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# Inhibition of Protein and Lipid Oxidation in Liposomes by Berry Phenolics

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The antioxidant activity of berry phenolics (at concentrations of 1.4, 4.2, and 8.4 $\mu$g of purified extracts/mL of liposome sample) such as anthocyanins, ellagitannins, and proanthocyanidins from raspberry (*Rubus idaeus*), bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), and black currant (*Ribes nigrum*) was investigated in a lactalbumin−liposome system. The extent of protein oxidation was measured by determining the loss of tryptophan fluorescence and formation of protein carbonyl compounds and that of lipid oxidation by conjugated diene hydroperoxides and hexanal analyses. The antioxidant protection toward lipid oxidation was best provided by lingonberry and bilberry phenolics followed by black currant and raspberry phenolics. Bilberry and raspberry phenolics exhibited the best overall antioxidant activity toward protein oxidation. Proanthocyanidins, especially the dimeric and trimeric forms, in lingonberries were among the most active phenolic constituents toward both lipid and protein oxidation. In bilberries and black currants, anthocyanins contributed the most to the antioxidant effect by inhibiting the formation of both hexanal and protein carbonyls. In raspberries, ellagitannins were responsible for the antioxidant activity. While the antioxidant effect of berry proanthocyanidins and anthocyanins was dose-dependent, ellagitannins appeared to be equally active at all concentrations. In conclusion, berries are rich in monomeric and polymeric phenolic compounds providing protection toward both lipid and protein oxidation.

**KEYWORDS: Protein oxidation; tryptophan; protein carbonyls; anthocyanins; ellagitannins; proanthocyanidins; antioxidants; phenolic compounds; berries**

## INTRODUCTION

Polyphenols are widespread in the plant kingdom, and they can interact with proteins leading to the inhibition of enzymes, a decrease of protein digestibility, and protein precipitation. Interaction can also affect protein functionality (*1*). Because of the structure of polyphenols and many different functional groups in the proteins, the complex can be formed both by hydrogen bonding and by hydrophobic bonding but also covalent attachment can occur depending on the polarity of the polyphenol (*2*−*6*). In turn, polyphenol interactions with proteins influence the structure and the taste of foods. Through these complex formations, polyphenols are postulated to act as antioxidants toward protein oxidation as well (*2*, *7*).

In foods, lipid oxidation can cause protein oxidation due to close interactions between lipids and proteins. During oxidation, proteins can cross-link and therefore affect the texture of food, i.e., change the viscosity of solution (*8, 9*). Oxidation reactions affect the quality of food, but they also have an impact on the charge and conformation of protein three-dimensional structure (exposure of hydrophobic groups, changes in secondary structure and disulfide groups) and protein functionality such as changes in food texture, decrease in protein solubility (due to aggregation or complex formation), color changes (browning reactions), loss of enzyme activity, and changes in nutritive value (loss of essential amino acids) (*10*−*12*).

The aim of this study was to investigate the effects of different phenolic berry extracts and phenolic fractions such as anthocyanins, ellagitannins, and proanthocyanidins isolated from black currants, bilberries, raspberries, and lingonberries on the oxidative stability of both proteins and lipids in a liposome model system. Phenolic compounds have been shown to be effective antioxidants in inhibiting lipid oxidation (*13*−*18*) as well as potent radical scavengers (*17*−*19*). In addition, anthocyanins have been shown to chelate metal ions at moderate pH with their ionized hydroxyl groups of the B ring (*20*). Procyanidin−bovine serum albumin (BSA) complexes have been reported to act as free radical scavengers (*19*). BSA has also been reported to exert a synergistic effect with antioxidants because of the formation of protein−antioxidant adducts (*21*). It is postulated that phenolic compounds inhibit the oxidation of proteins both by retarding the oxidation reactions by binding to the proteins and by forming complexes between protein molecules (*2*, *7*). Phenolic compounds such as caffeic acid, malvidin, and quercetin have been shown to inhibit BSA oxidation in the liposome model system (*13*), and different anthocyanins have

