Promising results of cranberry in the prevention of oral Candida biofilms

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This study shows that in cranberry there are compounds, proanthocyanidins, that prevent biofilm formation by Candida albicans and Candida glabrata. Eating cranberry may prevent oral biofilm formation.

Keywords
oral yeasts; oral diseases; polyphenols; red berries; phytochemical study; functional food.

Abstract
In the context of dental caries prevention by natural foodstuff sources, antifungal and antibiofilm activities of dry commercial extracts of cranberry fruit (Vaccinium macrocarpon Aiton) and two other red fruits (Vaccinium myrtillus L. and Malpighia punicifolia L.) were assessed on Candida albicans and Candida glabrata yeasts. When added to the culture medium, the cranberry extract displayed a significant anti-adhesion activity against Candida spp. when used at low concentrations. In addition, the pretreatment of surfaces with this extract induced an anti-adhesion activity mainly against C. glabrata yeasts and an antibiofilm activity against C. albicans. This activity was dependent on concentration, species, and strain. A phytochemical investigation bioguided by anti-adhesion tests against the two Candida species was carried out on crude cranberry juice to determine the active fractions. Three subfractions enriched in proanthocyanidins showed an anti-adhesion activity at low concentrations. This study investigated for the first time the interest of crude extracts of cranberry and cranberry juice fractions to prevent biofilms of C. glabrata. It highlighted the potency of consuming this fruit and using it as a source of anti-adhesion agents.

Introduction
The foodstuff market of cranberry fruit is expanding throughout Europe. It is the fruit of a shrub of peatlands located in the colder regions of North America: Vaccinium macrocarpon Aiton and two other red fruits (Vaccinium myrtillus L. and Malpighia punicifolia L.) are usually commensal flora but can be involved in the formation of dental plaque and may be responsible for mucosal or systemic infections, thus acting as opportunistic pathogens. These fungi are able to colonize topical devices including dentures (Estivilla et al., 2011) and grow on these substrates as a biofilm which makes them more resistant to antimicrobials than planktonic cells (Niimi et al., 2010). Many existing antifungal agents exhibit reduced activity against adhesion and biofilm development of Candida species. Only two antifungal families (echinocandins and lipid formulations of amphotericin B) are active against Candida biofilms. Therefore, it is important to search for novel approaches to prevent adhesion and further biofilm development and to find new agents with other mechanisms of action to prevent resistance development.
First, to understand the impact of cranberry consumption on the oral and potentially pathogenic fungal flora, the activity of a dry extract of this fruit and two other edible red fruits rich in polyphenols (blueberry: *Vaccinium myrtillus* L. and acerola: *Malpighia punicifolia* L.) was investigated against *Candida* yeasts; the most common species isolated from human mouths were studied: *Candida albicans* and *Candida glabrata* (Niimi et al., 2010). The anti-adherent activity of cranberry was then studied and its persistence was evaluated; the influence of cranberry on the growth of planktonic yeasts was also investigated to better characterize the anti-*Candida* interest of cranberry.

A phytochemical investigation bioguided by anti-adhesion tests against the two *Candida* species was then carried out on crude cranberry juice to determine the active fractions which could represent future therapeutic and/or prophylactic approaches against biofilm related to oral infections.

Materials and methods

Plant material

Three commercial fruit extracts cranberry (*V. macrocarpon* Aiton, *Ericaceae*) (Ref 1079), acerola (*M. punicifolia* L., *Malpighiaceae*) (Ref 1087), and blueberry (*V. myrtillus* L., *Ericaceae*) (Ref 1085) were purchased from HYTECK Laboratories – Aroma-Zone (Clermont-Ferrand, France).

The cranberry juice was a pasteurized and clarified cranberry juice produced by Fruit d’Or (Notre-Dame-de-Lourdes, QC, Canada). The juice was stored at −28 °C and thawed out at 4 °C.

Cranberry juice fractionation

Crude cranberry juice was first freeze-dried. The freeze-dried juice (20 g) was solubilized in 15 mL of water and then subjected to Amberlite XAD 16 column (diameter 6 cm, high 25 cm).

