Antimicrobial and preservative effect of berries in food models.

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Abstract

Five different berries including *aronia*, blackcurrant, blueberry, cranberry and raspberry were examined for their antibacterial property against four different food-borne pathogens: *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella Typhimurium*. To implicit the experiment standard broth and broth supplemented with food minimizing the inhibitory effect of organic acids, all berry extracts were pH neutralized. To do the experiment berry extracts were added to bacterial cultures (~5×106 CFU/ml inoculum) and growth was observed over a 24h period. After the duration the determination of Minimal Inhibitory Concentrations (MIC), Minimal Bactericidal Concentrations (MBC) and log CFU/ml reductions, were noted. In addition, the content of the bioactive compounds; total anthocyanins and total polyphenols of all the berries were determined. *Aronia*, blackcurrant and blueberry had the highest antimicrobial activity and concentrations of polyphenols and anthocyanins. *S. aureus* and *L. monocytogenes* were more sensitive to the berry extracts than *E. coli* and *S. Typhimurium*. Considering the effect of *aronia*, blackcurrant and blueberry extracts against *S. aureus* and *L. monocytogenes* the antibacterial property remained significant (a=0.05) even at neutral pH and in presence of food constituents. However, the antimicrobial effects were influenced by food constituents with a major reducing effect likely mediated by proteins. Finally, extracts of berries with high content of polyphenols and anthocyanin's like *aronia*, blackcurrant and blueberry have a significant antimicrobial effect against some food-borne bacteria, even at neutral pH mimicking common food products. It should be noted that even though, food constituents significantly increased the inhibitory concentration of berries, still, berries kept their potential as natural preservatives against important pathogens in many types of foods.

Key points

- ✓ Berries have potential to be introduced as GRAS preservatives in food products.
- ✓ Inhibitory effect of berries on the growth of pathogens is not just based on their organic acids.
- ✓ Composition of media culture can inhibit the effect of testing compounds and therefore causes a false interpretation.

Keywords: Berry, Antimicrobial Preservatives, Aronia, Blackcurrant, Blueberry, Staphylococcus aureus, Listeria monocytogenes, Media composition.

Introduction

Toxin-producing, spoilage causing and infectious microorganisms are naturally found in the environment and can be transferred to food products. Addition of food additives and preservative are interesting methods to save the products and the health of the consumers from the threat of these microorganisms [1-3]. Chemical food preservatives are widely used by the food industry to efficiently prevent or delay the spoilage of foods. However, using these chemicals may cause long-term adverse effects such as allergies and cancer [3-5]. These adverse effects warrant continuous research to find GRAS food preservatives.

In this case, an edible plant with a high concentration of bioactive compounds, such as phenolic and flavonoids, has been found interesting to use in food industry due to their health-promoting and therapeutic effects [6-8]. Berries with red, blue or purple colours are known as rich and important sources of phenolic, flavonoid anthocyanin's and organic acids [9-11] Sadilova. Bilberry, blueberry species, black- and red currant, cowberry (lingonberry), chokeberry (*aronia*), cranberry, and raspberry are specified for their content of flavonoid anthocyanins [11-13].

The health-promoting properties of the anthocyanins, as main flavonoids in plants, are anti- inflammatory, anti-allergic, anti-carcinogenic, antihypertensive and antimicrobial which

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mediated by molecular mechanisms such as ant oxidation, and metal-chelating activity [8,14]. Hence, anthocyanins are introduced as a good candidate food additive and preservatives to use in food industry [8,14,15].

In addition, berries contain weak organic acids such as citric acid, which can inhibit bacterial growth by lowering ph. These components can also increase the sensitivity of Gram-negative bacteria to other antimicrobial substances by increasing permeability of their outer membrane [10].

The antimicrobial effects of the berries against foodborne human pathogens has been investigated intensively [7,11,14,16,17]. However, the impact of food constituents on the antimicrobial property of berries remains unclear. The aim of this work was to investigate the antimicrobial effects of berry extracts on *S. aureus* and *L. monocytogenes* in the presence of food elements such as oil, starch, casein, milk and meat extract. Ph.-neutralization of berry extracts was also investigated on their antimicrobial effects.

Materials and Methods

Bacterial species

The bacterial strains used are listed in (Table 1). *S. aureus* was cultured in Mueller-Hinton broth (MH), Mueller-Hinton-Agar (MHA) or Tryptone Soy broth (TSB) and Tryptone Soy-Agar (TSA); *L. monocytogenes* was cultured in Brain Heart Infusion broth (BHI) and Brain Heart Infusion -Agar (BHIA); *S. Typhimurium* and *E. coli* were cultured in Luria-Bertani broth (LB) and Luria-Bertani -Agar (LBA). All strains were incubated aerobically at 37°C.

Plant material

Freeze dried powders of *aronia* (*Aronia* melanocarpa), blackcurrant (Ribes nigrum L.), blueberry (EricaceaeVaccinium), cranberry (Vaccinium oxycoccus L.), and raspberry (Rosaceae Rubus) were purchased from Berrifine, Ringsted, Denmark.

