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Influence of elderberry and blackcurrant concentrates on the growth of microorganisms

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Abstract

The influence of concentrates from elderberry and blackcurrant and a purified anthocyanin mix from blackcurrants were tested on the growth of typical Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecium*) and a yeast (*Saccharomyces cerevisiae*).

Blackcurrant concentrates inhibited the growth of *Staphylococcus aureus* DSM 799 while elderberry concentrates exhibited a slight stimulatory effect. Only mild effects were observed on the growth of *Escherichia coli* DSM 498. Blackcurrant concentrates inhibited *Enterococcus faecium* DSM 2918, whilst *Saccharomyces cerevisiae* ATCC 9763 was slightly stimulated by the fruit concentrates. The purified anthocyanin mix from blackcurrant concentrate did not influence the growth of the tested microorganisms. Anthocyanins do not significantly effect the growth of microorganisms. Inhibitory effects may be attributed to other phytochemicals present in the concentrates.

Keywords: Anthocyanins; Antimicrobial activity; Fruit concentrates

1. Introduction

The chemical reactivity of phytochemicals like anthocyanin pigments has been recognized for a long time. Anthocyanins and their aglycones, the anthocyanidins, are a major group of flavonoid compounds present in various berries and grapes in relatively high concentrations. Especially during the 50th and 60th studies were undertaken to investigate whether anthocyanins possess activity towards bacteria. The results indicated that anthocyanins may have stimulatory as well as inhibitory effects on microbial growth.

Masquelier and Jensen (1953a, 1953b) observed that malvidin in aged red wine might have a bactericidal effect. Zimmermann (1957) detected an influence of strawberry and grape anthocyanins on the growth rates of *Lactobacillus acidophilus* and Cameron‘s Putrefactive Anaerobe No 3679 (P. A. 3679). He reported that the growth of P. A. 3679 was stimulated by anthocyanins. The growth rate of *Lactobacillus acidophilus* was stimulated when adding 0.008–0.025 mg% anthocyanin, while 2.5 mg% depressed the growth rate. Zimmermann found no differences in the influence of strawberry and grape anthocyanins. Also, Pratt, Powers, and Somaatmadja (1960) reported that anthocyanins from strawberries and grapes influenced the growth of *Escherichia coli* and *Lactobacillus acidophilus*. They identified the pigments as pelargonidin 3-monoglucoside, cyanidin 3-monoglucoside and delphinidin 3-monoglucoside. Pratt et al. (1960) found both an inhibitory and a stimulatory effect on the growth of certain bacteria. The pigments accelerated growth by reducing the lag phase of the bacteria directly proportional to the anthocyanin concentration. An inhibition was also noted, and the

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maximum growth was inversely correlated with the anthocyanin concentration. Concentrations of 10 mg % pelargonidin 3-monoglucoside and cyanidin 3-monoglucoside first stimulated the growth of *E. coli*, but after 28 h practically no differences in the growth of *E. coli* with and without the pigment were noticeable. The maximum growth of *Lb. acidophilus* was reduced by 30% in anthocyanin-fortified media. Powers, Somaatmadja, Pratt, and Hamdy (1960) stated that pelargonidin 3-monoglucoside and delphinidin 3-monoglucoside inhibited the growth of *E. coli*, whilst delphinidin and malvidin stimulated growth. Malvidin-3,5-diglucoside stimulated growth at some stages and inhibited it at others. Puupponen-Pimiä et al. (2001) reported that berry extracts in general inhibited Gram-negative but not Gram-positive bacteria, whereas blackcurrant extract was least active. Blackcurrant slightly stimulated the growth of *Lactobacillus rhamnosus* VTT E-97800 and GG VTT E-96666, and *Lactobacillus paracasei*. They also stated that anthocyanidins as a group of compounds and blackcurrant extract, both inhibited the growth of *E. coli* CM 871. Rauha et al. (2000) detected a slight antimicrobial activity of blackcurrant extract towards *Bacillus subtilis*, and a moderate antimicrobial activity towards *Micrococcus luteus*.

Other authors such as Blank and Suter (1948) also investigated the effect of anthocyanins on pathogenic bacteria, and reported that the tested range of concentrations had no bactericidal effect on pathogenic bacteria. Mandrik (1953) likewise could not detect any influence of anthocyanins on the growth of pathogenic bacteria.

Therefore, it was of interest to test the influence of different elderberry and blackcurrant concentrates with different amounts of anthocyanins and a purified anthocyanin mix from blackcurrants on the growth of several microorganisms.

2. Material and methods

Escherichia coli DSM 498, *Staphylococcus aureus* DSM 799, *Enterococcus faecium* DSM 2918, and *Saccharomyces cerevisiae* ATCC 9763, were incubated in their typical growth media for 24 h at 37 °C. 1 ml of the diluted microbial suspensions (in a range from 10^1 to 10^3) were incubated in culture tubes in 10 ml of their typical growth media (Tryptic soy broth 1.05459, Brain heart broth 1.10493 and Malt extract broth 1.05397, respectively; VWR International, Darmstadt) for five days at either 37 °C (for the bacterial strains) or 25 °C (for the yeast), with added fruit concentrates. The growth of the microorganisms was investigated every 24 h by direct plate counts on VRBD agar 1.10275 for *E. coli*, Baird-Parker agar 1.05406 for the *S. aureus* strain, Tryptic soy agar 1.05458 for the *Ent. faecium* strain, and Malt extract agar 1.05398 for *Sacch. cerevisiae* ATCC 9763. All media were obtained from VWR International, Darmstadt, Germany.

Three different blackcurrant concentrates and three elderberry concentrates were applied, as well as a purified anthocyanin mix (48% cyanidin 3-rutinoside, 21% delphinidin 3-rutinoside, 6% cyanidin 3-glucoside, 4% delphinidin 3-glucoside). The blackcurrant and elderberry samples were derived from the production process of anthocyanin rich fruit concentrates that can be used as natural food colorants (Dr. Marcus GmbH, Geesthacht, Germany). Juice concentrates display the source for the production. Successive application of enzymatic depectinisation, ultrafiltration, and pasteurisation result in liquid concentrates. Powdered concentrates correspond to spray-dried liquid concentrates using maltodextrin as a carrier.

The fruit concentrates as well as the anthocyanin mix were buffered with citrate buffer (citric acid monohydrate, disodiumhydrogenphosphate dihydrate) to pH 5. The fruit concentrates contained different amounts of anthocyanins and were added in different amounts to the inoculated media (Table 1).

The antioxidant activity was determined by the activity of the concentrates and the purified anthocyanin mix to reduce the α,α-diphenyl-2-picrylhydrazyl (DPPH) radical according to Schwarz et al. (2001). The antho-

Table 1
Concentrates used in the experiments, and amounts added

Concentrate	Amount of concentrate mg/ml sample solution	Anthocyanin concentration in the sample solution (mg/10 ml)
Blackcurrant juice concentrate	16	0.88
	8	0.44
	4	0.22
Blackcurrant concentrate liquid	12	0.90
	6	0.45
	3	0.225
Blackcurrant concentrate powder	8	1.224
	4	0.612
	2	0.306
Elderberry juice concentrate	2	0.826
Elderberry concentrate liquid	2	0.914
Elderberry concentrate powder	1	0.916
Purified anthocyanin mix from blackcurrants	5	50
	1	10
	0.5	5
	0.2	2
	0.05	0.5