Protein-Lipid Interactions during Oxidation of Liposomes

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Abstract: Oxidation of bovine serum albumin and its interaction with phenolic red raspberry and bilberry extracts (4.2 and 8.4 μg/ml) was investigated in a liposome system. Samples were incubated in the dark at 37°C with copper, and the extent of oxidation was measured by determing the loss of tryptophan fluorescence and the formation of protein carbonyls, conjugated diene hydroperoxides and hexanal. Both red raspberry and bilberry extracts inhibited lipid and protein oxidation. Red raspberry extract in 4.2 μg/ml concentration was the best inhibitor against both lipid and protein oxidation. In conclusion, oxidative deterioration due to protein-lipid oxidation is inhibited by phenolic compounds in berries.

Keywords: protein oxidation; liposome model; phenolic compounds; antioxidant

INTRODUCTION

In complex food matrices strong interactions between proteins and lipids have a significant effect on the oxidative reactions. The oxidation reactions can easily transfer from lipids to proteins. Phenolic compounds such as flavonoids and phenolic acids are effective antioxidants in lipid oxidation. Phenolic compounds can inhibit also oxidation of proteins by retarding the oxidation reactions, by binding to the proteins and by forming complexes between protein molecules. It has been shown, that phenolic compounds such as ferulic acid, malvidin and rutin inhibit the oxidation of bovine serum albumin (BSA) in liposome model system [1]. Anthocyanins from berries (bilberry, red raspberry, lingonberry and black currant) have been shown to prevent not only lipid oxidation but also oxidation of lactalbumin [2].

In meat unfavourable chemical reactions such as oxidation of meat phospholipids resulting in an unpleasant taste called warmed over flavor and formation of protein carbonyls may be retarded with phenolic compounds. Many raw materials, like berries, pine bark, vegetable peels and rapeseed contain phenolic compounds such as anthocyanins, ellagitannins and sinapic acid derivatives. For this reason they can prove to be very potent antioxidants in food applications. By studying the tryptophan content and the protein-lipid interactions in emulsions it is possible to evaluate protein modification and lipid oxidation end-products during processing and storage of food emulsions such as milk and sausages.

EXPERIMENTAL

Materials. Red raspberry and bilberry extracts in 70% ethanol were prepared according to method of Kähkönen *et al*. [3] in the Laboratory of Food Chemistry, University of Helsinki, Finland. Bovine serum albumin (BSA), L-α-phosphatidylcholine (lecithin from soybean) with a phosphatidylcholine (PC) content of ~40% were purchased from Sigma-Aldrich Co. (St. Louis, MO), copper(II) acetate from Merck (Darmstadt, Germany) and sinapic acid from Extrasynthèse (Genay, France). Ethanol of AAS grade was from Primalco (Rajamäki, Finland), and methanol of HPLC grade was from Rathburn Chemicals Ltd. (Walkerburn, Scotland). The citrate buffer was made of citric acid (Pharmia Ltd., Helsinki, Finland) and sodium hydroxide (Dilut-it, J.T. Baker, Deventer, Holland) adjusted to pH 6.6. Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA).

Protein-liposome oxidation system. The liposomes were prepared as described by Huang and Frankel [4] to a final PC concentration of 0.8 wt%.

The liposomes were incubated in the dark at 37°C with 3.0 μM cupric acetate for 6 days with 0.16% (i.e. 20% of the PC concentration) BSA by using 27.5 mM citrate buffer at pH 6.6. BSA oxidation in liposomes was also measured in the presence of 4.2 and 8.4 μg/ml berry extracts. The reference compound, sinapic acid, was tested only at concentration of 10 μM (2.2 μg/ml). All results are given as the mean values of triplicate analyses.

Protein oxidation. BSA oxidation was measured by fluorescence spectroscopy by following the formation of protein carbonyls and loss of natural tryptophan fluorescence. Samples (500 μl) were dissolved in citrate buffer (1 ml). Emission spectra of tryptophan were recorded from 300 to 400 nm with the excitation wavelength set at 283 nm (F-4010 Hitachi fluorescence spectrophotometer), and emission spectra of protein carbonyls were recorded from 400 to 500 nm with excitation wavelength set as 350 nm.