Preparation of the berry extracts

Aqueous extracts of the berry powders were produced by methanol or ethanol (50% v/v) after agitation for 24h at 40°C [18-20]. The initial extracts were filtered through Munktell G/3w paper under vacuum and the residue was repeatedly extracted with the same solvents until it was colorless [21]. Subsequently, extracts were passed through 0.45 µm sterile filter (Syringe Filter Q-Max 0.45µm CA membrane sterile, Frisenette ApS). The berry extracts were neutralized to pH 7.00. It carried out by adding 1M NaOH under continuous stirring (PHM210 Standard pH Meter, Meter Lab, France). Evaporation of the solvent of the neutralized extracts was performed at 40°C using a heater (RCT basic, IKAMAG). The remaining of extracts was diluted into phosphate buffered saline (PBS). The diluted components were collected in sterile screw cap tubes and stored at 4°C.

Determination of Total Anthocyanin Content (TAC)

The content of monomeric anthocyanin was measured using a spectrophotometric pH differential protocol with slight modifications (AOAC 2006).

Each of initial extracts was added to two different cuvettes (1cm) in the same volume. Up to 1 ml of potassium chloride (0.025M, pH 1.0) was added to one of the cuvettes and sodium acetate buffer (0.4 M, pH 4.5) to another one. After 2 hours of incubation at room temperature, the absorbance of solutions at 520 nm and 700 nm were recorded. The total content of anthocyanin was calculated by following equations;

MW is the molecular weight of the predominant anthocyanin (449.2 g/mol for cyanidin-3-glucoside). DF is the dilution factor. 103=factor for conversion from g to mg. ε is the molar extinction coefficient (26,900 in L/mol/cm, for cyanidin-3-glucoside) and D is the path length in cm (1cm).

Determination of Total Phenolic Content (TphC)

The total phenolic content was determined by the Folin-Ciocalteu method [22]. One hundred and twenty-five microliter of Folin-Ciocalteu reagent were added to 1500l of diluted sample in a cuvette and mixed. Next, 375 1 of saturated sodium carbonate solution (75g/l) was added to the cuvette and mixed. After 2 hours of incubation at room temperature, in the dark, the absorbance at 765nm of berry extracts were measured (Diode Array UV-Vis Spectrophotometer Model 8453, Hewlett Packard). Gallic acid (0-500mg/l) was used for calibration of the standard curve. The results were expressed as milligram Gallic acid (see Figure 1.)

Determination of Total Protein Content (TPC) of media

The protein content of media was measured using the Bradford total protein assay with some modifications [23]. 200µl of diluted sample in PBS were added to 50µl of Bio-Rad protein dye in 96-well microtiter plate in 3 replicates. After 5 min, the absorbance at 595nm was measured. Bovine serum albumin (BSA; 0-5µg per well) was used for calibration of the standard curve. The total protein content of the media expressed as µg of BSA per ml (see Figure 2.)

Antimicrobial methods

Determination of MIC and MBC for neutralized berry extracts

MICs were determined using a 2-fold micro-dilution method in broth. Bacterial cultures (~5×106 CFU/ml) in fresh broth or

Table 1. *Bacterial species used in this study.*

Strain	Agar	Broth	Source
Gram-positive bacteria			
Listeria monocytogenes EGD-e (serovar1/2a)	BHI	BHI	(Glaser et al. 2001)
Staphylococcus aureus strain Newman (NCTC 8178)	MH and TSA	MH	(Duthie and Lorenz 1952)
Gram-negative bacteria			
Escherichia coli Serotype O157:H7	LB	LB	Danish beef meat (D3423) (Breum and Boel 2010)
Salmonella enterica subsp. Typhimurium strain 4/74	LB	LB	(Jelsbak et al. 2012)

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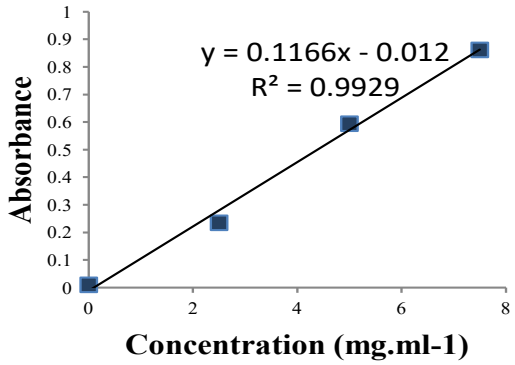

***Figure 1**. The standard curve for measuring the TPhC in samples Absorbance = 0.1166 × (Gallic acid (mg.ml-1)-0.012, (R2 = 0.9929).*

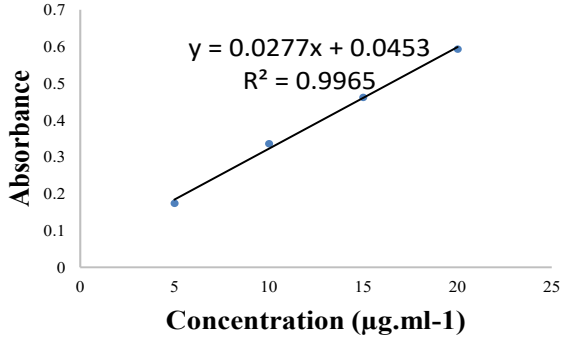

***Figure 2**. The standard curve used for measuring the total protein contents. Absorbance = 0.0277 × (protein (µg.ml-1)) + 0.0453, (R2 = 0.9965).*

fresh broth supplemented with food constituents were added to microtiter plates containing 2-fold dilution of berry extract and the plates were incubated at 37^{0}C for a 24h after incubation, the content of each well of microtiter plates was subjected to a CFU count [24]. Minimal Bactericidal Concentration (MBC) was considered as the lowest concentration (mg/ml) of berry extracts where no viable cells were detected after 24h of incubation on agar. Minimal Inhibitory Concentration (MIC) was considered as the lowest concentration of berry extracts (mg/ml) where the CFU/ml after 24 h of incubation was detected as less than or equal to the initial inoculum.

