration, was negatively influenced by ascorbic acid supplementation at the level of 605 mg/day. Serum Cp activity was significantly reduced during and even after 20 days after supplementation period.

Variety of plasma components of redox or free radical/reactive oxygen species scavenger properties can probably affect the concentration of Fe(II). The reason to study the relationship between Cp and RSE prior to the effect of the whole blood or plasma was the fact that albumin and other plasma proteins may affect the competition between Cp and phenolic present in RSE.

In the present study the removal of free Fe(II) ions, potential oxidative component of biological fluids, from the sample was found to be dependent on the level of Cp and RSE, two efficient antioxidants. The smaller amounts of RSE and Cp in the studied samples were more efficient in lowering Fe(II) concentration than their higher amounts. Moreover, both RSE and Cp at highest levels produced just insignificant changes in Fe(II) concentration in the experimental model.

Differences in the ability to eliminate Fe(II) was shown also by calculation of the theoretical values of ΔFe(II) for samples with Cp and RSE studied separately and compared with the values of ΔFe(II) found in a mixture of both components. The decrease of Fe(II) concentration in samples containing both Cp and RSE is lower in comparison with the calculated sum of ΔFe(II) for separate samples of Cp and RSE. The difference between experimental and calculated data is more significant for higher concentration of RSE. This difference suggests the competition for the substrate and higher ability of RSE to eliminate Fe(II) in the absence of Cp.

The effect of RSE on Cp activity observed in the experimental model may be estimated as the maximal value of ΔFe(II) that could be reached at the highest RSE concentration. The maximum of Fe(II) concentration which can be oxidized to Fe(III) was found by using the double reciprocal plot of [1/ΔFe(II)] vs. [1/RSE]. The RSE is more effective in the elimination of Fe(II) (almost 2 times) in samples without Cp. It means that Cp and RSE present in the same mixture could compete for the substrate, limited in the experiment model.

The results of recent studies suggest that polyphenols may act as antioxidants by metal chelation and/or scavenging of free radicals and reactive oxygen species. The relationship between the structure and the activity emphasized the presence of *ortho*-dihydroxy group in a polyphenolic compound. Polyphenols without catechol or galloyl moiety, i.e. vanilic acid, syringic acid and ferulic acid did not show any complex formation. Andjelković studied the capacity of polyphenolic acids bearing catechol and galloyl groups for complex formation with iron ions (Andjelković et al., 2006). The present study showed that the affinity of RSE components to Fe(II)/Fe(III) ions and the stability of formed chelates seem to be higher than the oxidative activity of Cp towards Fe(II).

The results of phenolic analysis of different fruits are widely published but only scant data are concerning the content of phenolic compounds in fruit seeds. Results obtained by Bushman (Bushman, Phillips, Isbell, Ou, Crane, & Knapp 2004) showed the presence of 8.7 mg of ellagic acid in 1 g of red raspberry seeds. HPLC analysis of raspberry seed extract isolated from the waste of fruit processing provided in Poland, revealed that 20% of phenolic fraction of raspberry seed ethanolic extract was covered by flavonol and almost 5% by ellagic acid and its derivatives (Klimczak, Małecka, Szlachta, & Gliszczyńska-Świgło, 2007; Pachołek, 2003).

The complex formation may be hindered by cellular iron chelators such as citric acid or malic acid present in fresh fruits. Cheng demonstrated that citric acid can delay and reverse formation of ferric-anthocyanin complex (Cheng & Crisosto, 1997).

The antioxidant activity of polyphenols has also been attributed to scavenging of hydroxyl radical HO. However, they rather act as ligands forming stable complexes with Fe(II) that cannot participate in Fenton reaction, ie generate hydroxyl radicals and Fe(III). The competition for Fe(II) was observed between tannic acid and H_2O_2, and the antioxidant activity of tannic acid examined by Lopes was inversely proportional to Fe(II) concentration (Lopes et al., 1999). The Fe(II)-tannic acid complexes would protect DNA against iron-mediated oxidative damage and be proposed for prevention of disorders related to iron-mediated diseases.

5. Conclusion

The present study shows that Cp and raspberry seed extract demonstrate the ferroxidase activity or chelating property in dose-dependent way. The presence of RSE in samples containing varied concentrations of Cp brings about the increase in Fe(II) elimination. However, the effect of RSE at higher levels of both RSE and Cp is limited by their competition for accessible amount of Fe(II) ions.

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