Amberlite XAD 16 resin (200 g) was soaked overnight in methanol. Then, the column was packed the day after and first washed with deionized water (2 L) before injection of the sample. First elution step was performed using deionized water (3 L) to elimate sugars and nonphenolic compounds. Then, methanol (2 L) was used to elute phenolics from the column. Resulting fractions, aqueous one (18.9 g) and methanolic one (1.1 g), were concentrated under reduced pressure and then freeze-dried.

Methanolic fraction was fractionated using LH-20 resin column using a protocol derived from Prior et al. (2001). First elution steps were reproduced, and a last elution using acetone/water (70 : 30 v/v) was added to the protocol. Six fractions were obtained (noted F1 to F6). Elution solvent, weight, and composition are detailed in Table 1.

HPLC analysis

HPLC analysis were performed on a Dionex UHPLC U3000RS system equipped with a LPG-3400RS quaternary pump, a RSLC WPS-300T RS automated injector, a TCC-300SD column oven, and a UHPLC+ DAD-3000 diode array detector (Thermo Fisher SA, Voisins le Bretonneux, France). The system was fitted with an Accucore aQ (150 x 3 mm i.d., 2.6 μm particle size) column, itself protected by an Accucore aQ Unigard (Thermo Scientific, Courtaboeuf, France). The mobile phases were solvent A 0.01% TFA in water and solvent B acetonitrile. The gradient was set as follows: initial acetonitrile content was 0%, it was raised to 60% in 7.6 min, then raised to 100% in 0.31 min and maintained for 1 min. Oven temperature was set at 40 °C, UV detection was monitored at λ 210, 254, 280, and 520 nm. Samples were injected at 5 mg mL⁻¹.

BL-DMAC assay

The DMAC protocol used was previously described by Prior et al. (2010). Samples were prepared at 10 or 5 mg mL⁻¹, in water or methanol, as convenient.

Organisms and growth conditions

Twelve *Candida* isolates were studied. *Candida albicans* ATCC 66396 and ATCC 3153 strains were purchased from the American Type Culture Collection. One strain of *C. glabrata* was obtained from Biomedical Fungi and Yeasts Collection (Brussels, Belgium): IHEM 9556. The other strains were isolated from patients at the laboratory of parasitology and medical mycology of the hospital of Poitiers (France): *C. albicans* Apu1 (pulmonary origin), 1202-081485 (mouth origin), 1202 120598 1021 (throat origin), 1202 210920 (tongue origin), and *C. glabrata* Gpu1 (pulmonary origin), 788, 924, 767, and Gsep1 (septicemia origin).

Yeasts were first grown for 48 h at 37 °C on Sabouraud agar slants (Sanofi Diagnostics Pasteur, Marne-la-Coquette, France). To obtain a biofilm, four loopfuls of this culture were carefully transferred to 30 mL of Yeast Nitrogen Base medium (Difco, Detroit, MI), supplemented with 50 mM glucose (Sigma, St Louis, MO) (YNB-Glc) and incubated overnight at 37 °C without shaking.

Table 1 Weight, elution solvent, and composition of the six obtained fractions (F1 to F6)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight (mg)</th>
<th>Solvent of elution</th>
<th>Components</th>
<th>Global PACs amount % (w/w) (DMAC assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>732.0</td>
<td>MeOH/water (20 : 80, v/v)</td>
<td>Phenolic acids, remaining sugar</td>
<td>0.13</td>
</tr>
<tr>
<td>2</td>
<td>150.0</td>
<td>MeOH/water (60 : 40, v/v)</td>
<td>Anthocyanin, flavonol</td>
<td>1.94</td>
</tr>
<tr>
<td>3</td>
<td>46.6</td>
<td>MeOH/water (60 : 40, v/v)</td>
<td>Low molecular weight PACs</td>
<td>3.45</td>
</tr>
<tr>
<td>4</td>
<td>36.6</td>
<td>MeOH</td>
<td>PACs and flavonoids</td>
<td>3.90</td>
</tr>
<tr>
<td>5</td>
<td>57.0</td>
<td>MeOH</td>
<td>Flavonoids and PACs</td>
<td>2.69</td>
</tr>
<tr>
<td>6</td>
<td>30.7</td>
<td>Acetone/water (70 : 30, v/v)</td>
<td>High molecular weight PACs</td>
<td>3.15</td>
</tr>
</tbody>
</table>
Minimum inhibitory concentrations of fruit extracts and cranberry fractions