Effect of food constituents

To examine the potential application of berries as a natural preservative in food products, berry extracts were tested in the presence of different food constituents. Food ingredients assessed were meat extract (10%, w/v), acid hydrolyzed casein (10%, w/v), sunflower oil (5%, v/v), starch (2%, w/v) and UHT milk with 1.5% fat. Ingredients were individually supplemented to the media, except for milk, which was used as a medium without any supplements. When adding oil to the medium, emulsifier Tween 80 was added at 0.1% [25]. All the supplemented media were autoclaved or sterile filtered prior to use. The experiment was based on the method used for determination of MICs and MBCs as described earlier. Supplemented media with and without inoculation served as positive and negative controls and inoculated media without any food ingredients. Two technical and biological replicates were also included. The biological replicates were based on extracts from different extraction batches.

Statistical analysis

Statistical analysis of the antimicrobial effect of berry extracts on the CFU counts of *S. aureus* and *L. monocytogenes* were performed using the LSMeans Tukey HSD test, using JMP software (Ver. 9.0.2). The significance was determined using least significant difference (LSD) (a=0.05)

Results

Preliminary antimicrobial screening of neutralized berry extracts

The results from the preliminary screening are presented in Table 2 and were used to select the more bioactive berries and more sensitive pathogens for further experiments. From the results it can be seen that each berry extract produced antimicrobial effects at neutralized pH on each of the tested pathogens. Extracts of *aronia*, blackcurrant, and blueberry demonstrated bactericidal activity against *S. aureus* (MBCs of 10-20 mg/ml) and bacteriostatic activity against *L. monocytogenes* (MICs of 20-78 mg/ml). These extracts, however, demonstrated less activity against the gram-negative *E. coli* (MBC of 78-313 mg/ml) and *S. Typhimurium* (MIC of 78-313 mg/ml). Cranberry extracts were bactericidal against *S. aureus* (MBC of 20 mg/ml), but had only inhibited growth of the Gram-negatives at the highest concentration. Raspberry extract demonstrated least antibacterial activity, with activity only at the highest concentration.

Antimicrobial activity of selected berry extracts in the food-constituent-supplemented media

The antibacterial efficacy against *S. aureus* and *L. monocytogenes* of berry extracts in media supplemented with food constituents is presented in (Table 3 & 4).

Effect of culture media on bacterial sensitivity to the berry extracts

The results from the prior experiments showed that the variation in the content of the media especially protein) influences the antimicrobial effect of the berries. To investigate if the use of different standard media (e.g. MH, BHI or LB) had also an indicating effect the prior experiments were repeated in a different manner. In the new experiment instead of standard media the bacteria were suspended in sterile 0.1% (w/v) peptone saline (FKP). Then different concentrations of blueberry extract were added to the solution. The experiment was repeated twice. The results from one of the repetitions are presented in (Figure 3).

In FKP the blueberry extract had MBC of 10 mg/ml for both *S. aureus* and *L. Monocytogenes*. In addition from the growth curves it can be seen that the CFU of *L. monocytogenes* from about 2×105 reduced to the under detectable limit (3×101) at concentration of 156-39 mg/ml of blueberry during the 1st to 5th h of the experiment. In this experiment blueberry extract had the MBC of 156 mg/ml on *E. coli* and 78 mg/ml on *S. Typhimurium*.

Results from this experiment on inhibitory effect of blueberry extracts on *S. aureus* and *S. Typhimurium* in FKP are similar

***Table 2**. Screening of antibacterial activity of 5 different berry extract on S. aureus NM, L. monocytogenes, E. coli O157:H7 and S. Typhimurium grown for 24h at 37°C. Results are given as mean log CFU/ml ± SEM and represent the average of 2- 13 repeats.*

Aronia; Viable cell counts (log CFU.ml⁻¹)*					
Bactria:		***S.a.***	***L.m.***	***E.c.***	***S.T.***
Inoculum CFU:		6.86 ± 0.09	6.41 ± 0.04	6.73	6.72
Concentration mg.ml⁻¹	313	0.00 ± 0.00	0.00 ± 0.00	0.00 ± −	0.00 ± −
	156	0.00 ± 0.00	0.00 ± 0.00	0.00 ± −	0.00 ± −
	78	0.00 ± 0.00	0.00 ± 0.00	9.34 ± +	9.79 ± +
	39	0.00 ± 0.00	1.13 ± 0.43	9.73 ± +	9.61 ± +
	20	0.00 ± 0.00	6.36 ± 0.40	10.24 ± +	10.34 ± +
	10	0.00 ± 0.00	8.36 ± 0.14	10.11 ± +	10.42 ± +
	5	5.36 ± 1.23	8.55 ± 0.03	10.13 ± +	10.30 ± +
	0	9.87 ± 0.04	9.17 ± 0.03	9.98 ± +	10.11 ± +

Cranberry; Viable cell counts (log CFU.ml⁻¹)*		
S.a.	***E.c.***	***S.T.***
7.07 ± 0.08	~ 6	6.66
0.00 ± 0.00	0.00 ± 0.00	4.06 ± 0.11
0.00 ± 0.00	5.42 ± 2.72	9.38 ± 0.06
0.00 ± 0.00	9.16 ± 0.16	9.40 ± 0.11
0.00 ± 0.00	9.20 ± 0.38	9.44 ± 0.08
0.00 ± 0.00	9.18 ± 0.42	9.45 ± 0.00
8.53 ± 0.32	9.12 ± 0.15	10.13 ± 0.18
9.69 ± 0.03	9.42 ± 0.22	10.07 ± 0.01
9.97 ± 0.19	9.45 ± 0.17	9.81 ± 0.03

