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Kaisu Ristiina Riihinen, Anu Ryynanen, Marko Toivanen, Eija Kononen, Riitta Torronen, Carina Tikkanen-Kaukanen

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Complete List of Authors:	Riihinen, Kaisu; University of Eastern Finland, Department of Biosciences; University of Illinois at Chicago, College of Pharmacy Ryynanen, Anu; University of Eastern Finland, Clinical Nutrition Toivanen, Marko; University of Eastern Finland, School of Pharmacy Kononen, Eija; University of Turku, Institute of Dentistry Torronen, Riitta; University of Eastern Finland, Clinical Nutrition Tikkanen-Kaukanen, Carina; University of Eastern Finland, Institute of Public Health and Clinical Nutrition
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Antiaggregation potential of berry fractions against pairs of *Streptococcus mutans* with *Fusobacterium nucleatum* or *Actinomyces naeslundii*

Kaisu Riihinen[a], *Anu Ryynänen*[b], *Marko Toivanen*[c], *Eija Könönen*[d,e], *Riitta Törrönen*[b] *and Carina Tikkanen-Kaukanen*[c,f]

[a]Department of Biosciences, [b]Institute of Public Health and Clinical Nutrition, Department of Clinical Nutrition, Food and Health Research Centre, [c]Department of Pharmaceutical Chemistry, University of Eastern Finland, P.O.Box 1627, FI-70211 Kuopio, Finland, [d]Department of Infectious Disease Surveillance and Control, National Institute for Health and Welfare (THL), P.O.Box 30, FI-00271 Helsinki and [e]Institute of Dentistry, University of Turku, FI-20520 Turku, Finland, [f]Institute of Public Health and Clinical Nutrition, Department of Public Health, University of Eastern Finland, P.O.Box 1627, FI-70211 Kuopio, Finland

Short title: Berry fractions with antiaggregation potential

*Corresponding author. Tel.: +1-708-209 9091; fax: +358-17-163322

E-mail address: Kaisu.Riihinen@uef.fi

ABSTRACT

Coaggregation is an interspecies adhesion process, which is essential to the development of dental plaque. Here we studied *in vitro* the composition of the soluble solids in the berry juice molecular size fractions [<10 kDa, FI; 10–100 kDa, FII; >100 kDa, FIII] derived from apple, bilberry, blackcurrant, cloudberry, crowberry, and lingonberry and their ability to inhibit and reverse coaggregation of the pairs of common species in dental plaque: *Streptococcus mutans* with *Fusobacterium nucleatum* or *Actinomyces naeslundii*. Inhibitory and reversal activity was found in the molecular size fractions FII and FIII of bilberry, blackcurrant, crowberry, and lingonberry. The active fractions contained higher amounts of polyphenols (5-12% of soluble solids) than those without activity (<2% of soluble solids). Proanthocyanidins dominated in the active lingonberry juice fractions FII and FIII and also small amounts of anthocyanins were detected. Anthocyanins, proanthocyanidins, and flavonol glycosides were prevalent in FII and FIII fractions of bilberry, blackcurrant, and crowberry juices. Comparable amounts of sugars and titratable acids were present in the latter three berry juice fractions of different size. The results indicate that the high molecular size fractions of lingonberry, bilberry, blackcurrant, and crowberry juices have antiaggregation potential on common oral bacteria, the potential being associated to their polyphenolic content.