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**Table 1.** Phenolic Profiles of Berry Extracts (Expressed as Percent of Total Phenolics Measured Using HPLC; ND, Not Detected)

| extract | As[a] | flavonols[b] | OH–C[c] | OH–B[d] | ProAs[e] | ET[f] | EA[g] | mg phenolic compounds/ g dry weight |
|---|---|---|---|---|---|---|---|---|
| black currant | 92.3 | 3.9 | 3.0 | ND | 0.8 | ND | ND | 233 |
| bilberry | 94.8 | 0.9 | 4.2 | 0.1 | ND | ND | ND | 572.8 |
| lingonberry | 75.0 | 6.9 | 2.8 | 1.4 | 13.9 | ND | ND | 72 |
| raspberry | 30.8 | 0.7 | ND | 0.4 | 8.2 | 50.6 | 9.3 | 182 |

[a] As, anthocyanins; amount based upon cyanidin-3-glucoside as standard. [b] (+)-Catechin as standard. [c] Chlorogenic acid as standard. [d] Gallic acid as standard. [e] ProAs, proanthocyanidins; flavan-3-ol as standard (*16*); for lingonberries, flavan-3-ols were further fractioned to two proanthocyanidin fractions: fraction I consisting of proanthocyanidin monomers (92%) and dimers (8%) and fraction II consisting of procyanidin dimers (73%) and trimers (27%) (*24*). [f] ET, ellagitannins; ellagic acid as standard. [g] EA, ellagic acid.

reported to be efficient antioxidants in inhibiting both protein and lipid oxidation in the lactalbumin−liposome system (*22*).

## MATERIALS AND METHODS

**Materials.** Lactalbumin, L-α-phosphatidylcholine (lecithin from soybean) with a phosphatidylcholine (PC) content of ∼40%, Amberlite XAD-7 nonionic polymeric absorbent, and lipophilic Sepadex LH-20 absorbent were purchased from Sigma Chemical Co. (St. Louis, MO), copper(II) acetate was purchased from Merck (Darmstadt, Germany), ethanol of AAS grade was purchased from Primalco (Rajamäki, Finland), and all other solvents of high-performance liquid chromatography (HPLC) grade were purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). The 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. The water used was purified by passing through a Milli Q system (Millipore Corp., Bedford, MA.). All berries (raspberry, bilberry, lingonberry, and black currant) were purchased from a market place. The leaves and branches were picked from berry samples, and samples were packed immediately into a vacuum and stored in a freezer at −18 °C until use.

**Extraction and Isolation of Phenolic Compounds.** The berry phenolic extracts and anthocyanin fractions from bilberry (*Vaccinium myrtillus*), black currant (*Ribes nigrum* var. Öjebyn), raspberry (*Rubus idaeus*), and lingonberry (*Vaccinium vitis-idaea*) were produced as described by Kähkönen et al. (*15*, *23*). Extraction of phenolic compounds was carried out by homogenizing (Ultra-Turrax T25 mixer, Janke & Kunkel, Germany) for 1 min 2 g of berries with 20 mL of solvent (acetone−water 70:30 v/v) in a centrifuge tube. The tubes were centrifuged (4000 rpm, 15 min) (RC5C, Sorvall Instruments, DuPont), and the clear supernatant was collected. The procedure was repeated twice with another 20 mL of solvent. The supernatants were combined and taken to dryness (berry phenolic extracts). The solid residues were dissolved in Milli Q purified water for further purifications to different phenolic fractions.

Berry anthocyanins and raspberry ellagitannins were isolated by the method described by Kähkönen et al. (*23*) with both Amberlite XAD-7 (diameter 40 mm, length 300 mm) and Sephadex LH-20 column chromatography (diameter 40 mm, length 300 mm). First, free sugars and organic and phenolic acids were eluted from berry phenolic extract with 6% $CH_3CN$ ($CH_3CN$:TFA:$H_2O$ 6.0:0.5:93.5 v/v/v) in Amberlite XAD-7 column. After that, the remaining phenolics in the column were eluted with 100% $CH_3CN$. The solvent was then evaporated, and residual phenolic extract was dissolved in 50% methanol (methanol−water 50:50 v/v). After that, anthocyanins were eluted with 50% methanol (methanol−water 50:50 v/v) in a Sephadex LH-20 column, and finally, ellagitannins were eluted with 70% acetone (acetone−water 70:30 v/v). The ellagitannin-containing fraction was then taken to dryness, and the anthocyanin-containing fraction was further purified by preparative HPLC as described by Kähkönen et al. (*23*). Lingonberry proanthocyanidins were isolated according to Määttä et al. (*24*) resulting in two fractions differering in their molecular weight. Proanthocyanidins were further fractioned to two proanthocyanin fractions: fraction I consisting of proanthocyanin monomers (92%) and dimers (8%) and fraction II consisting of proanthocyanidin dimers (73%) and trimers (27%).