Blackcurrant; Viable cell counts (log CFU.ml⁻¹)*					
Bactria:		***S.a.***	***L.m.***	***E.c.***	***S.T.***
Inoculum CFU:		6.89 ± 0.12	6.41 ± 0.04	6.68	~ 6
Concentration mg.ml⁻¹	313	0.00 ± 0.00	0.00 ± 0.00	0.00 ± −	− ± −
	156	0.00 ± 0.00	2.18 ± 0.40	9.45 ± +	+ ± +
	78	0.00 ± 0.00	1.45 ± 0.40	9.73 ± +	+ ± +
	39	0.00 ± 0.00	7.92 ± 0.19	9.63 ± +	+ ± +
	20	0.00 ± 0.00	7.85 ± 0.14	9.73 ± +	+ ± +
	10	0.00 ± 0.00	8.52 ± 0.18	9.57 ± +	+ ± +
	5	8.62 ± 0.69	8.98 ± 0.07	10.04 ± +	+ ± +
	0	9.88 ± 0.03	9.24 ± 0.08	10.15 ± 0.08	+ ± +

Raspberry; Viable cell counts (log CFU.ml⁻¹)*		
S.a.	***E.c.***	***S.T.***
7.07 ± 0.08	6.40	6.66
0.00 ± 0.00	0.00 ± 0.00	2.38 ± 1.69
5.59 ± 1.14	9.08 ± 0.06	9.48 ± 0.05
8.78 ± 0.38	9.25 ± 0.06	9.67 ± 0.00
9.00 ± 0.26	9.54 ± 0.10	9.73 ± 0.04
9.48 ± 0.34	9.27 ± 0.12	9.69 ± 0.13
9.62 ± 0.26	9.39 ± 0.06	9.94 ± 0.04
10.24 ± 0.12	9.56 ± 0.04	9.76 ± 0.02
10.01 ± 0.27	9.64 ± 0.02	9.88 ± 0.04

Blueberry; Viable cell counts (log CFU.ml⁻¹)*					
Bactria:		***S.a.***	***L.m.***	***E.c.***	***S.T.***
Inoculum CFU:		6.81 ± 0.07	6.41 ± 0.04	6.68	~ 6
Concentration mg.ml⁻¹	313	0.00 ± 0.00	0.00 ± 0.00	0.00 ± −	− ± −
	156	0.00 ± 0.00	1.30 ± 0.32	0.00 ± −	− ± −
	78	0.00 ± 0.00	0.00 ± 0.00	0.00 ± −	− ± −
	39	0.00 ± 0.00	1.03 ± 0.23	9.72 ± +	+ ± +
	20	0.00 ± 0.00	5.17 ± 0.56	9.71 ± +	+ ± +
	10	0.00 ± 0.00	7.85 ± 0.27	9.79 ± +	+ ± +
	5	7.28 ± 0.76	8.17 ± 0.18	10.02 ± +	+ ± +
	0	9.92 ± 0.06	9.23 ± 0.08	10.15 ± 0.08	+ ± +

Buffer (PBS 50% v/v); Viable cell counts (log CFU.ml⁻¹)*		
S.a.	*E.c.*	*S.T.*
6.99	6.68	6.66
9.87	10.57 ± 0.07	9.83

*: Data are expressed as mean ± standard error of the mean.

~ : Approximate. Diluted overnight culture (10^{-3}).

S.a. : S. aureus NM.

L.m.: L. monocytogenes.

E.c. : E. coli O157:H7.

S.T. : S. Typhimurium.

0.00 : Below detectable limits (<3×10^{1} CFU / ml).

− : No Viable cells were observed.

+ : Viable cells were observed.

:Inoculum.

: 0.00.

: ~ five log reduction in initial CFU.

: ~ four log reduction in initial CFU.

: ~ three log reduction in initial CFU.: Maximum growth

: ~ two log reduction in initial CFU.

: ~ one log reduction in initial CFU.

: No change in initial CFU.

: ~ one log increase in initial CFU.

: ~ two log increase in initial CFU.

: ~ three log increase in initial CFU.

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Table 3. *Antimicrobial effect of aronia, blackcurrant and blueberry extracts on S. aureus NM in broth supplemented with different food constituents. Cultures were grown for 24 h at 37°C and the results are given as mean log CFU/ml ± SEM and represent the average of 2- 13 repeats.*