Keywords: berries; dental biofilms; antiadhesion; coaggregation; antiaggregation; polyphenols

INTRODUCTION

Streptococcus, *Actinomyces*, and *Fusobacterium* are among the major bacterial genera, which colonize the oral cavity early in life (Könönen, 2000) and form crucial constituents of dental plaque, i.e., dental biofilms accumulating on non-shedding tooth surfaces (Kolenbrander *et al.*, 2006; Rosan and Lamont, 2000). Dental biofilms play a crucial role in the pathogenesis of the two most common oral infections, caries and periodontal diseases, where intergeneric coaggregation contributes to the development of cariogenic and periodontopathogenic communities (Kolenbrander *et al.*, 2006; Sbordone *et al.*, 2003). The first step in the formation of dental plaque is the adhesion of early colonizers (streptococci and *Actinomyces*) to the pellicle-coated tooth surface, while *Fusobacterium nucleatum* acts as a bridge between early and late colonizers, coaggregating with most oral bacteria examined (Al-Ahmad *et al.*, 2007; Kolenbrander *et al.*, 2006). Coaggregation is a further adhesion process by which two genetically distinct bacteria become attached to one another via specific molecules present in the biofilms. The basics of coaggregation have been examined *in vitro* with the simple method of mixing bacterial suspensions together (Kolenbrander *et al.*, 2006).

While antimicrobial agents inhibit the survival and growth of bacteria, antiadhesion and antiaggregation agents affect the formation of bacterial biofilms on oral surfaces. According to Weiss *et al.* (2002), the American cranberry (*Vaccinium macrocarpon*) constituents are able to inhibit and reverse the coaggregation of dental plaque bacteria (antiaggregation). In this context, information on other berries is still limited. Also, cranberry has been demonstrated to inhibit oral bacterial adhesion in artificial biofilms (Steinberg *et al.*, 2005; Yamanaka *et al.*, 2004; 2007). Polyphenols are considered to be involved in nonspecific antiadhesion effects against oral bacteria (Duarte *et al.*, 2006; Ferrazzano *et al.*, 2009;

Yamanaka *et al.*, 2007). The most predominant polyphenols in berries are anthocyanins (anthocyanidin glycosides) (Määttä-Riihinen *et al.*, 2004a; 2004b; Määttä *et al.*, 2001), which are seen as light red to bluish black pigments. Flavonol glycosides and proanthocyanidins are other abundant polyphenols in berries (Hellström *et al.*, 2009; Määttä-Riihinen *et al.*, 2004b). Recently, we reported that juice fractions of different molecular size prepared from bilberry (*Vaccinium myrtillus*), blackcurrant (*Ribes nigrum*), cranberry (*Vaccinium oxycoccus*), crowberry (*Empetrum nigrum & hermaphroditum*), and lingonberry (*Vaccinium vitis-idaea*) exhibit antiadhesion activity against a Gram-negative human pathogen *Neisseria meningitidis* (Toivanen *et al.*, 2009). We have also shown binding or inhibitory activities of molecular size fractions prepared from *Vaccinium* berries and crowberry against different pathogenic streptococcal strains of human or animal origin (Toivanen *et al.*, 2010).

The aim of the present study was to investigate *in vitro* the inhibition and reversal ability of apple, bilberry, blackcurrant, cloudberry (*Rubus chamaemorus*), crowberry, and lingonberry juice fractions against two pairs of oral bacterial species, namely, *Streptococcus mutans* with either *F. nucleatum* or *Actinomyces naeslundii*. In addition, the amount of titratable acids, sugars, proanthocyanidins, anthocyanins, and flavonol glycosides in the berry juice fractions was measured.

MATERIALS AND METHODS

Juice fractions. Juice concentrates of apple (mix of cultivars) (70ºBrix, content of soluble solids g/100 g of solution) and blackcurrant (65ºBrix) were obtained from VIP-Juicemaker Ltd (Kuopio, Finland). Bilberry, lingonberry, and crowberry (berry juice concentrates (65°Brix) and cloudberries were purchased from Kiantama Ltd (Suomussalmi, Finland). Cloudberry juice was prepared as described by Toivanen *et al.* (Toivanen *et al.*, 2009). Berry juices were stored at –20°C until used. The juices were fractionated into three different fractions, <10 kDa fraction (FI), 10–100 kDa fraction (FII), and >100 kDa fraction (FIII), through a molecular sieve by centrifugal filtering, as previously described (Toivanen *et al.*, 2009; 2010). The soluble solids (SS) in the fractions were analyzed as ºBrix values (SS g/100 g) using a digital refractometer (ATAGO PR-32, Tokyo, Japan) and diluted to the fixed SS content of 12 g/100g, except for the cloudberry juice fractions, which were analyzed as such.