**HPLC Determination of Phenolic Profiles.** Phenolic profiles of berry extracts were determined using an analytical HPLC method described by Kähkönen et al. (*16*). The separation of phenolic compounds was carried out on a Nova-Pak C18 column (150 mm × 3.9 mm, 4 μm; Waters) with a WISP 712 autosampler, three 501 pumps, a PDA996 diode array detector, and a Millennium 2020C/S software data module (Waters, Milford, MA). On the basis of spectral identification, phenolics were quantified in seven subclasses as shown in **Table 1**.

**Lactalbumin−Liposome Oxidation System.** The liposomes were prepared as originally described by Huang and Frankel (*25*) to a final PC concentration of 0.8 wt %. The liposomes were incubated in the dark at 37 °C with 3 μM cupric acetate for 7 days with 0.16% lactalbumin (i.e., 20% of the PC concentration) by using 27.5 mM citrate buffer at pH 6.6 in the presence of raspberry, lingonberry, bilberry, and black currant extracts or anthocyanin fractions, in the presence of raspberry ellagitannin fraction or in the presence of lingonberry proanthocyanin fractions (1.4, 4.2, and 8.4 μg/mL).

All tested berry extracts and fractions were dissolved in ethanol. Ethanolic solutions were pipetted into Erlenmeyer flasks (100 mL), and the solvent was then evaporated with nitrogen. The PC−lactalbumin solution was then added into the flask, and liposomes were prepared by sonicating the solution for 3 min with a U 50 Control Ikasonic Sonicator (Janke & Kunkel GmbH & Co. KG), and the flasks were sealed before oxidation. The tocopherol content of PC was investigated by using HPLC (*26*). PC contains residual amounts of α-, γ-, and δ-tocopherols: 19, 117, and 59 μg/g, respectively (1.6 ppm total tocopherols in the final liposome solution).

**Protein Oxidation.** Lactalbumin oxidation was measured by fluorescence spectroscopy by following both the formation of protein carbonyls or the loss of natural tryptophan fluorescence (*13*, *22*, *27*). 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). In addition, emission spectra of later products of oxidation (protein carbonyls) were recorded from 400 to 500 nm with the excitation wavelength set at 350 nm. The percent inhibition against loss of tryptophan fluorescence was calculated at day 6 as $[(C_0 - C_t) - (S_0 - S_t)]/(C_0 - C_t) \times 100$, where $C_0$ is the initial fluorescence of the control sample, $C_t$ is the fluorescence of the control sample at time $t$, $S_0$ is the initial fluorescence of the antioxidant sample, and $S_t$ is the fluorescence of the antioxidant sample at time $t$. The percent inhibition of protein carbonyls was calculated at day 6 as $[(C_t - S_t)/C_t] \times 100$, where $C_t$ is the fluorescence of protein carbonyls in control sample at time $t$ and $S_t$ is the fluorescence of protein carbonyls in antioxidant sample at time $t$. All results are given as the mean values of triplicate analyses.