Aronia - *S. aureus* NM; Viable cell counts (log CFU.ml⁻¹)*-A B							
Media: ►		MH C D	Starch D	Oil B C	Casein B	Meat A	Milk A
Inoculum CFU:		6.86 ± 0.09▼	6.74 ± 0.01 ▼	6.98 ± 0.00 ▼	6.77 ± 0.02 ▼	6.92 ± 0.15 ▼	6.67 ± 0.23 ▼
Concentration mg.ml⁻¹	313	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	0.00 ± 0.00 D	5.73 ± 0.15 E	5.76 ± 0.11 C
	200	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	0.00 ± 0.00 D	3.40 ± 0.00 F	8.50 ± 0.07 B
	156	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	0.00 ± 0.00 D	8.58 ± 0.14 D	8.83 ± 0.09 AB
	100	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	0.00 ± 0.00 D	8.92 ± 0.10 ABCD	9.10 ± 0.10 AB
	78	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	1.94 ± 1.24 CD	8.85 ± 0.16 CD	9.17 ± 0.05 AB
	50	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	3.79 ± 0.03 BC	8.96 ± 0.04 ABCD	9.03 ± 0.03 AB
	39	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 C	4.01 ± 0.03 BC	8.95 ± 0.06 BCD	9.19 ± 0.18 AB
	25	1.10 ± 0.40 C	0.00 ± 0.00 D	3.70 ± 0.26 B	3.94 ± 0.34 BC	9.05 ± ,0.00 ABC	8.91 ± 0.09 AB
	20	0.00 ± 0.00 C	0.00 ± 0.00 D	1.70 ± 1.00 C	3.62 ± 0.22 BC	9.24 ± 0.05 AB	9.02 ± 0.11 AB
	13	0.00 ± 0.00 C	0.00 ± 0.00 D	8.50 ± 0.06 A	3.86 ± 0.01 BC	9.13 ± 0.02 ABC	9.11 ± 0.11 AB
	10	0.00 ± 0.00 C	0.00 ± 0.00 CD	7.98 ± 0.07 A	6.05 ± 1.18 B	9.34 ± 0.07 A	9.27 ± 0.06 A
	6	4.12 ± 0.23 B	2.70 ± 0.00 B	9.24 ± 0.12 A	9.40 ± 0.02 A	9.16 ± 0.06 ABC	9.07 ± 0.03 AB
	5	5.36 ± 1.23 B	3.53 ± 0.83 BC	9.25 ± 0.02 A	9.29 ± 0.07 A	9.22 ± 0.07 ABC	9.32 ± 0.02 A
	3	9.58 ± 0.05 A	9.40 ± 0.25 A	9.14 ± 0.16 A	9.50 ± 0.03 A	9.35 ± 0.00 AB	9.13 ± 0.09 AB
	0	9.80 ± 0.05 A	9.85 ± 0.02 A	9.46 ± 0.33 A	9.15 ± 0.11 A	9.32 ± 0.05 A	9.14 ± 0.22 A

Blackcurant - *S. aureus* NM; Viable cell counts (log CFU.ml⁻¹)*-A							
Media: ►		MH D	Starch D	Oil B C	Casein C	Meat A	Milk A B
Inoculum CFU:		6.89 ± 0.12 ▼	6.74 ± 0.01 ▼	6.69 ± 0.29 ▼	6.81 ± 0.03 ▼	6.92 ± 0.15 ▼	6.67 ± 0.23 ▼
Concentration mg.ml⁻¹	313	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 C	5.82 ± 0.22 B	3.94 ± 3.25 B
	156	0.00 ± 0.00 C	0.00 ± 0.00 B	2.09 ± 1.39 B	2.09 ± 1.39 BC	9.02 ± 0.03 A	9.08 ± 0.03 A
	78	0.00 ± 0.00 C	0.00 ± 0.00 B	1.70 ± 1.00 B	2.73 ± 2.04 BC	9.17 ± 0.06 A	8.92 ± 0.06 A
	39	0.00 ± 0.00 C	0.00 ± 0.00 B	8.08 ± 0.07 A	4.54 ± 0.20 B	9.20 ± 0.07 A	8.90 ± 0.06 A
	20	0.00 ± 0.00 C	0.00 ± 0.00 B	9.57 ± 0.08 A	8.70 ± 0.07 A	9.13 ± 0.08 A	8.92 ± 0.02 A
	10	0.00 ± 0.00 C	1.35 ± 0.65 B	9.61 ± 0.08 A	8.96 ± 0.04 A	9.08 ± 0.06 A	9.03 ± 0.08 A
	5	8.62 ± 0.69 B	8.84 ± 0.14 A	9.77 ± 0.31 A	9.24 ± 0.16 A	9.26 ± 0.02 A	9.12 ± 0.04 A
	0	9.88 ± 0.03 A	9.78 ± 0.03 A	9.77 ± 0.21 A	9.06 ± 0.08 A	9.33 ± 0.04 A	9.05 ± 0.13 A

Blueberry - *S. aureus* NM; Viable cell counts (log CFU.ml⁻¹)*-B							
Media: ►		MH C	Starch C	Oil B C	Casein B C	Meat A	Milk A B
Inoculum CFU:		6.81 ± 0.07 ▼	6.74 ± 0.01 ▼	6.40 ± ▼	6.81 ± 0.03 ▼	6.86 ± 0.18 ▼	6.67 ± 0.23 ▼
Concentration mg.ml⁻¹	313	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 B	1.81 ± 1.11 C	0.00 ± 0.00 C
	156	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 B	4.07 ± 1.69 B	3.20 ± 2.50 BC
	78	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 B	8.78 ± 0.15 A	5.64 ± 0.63 AB
	39	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	1.98 ± 1.28 B	9.09 ± 0.09 A	5.90 ± 0.80 AB
	20	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	7.01 ± 1.39 A	9.28 ± 0.11 A	6.32 ± 2.85 AB
	10	0.00 ± 0.00 C	0.00 ± 0.00 C	6.88 ± 1.66 A	6.56 ± 2.32 A	9.38 ± 0.06 A	9.23 ± 0.08 A
	5	7.28 ± 0.76 B	8.10 ± 0.09 B	9.06 ± 0.00 A	9.38 ± 0.18 A	9.45 ± 0.20 A	9.27 ± 0.13 A
	0	9.92 ± 0.06 A	9.84 ± 0.02 A	10.12 ± 0.08 A	9.37 ± 0.1 A	9.39 ± 0.09 A	9.01 ± 0.01 A

Levels not connected by the same letter are significantly different.