Analyses of titratable acids and sugars. Titratable acids and sugars were analyzed based on the methods developed for various foods (Anon., 2000). Titratable acids were measured from the juice fractions diluted in water by titrating with alkaline solution to their potentiometric endpoint. The amounts of titratable acids were calculated by using the equivalent weights of the major acids in berries (malic acid in apple, cloudberry, and crowberry, and citric acid in bilberry, blackcurrant, and lingonberry). Sucrose was inverted with acid hydrolysis prior to the analysis of reducing sugars with the Schoorl method (Anon., 2000). Reducing sugars were determined by using stabilized alkaline solution of a copper salt (two Fehling's solutions). In the reaction, the amount of reduced copper is proportional to the amount of reducing sugars in the sample. The determination was continued by immediate titration with standard sodium thiosulphate solution until the end

point. Water was used as blank for the samples. The sugar content was read from a conversion table based on the titer difference of the sample from the blank.

Analysis of proanthocyanidins. Proanthocyanidin contents in the fractionated juices was estimated by a spectrophotometric procedure (Rohr *et al.*, 2000), which was modified as described by Toivanen *et al.* (2009). Briefly, juice fractions were diluted in acidified methanol (0.6 M HCl) and the absorbance values of the samples were measured at 520 nm with a UV-visible spectrophotometer (Shimadzu UV-1650PC; Kyoto, Japan) before and after incubation at +70ºC (Memmert ULE 500 oven, Schwabach, Germany) for 3 h. The absorbance differences of incubated and nonincubated samples were used for semiquantification of proanthocyanidins as released anthocyanidins. The calibration curves of proanthocyanidins were constructed by using depolymerized lingonberry dimer and trimer isolate with purity of 80% (Määttä-Riihinen *et al.*, 2005). The range of the calibration curve corresponded to 0.6-30% of proanthocyanidins of SS in the juice samples.

Analyses of anthocyanins and flavonol glycosides. In order to analyze anthocyanins and flavonol glycosides, the fractions were first diluted with methanol and acidified with concentrated HCl to 0.6 M and then filtered through a 0.45-µm syringe filter (Pall Life Sciences, Ann Arbor, MI) to the vials. High-performance liquid chromatography with diode array detection (HPLC-DAD) apparatus consisted of a Hewlett-Packard (HP) instrument with a 1100 series quaternary pump, an autosampler, and a diode array detector linked to an HP-ChemStation data handling system (Waldbronn Analytical Division, Germany). HPLC separation was achieved on a (150 x 4.6 mm i.d., 5 µm) Phenomenex Gemini reversed phased (RP) column (RP-18, Merck, Darmstadt, Germany) protected with a guard column of the same material (4 x 3 mm). The gradient program based on acetonitrile and methanol (ACN:MeOH, 85:15, v/v) as the organic phase and 8.5% formic acid as the water phase was modified from the procedure of Lätti *et al.* (2008). The gradual increase of the ACN-MeOH

at flow rate of 1 ml/min was arranged as follows: 4-10% (0-2 min), 10% (2-20 min), 10-15% (20-35 min), 15-35% (35-40 min), 35-80% (40-50 min), 80% (50-52 min), return to initial conditions in 8 min, and re-equilibration of the column for 10 min. Anthocyanins (at 520 nm) and flavonol glycosides (at 360 nm) were identified based on our earlier HPLC-DAD identification (Määttä-Riihinen *et al.*, 2004a; 2004b; Määttä *et al.*, 2001). The response factors for quantification of anthocyanins and flavonol glycosides were determined from freshly prepared solutions of cyanidin-3-glucoside and quercetin-3-glucoside in the concentration ranges 1.8-386 mg/l and 2-250 mg/l, respectively. Flavonol glycoside concentrations lower than 2 mg/l were considered as non-significant (NS) (Table 1). The cyanidin-3-glucoside was measured in acidified mixture of methanol and water by using the same solvent composition as used for the samples, since pH and water content affect the absorption coefficient of anthocyanins.