**Lipid Oxidation.** Lipid (liposome) oxidation was followed by formation of conjugated diene hydroperoxides and formation of hexanal. Samples (50−100 μL) were dissolved in methanol (5 mL), and conjugated diene hydroperoxides were analyzed spectrophotometrically at 234 nm (Lambda Bio UV/VIS Spectrophotometer, Perkin-Elmer). Hexanal (samples of 500 μL) was measured by using static headspace gas chromatography (Autosystem XL gas chromatograph equipped with an HS40XL headspace sampler, Perkin-Elmer, Shelton, CT; column

**Table 2.** Inhibition of Lipid and Protein Oxidation (after 6 Days of Oxidation) by Berry Phenolics Incorporated into Lactalbumin–Lecithin Liposomes (Percent Inhibition, Mean ± SD)[a]

| | conjugated diene hydroperoxides | | | hexanal | | |
|---|---|---|---|---|---|---|
| extract | 1.4 μg/mL | 4.2 μg/mL | 8.4 μg/mL | 1.4 μg/mL | 4.2 μg/mL | 8.4 μg/mL |
| raspberry | 56.3 ± 4.7a | 14.7 ± 1.5a | 24.9 ± 1.0c | –67.2 ± 17.7c | 48.1 ± 1.5ab | 85.2 ± 0.6b |
| lingonberry | –15.9 ± 0.8c | –2.2 ± 0.6b | 70.6 ± 1.3a | 71.2 ± 0.7a | 57.1 ± 2.7a | 98.7 ± 0.3a |
| bilberry | 19.4 ± 2.6b | –13.1 ± 0.3c | 66.5 ± 1.5a | 38.8 ± 0.5b | 57.3 ± 3.4a | 98.4 ± 0.1a |
| black currant | 22.5 ± 2.6b | –9.4 ± 0.7bc | 51.0 ± 1.7b | 45.1 ± 3.6b | 41.3 ± 2.8b | 93.7 ± 0.4ab |
| | | tryptophan fluorescence | | | carbonyl gain | |
| raspberry | 0.2 ± 3.3b | 10.7 ± 0.3a | 36.8 ± 8.5a | 91.7 ± 3.4a | –18.4 ± 1.1c | 64.7 ± 0.5b |
| lingonberry | –3.9 ± 0.1b | –1.7 ± 0.2b | 44.1 ± 0.3a | –17.9 ± 0.3c | –24.3 ± 0.1c | 52.8 ± 0.1c |
| bilberry | 22.9 ± 0.5a | 6.1 ± 0.2a | 27.9 ± 0.6b | 67.6 ± 0.1b | 49.1 ± 0.1a | 79.9 ± 0.3a |
| black currant | –4.7 ± 1.0b | –6.4 ± 2.1b | 20.1 ± 0.7c | –36.9 ± 0.7d | –8.6 ± 0.8b | 72.4 ± 0.2a |

[a] SD, standard deviation. Negative values indicate prooxidant activity. Values in the same column at the same concentration followed by different letters are significantly different ($p < 0.05$).

**Table 3.** Inhibition of Protein and Lipid Oxidation (after 6 Days of Oxidation) by Different Phenolic Fractions Isolated from Berries Incorporated into Lactalbumin–Lecithin Liposomes (Percent Inhibition, Mean ± SD)[a]