: Inoculum. : 0.00. : Maximum growth.

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Table 4. *Antimicrobial effect of aronia, blackcurrant and blueberry extract on L. monocytogenes in broth supplemented with different food constituents. Cultures were grown for 24 h at 37°C and the results are given as mean log CFU/mL ± SEM and represent the average of 2- 13 repeats.*

Aronia - *L. monocytogenes*; Viable cell counts (log CFU.ml^{-1})*-B							
Media: ►		**BHI B**	**Starch A B**	**Oil A B**	**Casein B**	**Meat A**	**Milk A B**
Inoculum CFU:		6.41 ± 0.04▼	6.33 ± 0.08 ▼	6.51 ± 0.01 ▼	6.42 ± 0.14 ▼	6.33 ± 0.11 ▼	6.19 ± 0.03 ▼
Concentration mg.ml^{-1}	313	0.0`0 ± 0.00 E	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 E	5.23 ± 0.18 CD	0.00 ± 0.00 H
	200	0.00 ± 0.00 E	0.00 ± 0.00 BC	0.00 ± 0.00 C	1.70 ± 1.00 DE	1.70 ± 1.00 E	5.13 ± 0.11 G
	156	0.00 ± 0.00 E	0.00 ± 0.00 C	0.00 ± 0.00 C	2.03 ± 1.33 DE	2.48 ± 0.69 E	5.70 ± 0.13 F
	100	0.00 ± 0.00 E	0.00 ± 0.00 BC	1.79 ± 1.09 C	3.09 ± 0.09CD	3.65 ± 0.47 DE	6.02 ± 0.00 EF
	78	0.00 ± 0.00 E	0.00 ± 0.00 C	3.50 ± 0.15 B	3.65 ± 0.18 CD	2.83 ± 0.63 E	6.37 ± 0.12 DE
	50	1.93 ± 0.00 D	0.00 ± 0.00 BC	8.54 ± 0.36 A	3.60 ± 0.05 CD	8.04 ± 0.08 AB	6.43 ± 0.07 DE
	39	1.13 ± 0.43 DE	3.72 ± 0.82 B	8.32 ± 0.04 A	3.89 ± 0.17 CD	7.14 ± 0.62 BC	6.55 ± 0.00 CD
	25	7.45 ± 0.45 BC	8.17 ± 0.12 A	8.51 ± 0.06 A	2.79 ± 0.09 CD	9.06 ± 0.03AB	6.55 ± 0.04 CD
	20	6.36 ± 0.40 C	8.41 ± 0.41 A	8.63 ± 0.02 A	3.53 ± 0.05 CD	9.14± 0.11 AB	6.74 ± 0.04 BCD
	13	8.48 ± 0.07 AB	8.41 ± 0.05 A	8.89 ± 0.09 A	7.45 ± 0.09 AB	9.49 ± 0.01 AB	6.80 ± 0.08 BCD
	10	8.36 ± 0.14 AB	8.51 ± 0.05 A	8.78 ± 0.00 A	4.84 ± 0.06 BC	9.37 ± 0.07 A	6.84 ± 0.06 BCD
	6	8.75 ± 0.07 AB	8.91 ± 0.18 A	9.00 ± 0.10 A	7.56 ± 0.00- AB	9.64 ± 0.08 AB	7.01 ± 0.14 ABC
	5	8.55 ± 0.03 AB	8.72 ± 0.06 A	8.91 ± 0.01 A	7.54 ± 0.05 AB	9.59 ± 0.07 A	7.20 ± 0.19 AB
	3	8.87 ± 0.07 AB	9.01 ± 0.06 A	9.04 ± 0.03 A	7.79 ± 0.01 A	9.77 ± 0.07 AB	6.98 ± 0.01 BC
	0	9.18 ± 0.01 A	9.09 ± 0.06 A	9.16 ± 0.01A	7.54 ± 0.06 A	9.77 ± 0.02 A	7.45 ± 0.07 A