Bacterial strains. Five clinical strains were selected, including Gram-positive *S. mutans* IH 113728, *A. naeslundii* AHP 28639 and AHP 28651, and Gram-negative *F. nucleatum* AHN 23952 and AHN 23937. The strains were grown in Brain Heart Infusion (BHI, LabM, England) broth with 0.5% yeast extract (Difco) under anaerobic conditions at 37ºC. The bacterial cells were harvested and washed with Dulbecco's phosphate-buffered saline (D-PBS) (10X stock solution, ECM4053, Eurolone) containing calcium and magnesium. The washed cells were then either suspended in coaggregation buffer D-PBS to an optical density of 1.5 at 400 nm (UV-Vis Spectrophotometer, GENESYS 10 Series, Thermo Electron Corporation) for the coaggregation assay or stored at -20ºC in 50% glycerol (99.5%, analytical grade, Normpur). The suspended cells were stored at 4ºC for the test period of two weeks.

Coaggregation and antiaggregation assay. The inhibition and reversal activities of the berry and fruit juice fractions against bacterial coaggregates were studied by using a

modification of the turbidimetric assay developed by McIntire et al. (Kolenbrander, 1995; McIntire *et al.*, 1982). The coaggregating bacteria used in the assays were *S. mutans* with either *A. naeslundii* (two strains) or *F. nucleatum* (two strains). In order to obtain four parallel results, the antiaggregation assays were carried out in two runs. Berry juice fractions were diluted in D-PBS to the concentrations of 12 and 48 mg/g. Coaggregation assays, inhibition and reversal of bacterial aggregates, and background measurements were run simultaneously. In the inhibition assays, a 25 µl aliquot of each berry juice fraction was mixed vigorously for 10 s with 25 µl of D-PBST (D-PBS with 0.05% (v/v) Tween20) and with 25 µl of the cell suspension of *S. mutans* and *A. naeslundii* or *F. nucleatum*. In the reversal test, 25 µl of the *S. mutans* cell suspension, 25 µl of the *A. naeslundii* or *F. nucleatum* cell suspension, and 25 µl of D-PBST were vigorously mixed for 10 s. To allow the formation of stable bacterial aggregates or bacterium-berry coupling, the mixtures were then agitated gently (100 revolutions per minute) in an orbital shaker (Orbital Shaker, Stuart Scientific, United Kingdom) for 30 min. Then the other bacterial pair (25 µl of the cell suspension) was added to the inhibition test mixtures and berry juice fraction (25 µl) was added to the reversal test mixtures. For background measurements, two different blanks were made. The blanks were prepared by mixing 25 µl of each bacterial cell suspension with 50 µl of D-PBST and 25 µl of the berry fraction as well as by mixing 25 µl of each cell suspension with 75 µl of D-PBST. The coaggregation score for each bacterial pair was measured from the mixture containing 25 µl of the both cell suspensions and 50 µl of D-PBST. All the prepared samples and blanks were mixed vigorously, agitated gently for 30 min, as described above, and then 200 µl of D-PBST was added. The mixtures were then centrifuged at 200 rpm (Eppendorf centrifuge 5415D, Hamburg, Germany) for 10 min to pack down the aggregates. The top part of the supernatant was transferred to a flat-bottomed

microtiter well. The turbidity was measured at 630 nm by using a microtiter plate reader (Victor2, 1420 Multilabel counter, Wallac, PerkinElmer LifeSciences, Boston, USA).