| | conjugated diene hydroperoxides | | | hexanal | | |
|---|---|---|---|---|---|---|
| fractions[b] | 1.4 μg/mL | 4.2 μg/mL | 8.4 μg/mL | 1.4 μg/mL | 4.2 μg/mL | 8.4 μg/mL |
| raspberry as | 25.5 ± 5.2c | –9.6 ± 3.6e | 54.0 ± 1.6c | 27.5 ± 0.0c | 49.6 ± 3.4c | 96.2 ± 0.1a |
| lingonberry as | 23.8 ± 3.9c | 37.1 ± 1.2c | 39.2 ± 7.9d | 63.0 ± 1.1ab | 68.0 ± 1.2b | 84.8 ± 3.4ab |
| bilberry as | 11.9 ± 2.1d | 12.7 ± 2.9d | 26.9 ± 6.9e | 58.2 ± 2.8ab | 87.0 ± 4.9a | 91.3 ± 3.6a |
| black currant as | –1.9 ± 1.7e | 22.3 ± 4.0d | 39.8 ± 5.8d | 52.6 ± 4.0b | 75.2 ± 2.6ab | 84.4 ± 3.5ab |
| raspberry et | 51.0 ± 0.4a | 58.6 ± 2.9b | 64.7 ± 2.1b | 55.2 ± 4.5b | 63.6 ± 1.8bc | 60.7 ± 3.5c |
| lingonberry pro I | 51.4 ± 0.8a | 68.7 ± 1.8a | 82.3 ± 1.9a | 55.3 ± 5.3b | 77.0 ± 0.4ab | 76.8 ± 3.0b |
| lingonberry pro II | 35.8 ± 2.2b | 78.0 ± 1.5a | 85.8 ± 0.7a | 70.4 ± 0.5a | 87.8 ± 1.0a | 94.5 ± 4.1a |
| | | tryptophan fluorescence | | | carbonyl gain | |
| raspberry as | 5.1 ± 0.3d | 7.5 ± 0.3d | 21.7 ± 0.1d | 33.1 ± 0.4e | 58.5 ± 0.2d | 73.8 ± 0.1d |
| lingonberry as | 18.8 ± 1.1bc | 25.6 ± 2.2c | 27.1 ± 2.1bcd | 56.6 ± 0.9bc | 71.9 ± 1.7b | 79.8 ± 0.9bcd |
| bilberry as | 13.7 ± 0.7c | 22.2 ± 1.5c | 24.8 ± 0.6cd | 59.6 ± 3.2b | 80.6 ± 1.0ab | 82.6 ± 1.6bc |
| black currant as | 5.3 ± 1.1d | 22.3 ± 0.6c | 27.9 ± 1.9bcd | 47.5 ± 4.9d | 82.2 ± 1.8a | 86.6 ± 0.6ab |
| raspberry et | 25.0 ± 1.2b | 49.8 ± 4.1b | 30.3 ± 2.9bc | 76.4 ± 1.0a | 56.2 ± 12.2d | 76.3 ± 4.0cd |
| lingonberry pro I | 24.1 ± 1.7b | 27.8 ± 2.6c | 34.2 ± 1.1b | 45.5 ± 1.8d | 83.9 ± 2.6a | 94.0 ± 0.4a |
| lingonberry pro II | 43.0 ± 3.2a | 64.4 ± 1.6a | 56.8 ± 4.9a | 50.6 ± 1.5cd | 70.1 ± 0.7c | 73.5 ± 1.8d |

[a] SD, standard deviation. [b] As, anthocyanin fraction; et, ellagitannin fraction; pro I, pro II, monomeric (pro I) and polymeric (pro II) proanthocyanidin fractions. Negative values indicate prooxidant activity. Values in the same column at the same concentration followed by different letters are significantly different ($p < 0.05$).

NB-54, Nordion) according to the method of Frankel et al. (*28*). The percent inhibition against liposome oxidation was calculated at day 6 using the same formula as for inhibition of protein carbonyls [$(C_t - S_t)/C_t] \times 100$, where $C_t$ is the amount of conjugated diene hydroperoxides or hexanal in control sample at time $t$ and $S_t$ is the amount of conjugated diene hydroperoxides or hexanal in antioxidant sample at time $t$. The results are given as the mean values of triplicate analysis.

**Statistical Analysis.** Differences among antioxidant activities were tested by multivariance analysis using Statgraphics Plus (STCC Inc., Rockville, MD). The significance level was $p < 0.05$.

## RESULTS

**Phenolic Profiles of Berry Extracts.** The phenolic composition of berry extracts is shown in **Table 1**. Black currant and bilberry extracts consisted mainly of anthocyanins (92−95%) with only modest amounts of other phenolics present. The main phenolics in lingonberries were also anthocyanins (75%), but in addition, lingonberries contained significant amounts of proanthocyanidins (14%). The main groups of phenolics in raspberries were ellagitannins (51%), although anthocyanins (31%) and proanthocyanidins (8%) were also present.