Blackcurant - *L. monocytogenes*; Viable cell counts (log CFU.ml^{-1})*-A							
Media:►		**BHI C**	**Starch A B**	**Oil A**	**Casein B C**	**Meat A**	**Milk A B**
Inoculum CFU:		6.41 ± 0.04 ▼	6.33 ± 0.08 ▼	6.51 ± 0.01 ▼	6.42 ± 0.14 ▼	6.33 ± 0.11 ▼	6.19 ± 0.03 ▼
Concentration mg.ml^{-1}	313	0.00 ± 0.00 D	4.14 ± 0.08 C	4.07 ± 0.19 E	0.00 ± 0.00 C	5.39 ± 0.16 C	4.77 ± 0.53 D
	156	2.18 ± 0.40 C	3.68 ± 0.22 C	8.28 ± 0.06 D	3.14 ± 2.44 BC	4.79 ± 0.77 C	8.32 ± 0.34 AB
	78	1.45 ± 0.40 CD	6.94 ± 0.83 B	8.35 ± 0.03 CD	5.48 ± 0.15 AB	8.70 ± 0.13 B	8.48 ± 0.07 A
	39	7.92 ± 0.19 B	8.67 ± 0.08 A	8.71 ± 0.23 BC	5.58 ± 1.41 AB	9.14 ± 0.06 AB	8.01 ± 0.15
	20	7.85 ± 0.14 B	8.55 ± 0.05 A	8.95 ± 0.03 AB	7.77 ± 0.13 A	9.50 ± 0.02 AB	7.90 ± 0.06
	10	8.52 ± 0.18 AB	8.75 ± 0.03 A	9.09 ± 0.11 AB	7.94 ± 0.07 A	9.53 ± 0.02 AB	7.79 ± 0.15 AB
	5	8.98 ± 0.07 A	8.97 ± 0.04 A	9.10 ± 0.04 AB	7.98 ± 0.02 A	9.72 ± 0.02 A	7.53 ± 0.18 BC
	0	9.24 ± 0.08 A	9.11 ± 0.07 A	9.23 ± 0.11 A	7.63 ± 0.07 A	9.79 ± 0.03 A	7.35 ± 0.13 C
Blueberry - *L. monocytogenes*; Viable cell counts (log CFU.ml^{-1})*-B							
Media: ►		**BHI C**	**Starch A B**	**Oil A B**	**Casein B C**	**Meat A**	**Milk B C**
Inoculum CFU:		6.41 ± 0.04 ▼	6.33 ± 0.08 ▼	6.51 ± 0.01 ▼	6.42 ± 0.14 ▼	6.40 ± 0.15 ▼	6.19 ± 0.03 ▼
Concentration mg.ml^{-1}	313	0.00 ± 0.00 D	0.00 ± 0.00 C	0.00 ± 0.00 D	0.00 ± 0.00 D	0.00 ± 0.00 D	0.00 ± 0.00 E
	156	1.30 ± 0.32 D	2.41 ± 0.51 B	2.16 ± 1.46 CD	0.00 ± 0.00 D	3.63 ± 1.54 C	0.00± 0.00 E
	78	0.00 ± 0.00 D	2.80 ± 1.19 B	5.03 ± 2.27 BC	0.00 ± 0.00 D	8.31 ± 0.40 B	0.00 ± 0.00 E
	39	1.03 ± 0.23 D	8.05 ± 0.69 A	7.83 ± 0.02 AB	3.62 ± 0.59 C	9.14 ± 0.08 A	4.22 ± 0.36 D
	20	5.17 ± 0.55 C	8.76 ± 0.18 A	8.57 ± 0.02 A	6.28 ± 1.44 B	9.37 ± 0.07 A	5.32 ± 0.28 C
	10	7.85 ± 0.27 B	8.70 ± 0.10 A	9.01 ± 0.22 A	7.74 ± 0.09 A	9.37 ± 0.07 A	5.89 ± 0.07 C
	5	8.17 ± 0.18 AB	8.73 ± 0.05 A	8.87 ± 0.18 A	7.97 ± 0.04 A	9.63 ± 0.13 A	6.74 ± 0.07 B
	0	9.23 ± 0.08 A	9.16 ± 0.02 A	9.21 ± 0.22 A	7.54 ± 0.02 A	9.68 ± 0.08 A	7.45 ± 0.13 A