The coaggregation percent was calculated as follows:

$$Coaggregation\% = \frac{(A_1 + A_2) - (A_{1+2})}{(A_1 + A_2)} * 100 \quad ,$$

where A_1 and A_2 present absorptions of the bacterial cell suspensions and A_{1+2} presents absorption of the coaggregation mixtures. The inhibition and the reversal activity induced by the juice fractions (F) was expressed as percents and calculated as follows:

$$CoaggregationF\% = \frac{(A_{1+F} + A_2) - (A_{1+2+F})}{(A_{1+F} + A_2)} * 100$$

$$Inhibition/reversal\% = \frac{Coaggregation\% - CoaggregationF\%}{Coaggregation\%} * 100$$

RESULTS

Composition of the juice fractions. The contents of proanthocyanidins and anthocyanins in the juice fractions varied between different berry species (Table 1). The low molecular size fractions (<10 kDa in FI) and all the three fractions of apple and cloudberry displayed low amounts of proanthocyanidins and no anthocyanins. The anthocyanin concentration was high in bilberry FII and FIII (3.99 % and 5.21 % of SS, respectively) and in crowberry FII and FIII (2.80% and 5.18 %, respectively). The proanthocyanidin concentration was high in blackcurrant FII and FIII (8.5% and 6.2%, respectively), in crowberry FII and FIII (5.8% and 6.3 %, respectively), and in lingonberry FII and FIII (5.8% and 4.7%, respectively). Although the anthocyanin contents were lower in fractions FI, the chromatographic profiles of anthocyanins in the three fractions of the bilberry, blackcurrant, crowberry, and lingonberry juices were identical in RP-HPLC-DAD analysis, as shown for crowberry in Figure 1. Flavonol glycosides were distributed mainly in fractions FII and FIII and major contents were 0.14% of SS in bilberry FIII, 0.09% of SS in blackcurrant FII, 0.05% of SS in blackcurrant FIII, and 0.07% of SS in crowberry FIII (Table 1). Titratable acids and sugars were detected in comparable amounts in all the three fractions (Table 1).

Coaggregation and antiaggregation assays. The degree of coaggregation was relatively low, being equal between *S. mutans* IH 113728 and the two strains of *A. naeslundii* (AHP 28639 and AHP 28651) and between *S. mutans* IH 113728 and the two strains of *F. nucleatum* (AHN 23952 and AHN 23937) (Table 2). Inhibition and reversal activities less than 50% were ignored, as proposed by McIntire *et al.* (1982). All positive scores for inhibition and reversal (antiaggregation) activities displayed low standard deviations and were reproducible in the four experiments. The reversal and inhibitory activity was found in FII and FIII of bilberry, blackcurrant, crowberry, and lingonberry juices (Table 2). The antiaggregation activity was detected in all eight bacterial pairs with the fraction FII of

bilberry, crowberry, and lingonberry juices. In addition, the crowberry juice fraction FIII had antiaggregation activity to seven of the eight studied pairs. Of the blackcurrant juice fractions, FIII was the most active but the effect was limited to the four bacterial pairs of *S. mutans* and *A. naeslundii*. The blackcurrant FII fraction had effect only to one of the studied eight bacterial pairs. The fractions FIII of bilberry juice and lingonberry juice showed activity to three and one of the eight bacterial pairs, respectively. The antiaggregation was mainly achieved with the berry concentration of 48 mg/g of SS. The crowberry juice fraction FIII with the concentration of 12 mg/g of SS gave reversal activity to two of the bacterial pairs and that of blackcurrant induced aggregation inhibition of one bacterial pair. The inhibitory and reversal activity of 100% was detected in the FII fraction of bilberry juice with the concentration of 48 mg/g of SS. The fractions of apple and cloudberry juices exhibited no antiaggregation activity.

DISCUSSION

The fractions of bilberry, blackcurrant, crowberry, and lingonberry demonstrated antiaggregation activity against the oral bacterial pairs examined. As shown in the present study and previous studies using spectrophotometric and chromatographic or NMR analyses (Toivanen *et al.*, 2009, 2010), these berries possess high proportions of polyphenols. In the fractions without antiaggregation activity, the polyphenol content was very low or polyphenols were not detected.