**Effect of Berry Extracts on Lipid and Protein Oxidation.** The antioxidant protection toward lipid oxidation was best provided by lingonberry and bilberry phenolics followed by black currant and raspberry phenolics (**Table 2**). Bilberry and raspberry phenolics exhibited the best overall antioxidant activity toward protein oxidation. The antioxidant effect toward lipid oxidation was more pronounced than the effect on protein oxidation. In general, most berry phenolics inhibited the formation of hexanal efficiently, while at lower tested concentrations of 1.4 and 4.2 μg/mL, most berry phenolics exhibited either a pro-oxidant activity or only a weak antioxidant activity toward the formation of conjugated diene hydroperoxides or loss of tryptophan fluorescence. Raspberry phenolics were an exception in exhibiting a pro-oxidant effect toward the formation of hexanal but an antioxidant effect toward the formation of conjugated diene hydroperoxides as well as toward carbonyl formation at the lowest concentration. Lingonberry and raspberry phenolics at a concentration of 8.4 μg/mL inhibited more the loss of tryptophan fluorescence than bilberry and black currant phenolics, while bilberry and black currant phenolics were more efficient toward the formation of carbonyl compounds.

**Effect of Phenolic Fractions on Lipid and Protein Oxidation.** Lingonberry proanthocyanidins were among the most efficient antioxidants toward both lipid and protein oxidation at all concentrations (**Table 3**). The fraction (pro II) containing dimeric (73%) and trimeric (27%) proanthocyanins appeared more effective than the 92% monomeric (pro I) fraction in inhibiting lipid oxidation as well as loss of tryptophan fluorescence. All anthocyanin fractions isolated from berries were effective especially in inhibiting the formation of both hexanal and carbonyl compounds. The overall antioxidant effect of anthocyanins toward hexanal formation was most pronounced

with bilberry anthocyanins followed by raspberry, black currant, and lingonberry anthocyanins. Black currant and bilberry anthocyanins were the best berry anthocyanins in inhibiting carbonyl formation. The antioxidant activities of anthocyanin and proanthocyanidin isolates were dose-dependent as they were more potent antioxidants at a higher concentration than at a lower concentration. On the contrary, ellagitannins isolated from raspberries appeared equally active at all concentrations. At the lowest concentration of 1.4 $\mu$g/mL, raspberry ellagitannins were among the most efficient antioxidants especially toward the formation of conjugated diene hydroperoxides and carbonyl compounds.

## DISCUSSION

Berry phenolics isolated from lingonberries and bilberries and to a slightly lesser extent those from raspberries and black currants acted as antioxidants toward both protein and lipid oxidation. As to inhibition of lipid oxidation, this result confirms earlier findings by Kähkönen et al. (*16*) who reported that bilberry, black currant, lingonberry, and raspberry extracts are effective antioxidants during the oxidation of bulk lipids such as methyl linoleate. Phenolic berry extracts, such as bilberry, raspberry, and strawberry, have earlier been reported to act as antioxidants also in low-density lipoprotein and liposome oxidation models (*15*, *29*). In addition, raspberries, bilberries, and lingonberries scavenge oxygen radicals (*30*−*34*). The nature of the phenolic compounds in berries has an impact to their antioxidant activity. However, the antioxidant activity of berry phenolics depends not only on the phenolic composition but also on the oxidation model system of choice as well as the oxidation products monitored. In this study, the antioxidant effect of berry phenolics was more pronounced toward lipid oxidation, especially inhibition of formation of hexanal than toward protein oxidation. Inhibition of formation of lipid aldehydes such as hexanal generally resulted in inhibition of carbonyl formation due to reduced lipid protein interaction.

Isolated berry phenolics inhibited protein oxidation as measured loss of tryptophan fluorescence and formation of carbonyl compounds. In general, they prevented the formation of carbonyl compounds more efficiently than the loss of tryptophan fluorescence. The decrease of tryptophan fluorescence can be due also to other changes in the protein molecule than oxidation such as other conformational changes due to interaction with lipids and phenolic compounds. It is also postulated that the interaction of the aromatic ring of the polyphenols with aromatic residues such as tyrosine and tryptophan of the protein may be responsible for quenching of fluorescence intensity based on tryptophan fluorescence experiments (*35*−*37*). In foods, the antioxidant activity of phenolic compounds can be masked by interactions with different proteins (*38*, *39*). The masking is usually more pronounced with proteins containing more proline such as caseins and human saliva proteins. In this study, the bearing or quenching of tryptophan fluorescence by berry phenolics was studied measuring the tryptophan fluorescence at several time points during oxidation. In the beginning of the oxidation (at day 0 and day 1), the tryptophan fluorescence was the same in all samples (in the control sample and in the samples containing berry phenolics), and no quenching or additional fluorescence occurred.