Lipid oxidation. Lipid (liposome) oxidation was followed by formation of conjugated diene hydroperoxides and formation of hexanal. Samples (100 μl) were dissolved in methanol (5 ml), and conjugated diene hydroperoxides were analyzed spectrophotometrically at 234 nm (Lambda Bio UV-vis spectrophotometer, Perkin-Elmer, Norwalk, CT). Hexanal (samples of 500 μl) was measured using static headspace gas chromatography (Autosystem XL gas chromatograph equipped with an HS40XL headspace sampler; Perkin-Elmer, Shelton, CT; column NB-54, Nordion) according to method of Frankel *et al*. [5].

RESULTS AND DISCUSSION

In the liposome model system, the formation of conjugated dienes was inhibited by 41% with bilberry extract (4.2 and 8.4 μg/ml concentration) and with red raspberry extract (4.2 μg/ml concentration). Formation of hexanal was inhibited by 15% with bilberry extract (4.2 and 8.4 μg/ml concentration) and red raspberry extract (4.2 μg/ml concentration) (Table 1). Inhibition of tryptophan fluorescence was 40% with red raspberry extract (4.2 μg/ml concentration). Loss of tryptophan fluorescence is principally due to oxidative changes in tryptophan residues in protein. Formation of protein carbonyls was inhibited by 35% with red raspberry extract (4.2 μg/ml concentration) (Table 2). With sinapic acid the formation of conjucated diene hydroperoxides, formation of hexanal, tryptophan loss and carbonyl gain were inhibited by 41.1%, 15.3%, 31.0% and 26.7%, respectively.

The activity of antioxidants in foods and biological systems is dependent on a multitude of factors, including colloidal properties of the substrates, the conditions and stages of oxidation and the localization of antioxidants in different phases [6]. Anthocyanins are the main phenolic constituents in bilberry, whereas in red raspberry the main phenolics found are ellagitannins. Bilberry is also rich in flavonols, mainly quercetin, and hydroxycinnamic acid derivatives, while red raspberry has an especially high content of ellagic acid [3]. The *o*-dihydroxyphenolic acids interact more strongly than monohydroxyphenolic acids with BSA [7].

Table 1. Inhibition of lipid oxidation (after 6 days of oxidation) in BSA-lecithin liposome oxidation with 4.2 and 8.4 μg/ml phenolic extracts (percent inhibition)

Compound	Hydroperoxides		Hexanal	
	4.2 μg/ml	8.4 μg/ml	4.2 μg/ml	8.4 μg/ml
Bilberry extract	41.1	41.0	16.8	15.2
Red raspberry extract	41.1	26.7	14.4	7.8

Table 2. Inhibition of protein oxidation (after 6 days of oxidation) in BSA-lecithin liposome oxidation with 4.2 and 8.4 μg/ml phenolic extracts (percent inhibition)

Compound	Tryptophan loss		Carbonyl gain	
	4.2 μg/ml	8.4 μg/ml	4.2 μg/ml	8.4 μg/ml
Bilberry extract	26.9	29.3	29.0	27.0
Red raspberry extract	40.5	15.2	35.3	24.1

Thus, strong protein binding of phenolic acids or polyphenolic compounds may promote oxidation in the lecithin-liposomes [2]. Antioxidative activity of BSA may also be related to its ability to react with lipid oxidation products and to produce modified BSA with antioxidative activity [8]. In addition, Heinonen *et al*. [9] showed the stabilizing effect of BSA on liposome oxidation.

CONCLUSIONS

In this study, red raspberry extract at concentration of 4.2 μg/ml was the best inhibitor against both lipid and protein oxidation. In conclusion, oxidative deterioration due to protein-lipid oxidation is inhibited by phenolic compounds in berries.

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