Stock solutions of tested commercial extracts were prepared in sterile distilled water or DMSO (depending on their solubility) at 100 mg mL\(^{-1}\). These solutions were shaken and centrifuged for 15 min at 2647 \(g\), at 25 °C (Cr3i centrifuge Jouan). On the other hand, stock solutions of cranberry juice fractions were prepared at 500 or 50 mg mL\(^{-1}\) (depending on the available quantity of fractions) in sterile distilled water or DMSO (depending on their solubility) and shaken. The minimum inhibitory concentrations (MICs) of commercial extracts and cranberry fractions were determined by a broth microdilution method in order to comparatively study their antifungal and antibiofilm activities (CLSI reference M27-A3 micromethod adapted protocol). Briefly, yeasts were first grown for 24 h on Sabouraud agar slants, and \textit{Candida} sp. inocula were prepared by suspending yeasts in RPMI-MOPS and adjusting to a final concentration of \(10^6\) colony-forming units mL\(^{-1}\). Serial twofold dilutions of each supernatant of commercial extract or cranberry juice fraction were prepared in RPMI-MOPS. They were made in 96-well microtiter plates over the range 10 \(\mu\)g mL\(^{-1}\) to 100 mg mL\(^{-1}\) or 0.2–5 mg mL\(^{-1}\), respectively (final DMSO concentrations did not exceed 2% of the overall volume in wells). The same volume per well of yeast culture was added. Different controls were included in each experiment: non-treated yeasts; yeasts treated by DMSO 2% or distilled water; yeasts treated by amphotericin B (positive control). The MICs were determined after incubation for 24 and 48 h at 37 °C without shaking. All tests were performed in triplicate in at least two separate experiments. Fruit extracts with a MIC less than 100 \(\mu\)g mL\(^{-1}\) were regarded as potentially useful.

Saliva preparation

Non-stimulated saliva collected from 12 healthy volunteers was pooled, altered with dithiothreitol (2.5 mmol L\(^{-1}\)), shaken in the ice for 10 min, centrifuged for 20 min at 3000 \(g\) and 4 °C (Cr3i centrifuge Jouan), diluted in three volumes of distilled water (25%), and filtered through a 0.45 μm membrane. This stock solution was stored at −80 °C.

Anti-adhesion activity of experimental medium supplemented with fruit extracts or cranberry fractions

A stock solution of each commercial fruit extracts was prepared at 120 mg mL\(^{-1}\) in 10 mM phosphate-buffered saline (PBS, pH 7.4). It was shaken, sonicated, and centrifuged for 15 min at 2647 \(g\), at 25 °C (Cr3i centrifuge Jouan). Concerning cranberry juice fractions, stock solutions prepared for MIC assays were used. In both cases, a serial twofold dilution was carried out in YNB-Glc (final DMSO concentrations did not exceed 2% of the overall volume in wells).

Untreated 96-well tissue culture plates were pretreated with 200 μL of human saliva at 2%, obtained by dilution of the stock solution in distilled water, incubated for 2 h at 37 °C, and then completely aspirated prior biofilms production; this pretreatment allowed to mimic the natural coating of oral surfaces in the mouth. One hundred microliters of a serial twofold dilution in YNB-Glc of each fruit extract or cranberry juice fraction was added over the range 20 \(\mu\)g mL\(^{-1}\) to 40 mg mL\(^{-1}\) or 0.4 \(\mu\)g mL\(^{-1}\) to 10 mg mL\(^{-1}\), respectively. Each well then received 100 μL of the yeast suspension (\(2.10^7\) cells mL\(^{-1}\)). Some wells were reserved for PBS, distilled water, or DMSO 2% controls. After 2 h of incubation at 37 °C, spent media and free-floating microorganisms were removed by aspiration. The wells were washed twice with PBS, then observed under inverted optical microscope (IX51\(^{TM}\) inverted microscope, Olympus) prior to biofilm quantification using a previously described metabolic assay based on the reduction of a tetrazolium salt (XTT) (Cocuaud et al., 2005; Dalleau et al., 2008). Briefly, 300 mg L\(^{-1}\) XTT (Sigma) and 0.13 mM menadione (Sigma) were added in 200 μL of PBS in each well. Plates were incubated for 3 h at 37 °C without shaking, then gently agitated and XTT formazan was measured colorimetrically at 450 nm (microplate reader LP400; Sanofi Diagnostics Pasteur). Background formazan values were determined with plates containing PBS only or containing PBS, XTT, and menadione; these values did not exceed 0.005 absorbance units and therefore were not significant. All tests were performed in triplicate in at least two separate experiments.

