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Characterization of in vitro antifungal activities of small and American cranberry (*Vaccinium oxycoccos* L. and *V. macrocarpon* Aiton) and lingonberry (*Vaccinium vitis-idaea* L.) concentrates in sugar reduced fruit spreads

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ABSTRACT

In this study, cranberry and lingonberry concentrates were added to commercial sugar-reduced fruit spreads (raspberry–*Aloe vera*, strawberry–guava, and strawberry–lime), and tested for their antifungal activities. Selected strains of the species *Absidia glauca*, *Penicillium brevicompactum*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*, as well as xerophilic environmental isolates of the genera *Penicillium* and *Eurotium* were used for challenge testing. Initially, varying concentrations of synthetic antifungal agents, such as sodium benzoate, potassium sorbate and butyl 4-hydroxybenzoate were tested against these fungi on wort agar containing 31% fructose at different pH values. Subsequently, the experiments were conducted in fruit spreads containing different concentrations of cranberry and lingonberry concentrates. The results of this study demonstrate that these concentrates were able to inhibit growth of visible colonies of xerophilic and non-xerophilic fungi. Cranberry and lingonberry concentrates are interesting candidates for natural preservation against fungal growth in sugar reduced fruit spreads.

1. Introduction

There is a tremendous increase in consumer demand for low caloric, safe, and premium food products containing natural, safe and environmentally friendly food preservatives (Gould, 2001; Corbo et al., 2009; Vasantha Rupasinghe and Yu, 2012). Therefore, the food industry provides low caloric functional foods using additives from natural sources instead of artificial ones. Furthermore, novel concepts and products are being developed to meet these requirements. Council Directive 2001/113/EC (http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32001L0113) relates to fruit jams, jellies and marmalades, which are mixtures of sugars, fruit pulp and/or puree, brought to a suitable gelled consistency by the addition of pectin. Products defined in the Directive shall have a soluble dry matter content of 60% or more, except for low-caloric products, where sugars are totally or partially replaced by sweeteners. The upper and lower limits for the soluble solid content of reduced sugar products have been lowered to 50% and 25%, respectively. However, the above mentioned regulation does not apply to so-called fruit spreads, which are low-caloric jam-like products not containing additional sugar. Since the water activity (a_W) of such products having soluble solid contents less than 20% is above 0.8, they are prone to rapid spoilage without added preservatives. Although benzoic acid (500 ppm) and sorbic acid (1000 ppm) are permitted, the strategy of clean labeling would require the omission of chemical preservatives. Therefore, the aim of the present work was to investigate the antimicrobial effect of berry fruits being rich in natural preservatives which may be added to fruit spreads, thus enhancing their microbial stability.

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Fungi are an important cause of food spoilage. They are widespread and ubiquitous microorganisms (Yousef and Carlstrom, 2003). The growth of xerophilic fungi (i.e. *Zygosaccharomyces* spp., *Eurotium* spp.) due to lowered sugar content and high a_w values (>0.75) is among the most important factors affecting shelf-life and quality as well as safety of sugar reduced fruit preserves (Deak and Beuchat, 1996; Hocking and Pitt, 1979; Pitt and Hocking, 2009). Since living microbial cells are inactivated by pasteurization and hot filling during processing, contamination may occur due to germinating thermo-resistant fungal spores, and secondary contamination after opening the jar. According to Hocking and Pitt (1981), some species of the genera *Eurotium* (*Aspergillus*) and xerophilic *Penicillium* such as *Penicillium corylophilum* and *Penicillium brevicompactum* are usually responsible for spoilage in jams (Char et al., 2005).

Reduced sugar content is the main reason for growth of microorganisms in low-calorie jams (Pitt and Hocking, 2009). It is known that by adding more than 60% of sugar to food-water systems, the growth of most microbiological organisms is slowed down or even inhibited due to the limited availability of free water. Sugar concentration, oxygen tension, pH, storage temperature and time, initial cell concentration, type of contaminating species, and the presence of preservatives are further factors affecting microbial growth (Char et al., 2005; Defigueiredo and Splittstoesser, 1980).

Optimum design of the manufacturing process (i.e. applying the hurdle concept) may delay or even prevent fungal growth (Alzamora et al., 1995; Gould, 1995). Employing the existing synergy between two or more limiting parameters allows optimizing the growth/no growth (G/NG) ratio, thus substantiating the hurdle concept (Jay et al., 2005). The hurdles for sugar reduced fruit spreads may comprise hot filling, a_w-value, pH-value, and the undissociated forms of antifungal phenolic acids. Currently, salts of benzoic acid and sorbic acid in concentrations up to 0.2% are legally used in jams to control fungal growth (Jay et al., 2005). However, such antifungal agents are considered as food additives, and such are subject to labeling, and consumers disapprove such synthetic food additives as well as high-caloric food, whereas berry concentrates and minimally processed food having less calories are in demand. Using fruit concentrates displaying antimicrobial activity would therefore be a valuable alternative to resolve problems regarding product shelf-life of fruit spreads. Extracts from various parts of *Vaccinium* species contain high levels of phenolic compounds and benzoic acid derivatives that delay or even inhibit the growth of microorganisms, thus improving the shelf-life without altering the quality and safety (Gomez et al., 2012).