In this study, bilberry and raspberry phenolics exhibited the best overall antioxidant activity toward protein oxidation. At the highest concentration of 8.4 $\mu$g/mL, the best antioxidant activity in inhibiting the loss of tryptophan fluorescence was shown by lingonberry phenolics followed by raspberry, black currant, and bilberry phenolics. Phenolic compounds in bilberries and black currants were more potent than in raspberries and lingonberries in inhibiting the formation of protein carbonyls. Bilberries and black currants are rich in anthocyanins (94.8 and 92.3%, respectively), and they were the most active anthocyanins as compared to other anthocyanin fractions toward the formation of protein carbonyls. Black currant anthocyanin isolate contains delphinidin- and cyanidin-3-glucosides and -3-rutinosides (*23*). Especially cyanidin and its glucosides have been very potential antioxidants in the lactalbumin−liposome system (*22*). The anthocyanin composition of bilberry is more complex, but delphinidin-, cyanidin-, and petunidin-3-glucoside, -3-galactoside, and -3-arabinoside are the most dominant (*23*). Most likely, synergism exists between both black currant and bilberry anthocyanins because the typical delphinidin glycosides were not as potent antioxidants toward oxidation of lactalbumin as compared to other anthocyanins such as cyanidin glycosides (*22*).

Berry anthocyanins exhibited only a weak antioxidant activity toward the loss of tryptophan fluorescence. Thus, other phenolic constituents such as proanthocyanidins in lingonberries and ellagitannins in raspberries are mainly responsible for the inhibition of changes in the tryptophan residues of lactalbumin. Tannins such as hydrolyzable ellagitannins and proanthocyanidins (condensed tannins) are known to interact with proteins resulting in complex formation responsible for the astringent character of foods and beverages rich in tannins (*40*). Especially the dimeric and trimeric proanthocyanidins isolated from lingonberries were efficient in inhibiting the loss of tryptophan fluorescence. This confirms our earlier finding showing that dimeric proanthocyanidins B1 and B2 are potent antioxidants toward protein oxidation (*22*). Data of the antioxidant activity of lingonberry proanthocyanidins are not available to our knowledge, but cocoa proanthocyanidins have been reported to be very effective antioxidants toward liposome oxidation, and the antioxidant activity is dependent on the proanthocyanidin chain length (*41*).

Raspberry anthocyanins are constituted of cyanidin and pelargonidin glycosides with different sugar substituents (*23*), which have been shown to be efficient antioxidants in the lactalbumin−liposome model system toward both lipid and protein oxidation (*22*). In raspberries, the coexistence of anthocyanins (30.8%) and ellagitannins (50.6%) may not be the best combination for the inhibition of lipid oxidation as raspberry phenolics were among the least potential antioxidants. Raspberry ellagitannins were equally active or more potent antioxidants than anthocyanins, except in inhibiting the formation of hexanal at a concentration of 8.4 $\mu$g/mL. The ellagitannin fraction isolated from raspberry is a mixture of monomeric ($M_w \sim 936$ g/mol), dimeric (including sanquiin H and lambertianin C), and polymeric ellagitannins, and the purity of the fraction is 94% (*42*). As compared to anthocyanin structures, ellagitannins are larger molecules with considerably more hydroxyl groups readily interacting with proteins and lipids (*7*, *29*, *43*−*46*). Earlier, Mullen et al. (*17*) have reported that sanquiin H is a major contributor to the antioxidant capacity assessed by electron spin resonance spectroscopy of raspberries together with vitamin C and anthocyanins.

In conclusion, berry raw materials rich in phenolic compounds may provide antioxidant protection toward both lipid and protein oxidation resulting in improved quality of foods, especially of dairy origin. The antioxidative effect is, however, strongly dependent on the choice of berry raw material as the antioxidant

activity differs between the individual berry phenolic constituents, including anthocyanins, ellagitannins, and proanthocyanins.

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