Anti-adhesion and antibiofilm activities resulting from pretreatment of surfaces with cranberry extract

The supernatant of centrifuged stock solution at 120 mg mL\(^{-1}\) of commercial cranberry extract was diluted at 30 and 7.5 mg mL\(^{-1}\) using PBS.

Experiments were performed in untreated 96-well tissue culture plates pretreated for 2 h at 37 °C with 200 μL of saliva 2% and 100 μL of extract or PBS (control). They were then emptied by aspiration. Two hundred microliters of the yeast suspension (\(2.10^7\) cells mL\(^{-1}\)) was added to the wells, and the plates were incubated at 37 °C for 2 or 22 h to allow adhesion and biofilm formation, respectively. Then, half of the wells were washed twice with PBS to evaluate the metabolic activity of adherent population, whereas the second half permitted to assess the metabolic activity of the overall population, including planktonic yeasts. Cells were then observed under inverted optical microscope (IX51\(^{TM}\) inverted microscope, Olympus), and the metabolic activity was assessed using the XTT assay (Cocuaud et al., 2005; Dalleau et al., 2008). All tests were performed in triplicate in at least two separate experiments.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) (\(P < 0.05\)), and Scheffe’s tests were conducted to determine differences among the test groups (Statview, version 5.0.0.0; SAS Institute Inc, Berkeley, CA).
Results

Antifungal activity

The antifungal activity of the three commercial red berries extracts and all fractions obtained from cranberry juice was assessed on C. albicans ATCC 3153 and C. glabrata IHEM 9556. These extracts and fractions showed no relevant antifungal activity against the two species: all MICs were > 100 μg mL⁻¹, reaching > 100 mg mL⁻¹ in some cases (data not shown). All the studied strains were susceptible to amphotericin B (MIC < 2 μg mL⁻¹) as previously shown (Baissac et al., 2004).

Ability of yeasts to adhere to polystyrene surfaces in the presence of red fruit extracts

The tested fruit extracts (≥ 10 mg mL⁻¹) had a higher influence on adhesion of C. albicans vs. C. glabrata (Fig. 1). Cranberry extract significantly inhibited C. albicans adhesion (P < 0.05), regardless of the tested concentrations (Fig. 1). Candida glabrata species was more resistant: cranberry extract at concentrations ≥ 20 mg mL⁻¹ was necessary to inhibit their metabolic activity (P < 0.05). The blueberry extract had no effect on C. glabrata adhesion and only slightly inhibited C. albicans adhesion phases when used at 40 mg mL⁻¹. Finally, the acerola extract was poorly active on Candida spp. adhesion: a weak activity was observed on C. glabrata at 40 mg mL⁻¹, whereas it had no anti-adhesion activity against C. albicans adhesion phase (Fig. 1).

Influence of surface pretreatment with cranberry extract on biofilm growth

Six strains of C. albicans and C. glabrata were studied. The metabolic activity of sessile yeasts was evaluated after a 2 h (influence on early phase biofilm) or a 22 h (on maturation phase biofilm) incubation time in polystyrene wells pretreated with cranberry extract.

When yeasts were incubated for 2 h, the surface pretreatment using the extract at 10 mg mL⁻¹ or 40 mg mL⁻¹ resulted in the decrease (P < 0.05) of sessile yeast inoculum for three (50%) or four (66.7%) strains of C. glabrata, respectively (Fig. 2a). Such an activity was observed only for one strain (16.7%) of C. albicans and only using cranberry extract at 40 mg mL⁻¹.