Plant-derived substances can affect the formation of bacterial aggregates either by inhibition of their formation and by reversal of formed units, which mechanisms may be applied in the control of plaque-related diseases (Allaker *et al.*, 2009). In the present antiaggregation study, the berry fractions were selected based on our recent results achieved with berry juice fractions against pathogens, including Gram-positive *Streptococcus* strains, *S. pneumonia, S. agalactiae*, and *S. suis*, and a Gram-negative *N. meningitidis* (Toivanen *et al.*, 2009; 2010). In the present study, *S. mutans* was selected for coaggregation experiments, since it has been considered as one of the key organisms involved in caries (Kleinberg, 2002), while *A. naeslundii* and *F. nucleatum* are important species in the maturation process of dental biofilms (Al-Ahmad *et al.*, 2007). In general, oral streptococci are prominent early colonizers on tooth enamel and they form coaggregates with other early colonizers, such as *Actinomyces.* However, coaggregation scores between *S. mutans* and *A. naeslundii* have been found to be rather weak (Cisar *et al.*, 1979; Crowley *et al.*, 1987). Thus, the low coaggregation score found for the pairs of *S. mutans* with the two strains of *A. naeslundii* in the present study was expected. Furthermore, *S. mutans* coaggregated with *F. nucleatum* strains with a low score. The low coaggregation scores were taken into

consideration in calculations so that inhibition and reversal activities less than 50% were not considered significant.

It is notable that the antiaggregation activity may not be connected to a certain group of polyphenols. As shown in Table 1, the fractions FII and FIII of bilberry juice contained a relatively high amount of anthocyanins but a low amount of proanthocyanidins, while high amounts of both anthocyanins and proanthocyanidins were found in the fractions FII and FIII of crowberry juice. In contrast, in the fractions FII and FIII of lingonberry and blackcurrant juices, the anthocyanin concentration was low compared to the proanthocyanidin concentration. Flavonol glycosides were found predominantly in active fractions, which may have an impact on antiaggregation activity, however, their amount was lower compared to major proanthocyanidins and anthocyanins. We have recently demonstrated that polyphenol macromolecular complexes with proanthocyanidins and polyhydroxyflavonoids are present in the active berry juice fractions FII and FIII, while these complexes are absent in the inactive FI and in weakly effective lingonberry FIII fractions (Toivanen *et al.*, 2009).

According to our recent nuclear magnetic resonance (NMR) analysis (Toivanen *et al.*, 2010), high molecular size fractions contain low molecular weight sugars (mainly glucose) and benzoic acid and high molecular weight polyphenols. In the present study, acids and sugars were equally found both in active and in inactive fractions (Table 1), indicating that they are not the major promoters of the antiaggregation properties. This observation is in agreement with earlier studies (Johnson-White *et al.*, 2006; Steinberg *et al.*, 2005). Our finding on identical chromatographic profiles of anthocyanins (Figure 1) in the fractions FI, FII, and FIII of bilberry, blackcurrant, and crowberry juices indicates that the three fractions contained similar anthocyanin molecules. Although the antiaggregation activity seems to be

associated with polyphenols, it is not due to the specific anthocyanin structures in the studied bilberry, blackcurrant or crowberry juice fractions.

Among berries, there are two different types of proanthocyanidins: A-type (rare) and B-type (common). The rare A-types dominate in the Finnish *Vaccinium* berries (Määttä-Riihinen *et al.*, 2005). The type of proanthocyanidin can influence the antiaggregation ability (Foo *et al.*, 2000). This concerns especially the high molecular size fraction FII of lingonberry juice but also its weakly effective fraction FIII. In lingonberry and blackcurrant juice fractions, the antiaggregation activity may result from the high content of proanthocyanidins as well.