Puupponen-Pimiä et al. (2005) studied the effects of berries and berry phenolics on selected pathogenic gastrointestional bacteria. Extracts from berries containing high levels of benzoic acid derivatives were reported to inhibit the growth of Gram-positive and Gram-negative bacteria. According to their finding, cloudberry (*Rubus chamaemorus* L.) and raspberry (*Rubus idaeus* L.) were the most efficient berry fruits. Moreover, antimicrobial activity of American cranberry constituents against *Escherichia coli* O157:H7, and *Listeria monocytogenes* has been shown (Lacombe et al., 2013). Studies regarding the antifungal potential of berry extracts in sugar reduced fruit spreads are scarce, and therefore, their suitability as natural preservatives has been insufficiently demonstrated so far. The aim of the present study was to evaluate the antifungal potential of cranberry and lingonberry concentrates, both belonging to the family Ericaceae, particularly being rich in benzoic acid derivatives (Budavari et al., 1996; Hegnauer, 1966). Moreover, colony inhibition rates and minimum inhibitory concentrations that inhibit the growth of the tested fungi were determined.

2. Materials and methods

2.1. Fungal strains

P. brevicompactum (DSM 3825), *Absidia glauca* (DSM 811) *Saccharomyces cerevisiae* (DSM 1333) and *Zygosaccharomyces bailii* (DSM 70834) strains were obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig (DSMZ). Two additional fungal strains were isolated from fruit spreads exposed to the environment (see Section 2.5), and included in the experiments.

2.2. Media, chemicals and test substrates

Wort agar medium containing 15.0 g/L malt extract, 0.75 g/L universal peptone, 12.75 g/L maltose, 2.75 g/L dextrin, 2.35 g/L glycerol, 0.4 g/L KH_2PO4, 1.0 g/L NH_4Cl, and 20.0 g/L agar–agar was prepared according to the manufacturer's instructions (Merck, Darmsatdt, Germany), and used for the growth of mold spores and yeast cells. When needed, D(−) Fructose (Merck, Darmstadt, Germany, 99% pure, granular) was added to a final concentration of 31% (w/v) to lower the water activity (a_w). 1 N HCl was used to adjust the pH-value. Sodium benzoate (Sigma-Aldrich, Taufkirchen, Germany), potassium sorbate (Merck, Darmstadt, Germany), and butyl 4-hydroxybenzoate (butyl paraben) (Sigma-Aldrich, Germany) were used as antifungal preservatives. For this purpose, stock solutions (100 mg/mL) were prepared in ultrapure water (Gibco, Thermo Scientific Fisher, Schwerte, Germany) and sterilized by filtration with a 0.2 μm microfilter. For preparation of wort agar containing fructose, wort agar and fructose were prepared separately in a low volume of ultrapure water and autoclaved separately at 121 °C for 15 min. A fructose concentration of 31% was chosen since this is approximately the mean of the fructose content of the commercial fruit spreads used in later experiments. After cooling down to 48 °C, wort agar and fructose solution were mixed and adjusted to the final volume.

Commercial concentrates produced from lingonberry (*Vaccinium vitis-ideae* L.) and from both cranberries species (*Vaccinium macrocarpon* Aiton and *Vaccinium oxycoccos* L.) were supplied by Ernteband Fruchtsaft (Winnenden, Germany) and Rudolf Wild (Heidelberg, Germany). Commercial sugar reduced fruit spreads with the trade name "Schwartau Extra Wellness" with the flavors strawberry–lime, strawberry–guava, and raspberry–*Aloe vera* were obtained from the producer (Schwartauer Werke, Bad Schwartau, Germany). They did not contain chemical preservatives. The composition of these commercial products is given in Table 1. The production process includes basically the following steps: According to the industrial recipes (Table 1), fruits and fruit concentrates were mixed with fructose syrup (70%), nutriose® (Roquette, Cassano Spinola, Italy) and water. The mixture was heated to 75 °C. Subsequently, amidated pectin (Amid CF 020, Herbstreith & Fox, Neuenbürg, Germany) was added, and the mixture was heated under reduced pressure until 43 °Brix of dry matter was achieved. Subsequently, the mixture was adjusted to pH 3.0–3.1 with citric acid (40% w/v), heated to 85 °C (P ~ 0.08 min) and filled into glass jars under steam injection.

Table 1
Ingredients and conditions of the fruit spreads used in this study.

Ingredients	Red raspberry aloe vera	Strawberry guava (%)	Strawberry lime
Red raspberry preparation	25	–	–
Red currant preparation	15	–	–
Aloe-vera preparation	10		–
Acerola concentrate (45%)	1.5	1.5	1.5
Fructose syrup (70%)	44.03	42.75	45.13
Strawberries	–	40	45
Guava puree	–	10	–
Lime juice concentrate	–	–	1
Nutriose	6	6	6
T.P. Amid AF 005 A	1.2	1	1
Citric acid	0.7	1	0.5

Nutriose: prebiotic fibers; T.P. Amid AF005 A = pectin; pH of all fruit spreads = 3.0–3.1; dry solids = 43%: fructose content in all fruit spreads = 29.9–31.6%.