Considering 22-h-old biofilms, this pretreatment significantly decreased (P < 0.05) the biofilm of one (16.7%) of tested strains; extract at 2 mg mL⁻¹, two (33.3%; 10 mg mL⁻¹), or three (50%; 40 mg mL⁻¹) C. albicans strains, respectively (Fig. 2b). Such an inhibition was only observed for two strains (33.3%) of C. glabrata and only using cranberry extract at 40 mg mL⁻¹.

Influence of surface pretreatment with cranberry extract on total yeast inoculum

The metabolic activity of the whole microbial population, including both adherent and planktonic yeasts, was investigated 2 and 22 h after the addition of yeasts in the pretreated wells. In the case of a 2 h culture, the pretreatment of surfaces with cranberry extract caused a significant inhibition (P < 0.05) of the metabolic activity of three (50%) of strains; extract at 40 mg mL⁻¹ or one (16.7%; 10 or 2 mg mL⁻¹) strains of C. albicans (Fig. 3a). Such an activity was observed for two (33.3%; 2 mg mL⁻¹), three (50%; 10 mg mL⁻¹), or four (66.7%; 40 mg mL⁻¹) strains, respectively, of C. glabrata (Fig. 3b).

Considering the influence on yeasts cultured for 22 h, this pretreatment resulted in the inhibition (P < 0.05) of the metabolic activity of two (33.3% of strains; 2 mg mL⁻¹ or 10 mg mL⁻¹) or four (66.7%; 40 mg mL⁻¹) strains of C. albicans (Fig. 3b). At 40 mg mL⁻¹, it significantly inhibited the metabolic activity of all strains of C. glabrata, whereas the extract used at lower concentrations was able to inhibit the metabolic activity of only one (16.7% of strains; 2 mg mL⁻¹) or two (33.3%; 10 mg mL⁻¹) strains of C. glabrata (Fig. 3b).

Bioguided phytochemical investigation

A freeze-dried cranberry juice was fractionated on Amberlite XAD 16 resin. Two fractions were first obtained: aqueous and methanolic fractions (Table 2). Only the latter showed an anti-adhesion activity against C. albicans and C. glabrata at low concentrations (0.32 and 0.64 mg mL⁻¹ respectively vs. 10.5 mg mL⁻¹ for aqueous fraction). HPLC analysis of these two fractions confirmed the separation of sugars and nonphenolic compounds (aqueous fraction) from phenolic compounds (methanolic fraction) (Fig. S1). Then, the methanolic fraction was further fractionated on LH 20 resin column. Of the six subfractions obtained, three showed significant anti-adhesion activity at very low concentrations (< 0.25 mg mL⁻¹). F4 and F6 were active against the two tested species using concentrations ranging between 0.062 and 0.25 mg mL⁻¹, whereas F3 dem-
Cranberry inhibits adhesion of two Candida species

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Fig. 2 Effect of the cranberry extract at three concentrations on adherent yeasts of two Candida species, with surface pretreatment. Effect on (a): early step (2 h) or (b): later step (22 h). The experiments were carried out on six strains of C. albicans and C. glabrata. Results are expressed as metabolic activity values of the adherent yeasts (A450 nm) ± standard deviations. All experiments were carried out in triplicate. PBS was used as a control. *Significantly different from the control (0 mg mL⁻¹; PBS) (variance analysis and Scheffe’s test P < 0.05). Origin of the strains: C. albicans: a pulmonary isolate Apu1, b ATCC 3153, c ATCC 66396, d mouth isolate 1202-081485, e throat isolate 12021205981021, f tongue isolate 1202210920; C. glabrata: a’ pulmonary isolate Gpu1, b’ IHEM 9556, c’ septicemia isolate 788, d’ septicemia isolate 924, e’ septicemia isolate 767, f’ septicemia isolate Gsep1.

onstrated a significant activity (P < 0.05) only against C. glabrata (0.25 mg mL⁻¹). HPLC analysis revealed that the three active subfractions were enriched in low molecular weight proanthocyanidins (PACs), flavonoids and PACs and high molecular weight PACs, respectively (Figs. S2–S7). Subfractions constituted by phenolic acids and remaining sugar (F1) and anthocyanins and flavonols (F2) (Figs. S2–S7) were not active at low concentrations. BL-DMAC assay, used to evaluate global PACs amount, clearly showed a decrease in the amount of adherent yeasts in the wells treated with active compounds compared with non-treated wells (Fig. S8).