It has been demonstrated that the polyphenol fraction (0.25 mg/ml of which 62% were polyphenols) of American cranberry is able to inhibit the biofilm formation of two periodontitis-associated anaerobes, *Porphyromonas gingivalis* and *F. nucleatum* (Yamanaka *et al.*, 2007). In another study, cranberry proanthocyanidins (0.500 mg/ml) and flavonols (0.125 mg/ml), alone or in combination, reduced the formation and accumulation of *S. mutans* biofilms (Duarte *et al.*, 2006). In the present study, 0.6-1.4 mg/g of polyphenols in bilberry, blackcurrant, crowberry, and lingonberry juice fractions displayed inhibition and/or reversal activity against the studied coaggregates. The dilutions of the juice fractions used in our antiaggregation assay represented the concentrations present in a drinkable juice.

Berry juices can have a high content of fructose, glucose, citric acid, and malic acid (Viljakainen *et al.*, 2002). Therefore, a drinkable berry juice without further processing is unsuitable for oral hygiene purposes. In the present study, considerable amounts of sugars and titratable acids were present in all the three fractions examined. Low molecular weight

sugars are fermentable by acid-producing dental bacteria in plaque, which may increase the risk of dental caries. Moreover, the low pH value and titratable acidity are the major chemical risk factors for dental erosion (Lussi and Jaeggi, 2008). Therefore, for clinical studies or for possible clinical applications, antiaggregative berry polyphenols should be isolated from the active fractions, as described for the high molecular weight non-dialyzable material from cranberry juice concentrate (Weiss *et al.*, 2004). In the case of polyphenolic beverages, such as cocoa bean husk extract, red wine, cistus, and black tea, which have also shown their potential in prevention of biofilm-induced diseases in the oral cavity, no further processing is needed (Matsumoto *et al.*, 2004; Hannig *et al.*, 2009). There is preliminary *in vivo* evidence that daily usage of a mouthwash containing cranberry constituents or cacao bean husk can reduce mutans streptococcal counts in the mouth (Matsumoto *et al.*, 2004; Weiss *et al.*, 2004). It has recently been reported that oral rinses with red wine, cistus tea, and black tea can reduce the total count of adherent bacteria (Hannig *et al.*, 2009).

Our *in vitro* results suggest that polyphenol constituents in the high molecular size fractions of bilberry, blackcurrant, crowberry, and lingonberry juices may affect the coaggregation capabilities of oral bacteria. Anthocyanins, proanthocyanidins, and flavonol glycosides may be responsible for the observed antiaggregation activity. The susceptibility of oral biofilms to antimicrobial and antiaggregation agents is limited due to the properties of the microbial community (Sbordone *et al.*, 2003). Therefore, the results of the present coaggregation study may not be directly extrapolated as an evidence of the inhibitory effect of the berry components against dental biofilms. Although berry polyphenols have shown their antiaggregation potential, sugars and acids should be eliminated from berry material to get it more suitable for oral hygiene purposes. In order to evaluate the effect of various

berry components on oral pathogens, characterization of the antiaggregative molecules and *in vivo* studies will be needed.

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FIGURE LEGEND

Figure 1. HPLC-DAD chromatograms at 520 nm of different molecular size crowberry fractions: <10 kDa (FI), 10-100 kDa (FII), and >100 kDa (FIII). Identity of anthocyanins: (1) delphinidin 3-galactoside, (2) cyanidin 3-galactoside, (3) delphinidin 3-arabinoside, (4) petunidin 3-galactoside, (5) cyanidin 3-arabinoside, (6) peonidin 3-galactoside, and (7) malvidin 3-galactoside.