First, the ability of yeasts to adhere to polystyrene surfaces and to subsequently grow as a biofilm in the presence of commercial fruit extracts was investigated. The cranberry extract was the most anti-adhesion, as shown using concentrations ranging between 0.02 and 40 mg mL⁻¹; results demonstrated the significant anti-adhesion activity of this extract on C. albicans (P < 0.05) when used at concentrations ≥ 1.25 mg mL⁻¹ (data not shown). Considering these results, the cranberry extract was selected for further investigations.

A second approach was to pretreat polystyrene surfaces with cranberry extract (2–40 mg mL⁻¹) to simulate a mouthwash or to approach the foodstuff reality where the food or beverage is placed in the mouth and then swallowed; they are not present anymore in the oral sphere. First of all, some activities were observed which proved that the cranberry extract was not totally removed after supernatant aspiration. Some components from both cranberry extract and saliva would coat the surfaces. Besides, salivary proteins (especially proline-rich proteins) are known to bind polyphenols (Bennick, 2002).

Discussion

The evaluation of MICs demonstrated that tested commercial red berry extracts and cranberry juice fractions displayed no relevant activity against planktonic Candida spp. These results are consistent with available data obtained on C. albicans with the cranberry juice (Swartz & Medrek, 1968) or the A-type cranberry proanthocyanidins (Feldman et al., 2012). In that way, other studies reported no alteration in bacterial growth and viability in the presence of cranberry compounds (Labrecque et al., 2006; Koo et al., 2010; La et al., 2010; Maharshak et al., 2011).

Moreover, in this study, the activity of the fruit extracts and cranberry fractions was evaluated against Candida spp. biofilms, using a metabolic based method which is known to be simple, practical, and reproducible (Jin et al., 2004). However, results obtained with active extracts and fractions were also confirmed based on colony-forming unit (CFU) counts to check the absence of interference between red fruit compounds and XTT; CFU results coincide with those obtained by XTT method (data not shown). Beside, inverted optical microscopy observations made before XTT addition clearly showed a decrease in the amount of adherent yeasts in the wells treated with active compounds compared with non-treated wells (Fig. S8).

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**Table 2** Anti-adhesion activity of methanolic, aqueous, and F1 to F6 fractions against *Candida albicans* and *Candida glabrata*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Minimum anti-adhesion concentration (mg mL⁻¹)</th>
<th><em>C. albicans</em></th>
<th><em>C. glabrata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>0.32</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.062</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>&gt; 1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as minimum anti-adhesion concentration (*P* < 0.05). All experiments were performed in triplicate in at least two separate experiments. ND: not determined.

The activity of cranberry extract against adherent yeasts (Fig. 2) and the overall yeast population, including both adherent and planktonic yeasts (Fig. 3), was investigated in order to study the anti-adherent interest of this fruit and to evaluate whether cranberry acted specifically on adherent cells or was also able to influence the metabolic activity of planktonic yeasts.

The pretreatment of surfaces with the cranberry extract had a higher influence against adhesion of *C. glabrata* yeasts compared with that of *C. albicans* (Fig. 2a). The cranberry extract would be poorly able to prevent *C. albicans* adhesion contrary to those of *C. glabrata*, even if a strain-dependence was observed in this last case.

This pretreatment displayed a higher influence against *C. albicans* biofilm maturation compared with that of *C. glabrata*, unlike what was observed on adherence phase (Fig. 2b). This observation suggested a delayed and persistent effect on this species. Here again, the influence was strain and species-dependent. So, the cranberry extract was able to prevent early and maturation phases of fungal biofilms; however, its activity depended on both *Candida* species and strains.

Concerning the effect on the overall population, regardless of its adhered or planktonic status after 2 h (Fig. 3a), surface pretreatment with the commercial cranberry extract reduced the metabolic activity (*P* < 0.05) for only 50% of *C. albicans* strains vs. 100% of *C. glabrata* strains; these results confirmed the species, strain, and concentration-dependant activity of this cranberry extract. After 22-h incubation, this pretreatment still displayed a higher influence on the metabolic activity of *C. glabrata* yeasts compared with that of *C. albicans* (Fig. 3b), showing, however, an antimetabolic activity on 66.7% of *C. albicans* strains; the activity of cranberry extract on this species could be delayed. It was able to influence the metabolic activity of yeasts growing as mixed adherent and planktonic status and showed a persistent effect.

The observed ability of cranberry extract to reduce the metabolism and literature data suggest a fungistatic activity (Swartz & Medrek, 1968). Concerning the mechanism of action, studies reported a decrease in hydrophobicity of some bacteria such as *E. coli* or cariogenic *S. mutans*, and...
S. sobrinus due to the presence of cranberry extract or juice (Yamanaka et al., 2004; Wojnicz et al., 2012).

In parallel of these studies concerning the activity of a commercial cranberry extract, a crude cranberry juice was studied and a bioguided fractionation was made by anti-adhesion assays against the two reference species (C. albicans and C. glabrata) to determine the active fractions. The three subfractions enriched in low molecular weight PACs (F3), flavonoids and PACs (F4) and high molecular weight PACs (F6) showed an anti-adhesion activity at concentrations lower than those obtained for dry commercial extract and methanolic fraction, suggesting a real interest of cranberry juice and even more of fractions enriched in PACs (Table 2). Our results, concerning the improved activity of fractions, were in agreement with those of Shmueli et al. (2012) who demonstrated that the active ingredients were likely to be less than 3% of the dry solid. Our results were also in accordance with the marked inhibition of C. albicans adherence recently reported by Feldman et al. (2012) and Rane et al. (2014) in the presence of cranberry PACs. Results of Feldman et al. (2012) were obtained studying only one strain of C. albicans. Rane et al. (2014) studied several strains of C. albicans and reported active concentrations close to those observed in our study; however, they reported that cranberry reduced biofilm formation of all tested strains contrary to what we observed, suggesting a strain-dependence of cranberry activity. In addition, our results demonstrated that the anti-adhesion activity of cranberry extract and PACs fractions was not limited to C. albicans but was also observed with emerging fungal pathogen C. glabrata.

In addition to being empirically demonstrated, available data report that cranberry extract has no cytotoxicity toward human healthy cells, like human gingival HF-1 fibroblasts (Babich et al., 2012).

In conclusion, our results pointed out the in vitro anti-adhesion potential of three commercial edible red fruit extracts and cranberry juice fractions against two major species of Candida spp. and improved the knowledge regarding natural food and beverages, such as tea, coffee, milk, or wine that have previously demonstrated anti-adhesion properties in the oral cavity (Signoretto et al., 2012). These fruit extracts and fractions had no antifungal activity on C. albicans and C. glabrata as shown by MIC studies. The anti-adhesion property of the commercial cranberry extract was demonstrated when incubated together with Candida spp. yeasts but also when used to pretreat surfaces. This supports the potential oral health benefits associated with cranberry. However, the anti-adhesion and antibiofilm activities of this extract depended on the tested strains of Candida spp. This study investigated for the first time the interest of crude extracts of cranberry and cranberry juice fractions (methanolic and enriched in PACs) to prevent biofilms of different species of Candida spp. especially C. glabrata. These results encourage further fractionation of active fractions to improve the use of these foodstuff components, especially cranberry PACs and hopefully find new agents active against biofilm related to oral infections. In particular, the active compounds derived from cranberries have promising cariostatic properties and may potentially be used in supplement to fluoride.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. HPLC chromatogram of methanolic cranberry fraction at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S2. HPLC chromatogram of fraction F1 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S3. HPLC chromatogram of fraction F2 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S4. HPLC chromatogram of fraction F3 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S5. HPLC chromatogram of fraction F4 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S6. HPLC chromatogram of fraction F5 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S7. HPLC chromatogram of fraction F6 at 210 nm (black), 280 nm (pink) and 520 nm (blue).

Fig. S8. Adherent C. albicans without treatment (a) or after treatment with F6 at 250 µg mL⁻¹ (b) (magnification × 100).