434 TABLE 1. Composition of soluble solids (SS)[a] in indicated juice fractions.

		% of SS				
	SS g/100g	TA	Sugar	PA	AC	FG
Apple						
FI	22	4.5	70	<0.6	ND[c]	ND
FII	24	4.6	77	<0.6	ND	ND
FIII	24	4.5	77	<0.6	ND	ND
Bilberry						
FI	18	11.2	80	<0.6	0.34	ND
FII	24	11.9	63	1.9	3.99	NS
FIII	32	12.7	83	1.4	5.21	0.14
Blackcurrant						
FI	17	24.4	63	0.7	0.29	ND
FII	25	22.3	59	8.5	2.01	0.09
FIII	24	22.2	58	6.2	1.31	0.05
Cloudberry						
FI	3	18.9	57	<0.6	ND	ND
FII	4	19.1	65	<0.6	ND	ND
FIII	3	NA	NA	<0.6	ND	ND
Crowberry						
FI	16	7.4	65	0.7	0.29	ND
FII	22	7.6	55	5.8	2.80	NS
FIII	32	7.5	48	6.3	5.18	0.07
Lingonberry						
FI	19	17.4	52	1.2	0.09	NS
FII	25	16.4	53	5.8	0.32	NS

FIII	25	15.4	63	4.7	0.21	NS

[a]Abbreviations: titratable acids (TA), proanthocyanidins (PA), anthocyanins (AC), flavonol glycosides (FG), not analyzed (NA), not detected (ND), and not significant (NS). [b]Juice concentrates were fractionated into three different fractions according to their molecular size, i.e. <10 kDa fraction (FI), 10–100 kDa fraction (FII), >100 kDa fraction (FIII). [c]No peaks were detected at 520 nm for AC and at 360 nm for FG in HPLC-DAD.

TABLE 2. Inhibition (%) and reversal (%) of coaggregation between oral bacteria pairs with indicated juice fractions[a].

		S. mutans IH 113728 & *A. naeslundii* AHP 28639		*S. mutans* IH 113728 & *A. naeslundii* AHP 28651		*S. mutans* IH 113728 & *F. nucleatum* AHN 23952		*S. mutans* IH 113728 & *F. nucleatum* AHN 23937	
		Coaggr. 35 (±5)% n=22		Coaggr. 34 (±6)% n=22		Coaggr. 33 (±8)% n=34		Coaggr. 31 (±6)% n=34	
Fractions	SS mg/g	inhibition[b]	reversal	inhibition	reversal	inhibition	reversal	inhibition	reversal
Apple									
FI	48	< 50[b]	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FIII	48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
Bilberry									
FI	≤48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	12	< 50	< 50	< 50	< 50	< 50	<50	< 50	< 50
FII	48	95 (±9)	100 (±0)	100 (±0)	100 (±0)	93 (±9)	99 (±1)	93 (±15)	100 (±0)
FIII	12	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FIII	48	77 (±27)	< 50	< 50	79 (±24)	< 50	80 (±23)	< 50	< 50
Black currant									
FI	≤48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	12	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	48	< 50	< 50	< 50	84 (±17)	< 50	< 50	< 50	< 50

FIII	12	71 (±13)	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FIII	48	79 (±21)	81(±21)	87 (±13)	90 (±6)	< 50	< 50	< 50	< 50
Cloudberry									
FI	34	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	40	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FIII	33	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
Crowberry									
FI	≤48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	12	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	48	83 (±21)	93 (±8)	91 (±12)	90 (±12)	85 (±17)	98 (±1)	71 (±28)	95 (±8)
FIII	12	< 50	85 (±11)	< 50	82 (±21)	< 50	< 50	< 50	< 50
FIII	48	86 (±19)	94 (±9)	84 (±13)	80 (±30)	< 50	88 (±27)	74 (±9)	79 (±9)
Lingonberry									
FI	≤48	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	12	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FII	48	81 (±22)	100 (±0)	94 (±8)	97 (±7)	80 (±14)	88 (±14)	83 (±12)	99 (±1)
FIII	12	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
FIII	48	< 50	< 50	< 50	< 50	< 50	< 50	93 (±12)	< 50

[a]Descriptions of fractions in Table 1.

[b]Duplicates of bacterial pairs in inhibition and reversal assays were used and each experiment was repeated at least twice. Positive scores over the limit 50% in reversal and inhibition are shown. Standard deviation is shown in parentheses.

FIGURE 1