Levels not connected by the same letter are significantly different.

: Inoculum. : 0.00. : Maximum growth.

to those that were performed in standard media (MH and LB). However, the MBC of blueberry extract on *E. coli* was increased to 156 mg/ml from 78 mg/ml in LB. Finally, the MBC of blueberry extract on *L. monocytogenes* was decreased to10 mg/ml from 78 mg/ml in BHI. From the Table 5 it can be seen that the total protein content of BHI was almost 2 times more than MH and 4 times higher than LB.

The effect of protein content of media on Inhibitory Concentrations (IC) of different berry extracts on *S. aureus* NM and *L. monocytogenes* is presented in Figure 4. Data from the experiments with the food-constituents-supplemented media and total protein measurement were used for the graphs. Strength of relationship is measured by the coefficient of determination (R^2). Mean of inhibitory concentrations is an inhibitory concentration of berries between the berries MIC and MBC. The graphs show a relationship between the efficacies of berry extracts and protein content of media on specially S. aureus

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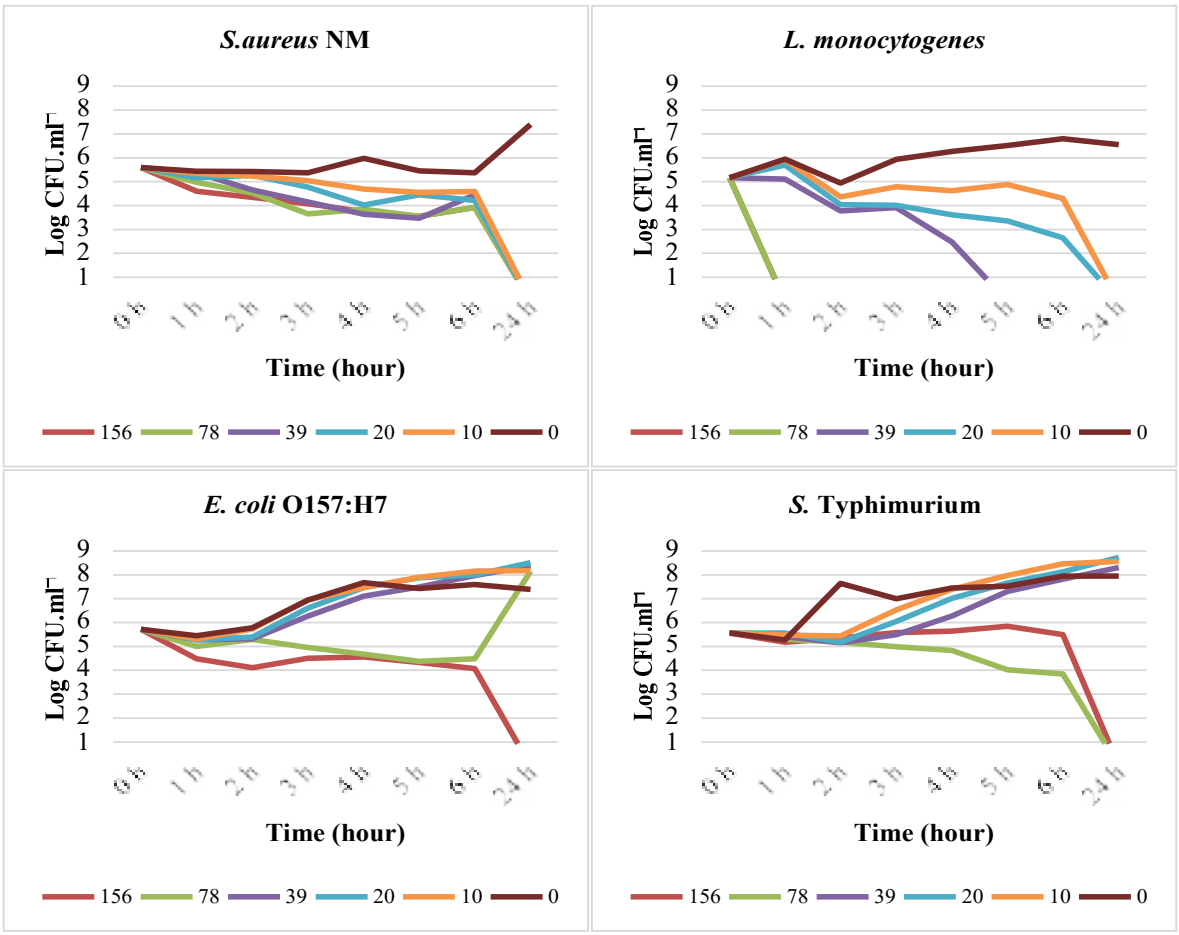

Figure 3. *Growth curves based on CFUs of S. aureus NM, L. monocytogenes, E. coli O157 and S. Typhimurium at presence of different concentrations of blueberry in FKP.*

Table 5. *Total Anthocyanin (TAC.) and Phenolic (TPhC) contents of berry extracts.*

Berry name	TAC mg.100 g-1 pwd*a			TPhC mg.100 g-1 pwd*b		
	Ave.		SEM	Ave.		SEM
Aronia	157	±	1	6920	±	3
Blackcurrant	1042	±	25	3594	±	2
Blueberry	2303	±	71	6973	±	3
Cranberry	197	±	1	2579	±	2
Raspberry	276	±	2	2800	±	2
Bilberry	19771	±	19	30499	±	9
Cranberry	3313	±	54	33015	±	11
Lingonberry	4315	±	30	30922	±	11

**: Powder (pwd)*

1a: the results are mean value of 3 repetitions of one representing experiment.

1b: the results are value of a representing experiment. SEM: standard error of the mean. The equation used for calculation of TPhC is presented in Figure2. TAC expressed as mg.

Table 6. *Total protein contents of different media.*

	TPC µg.ml-1*	SD	SEM
MH	33.5	1.4	1
MH + Meat	270.6	4.9	3.5
MH + Casein	72	4.8	3.4
LB	15.2	1.7	1.2
BHI	60.5	0.4	0.3
BHI + Meat	297.7		
BHI + Casein	99		
TSB	34.9	0.9	0.7

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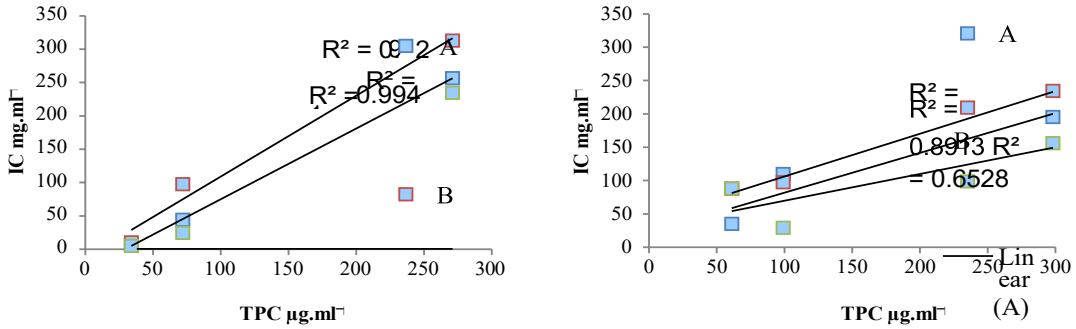

Figure 4*. Effect of concentration of protein in media on inhibitory concentrations (IC) of different berry extracts on S. aureus NM (left) and L. monocytogenes (right). The strength of the relationship is measured by the coefficient of determination (R²). A: mean of IC of aronia. B: mean of IC of blackcurrant. C: mean of IC of blueberry. Error bars show SEM. Total protein content measured by Bradford assay and expressed as µg of BSA per ml of PBS.*

Discussion

Antimicrobial property of active compounds from berries

Studied the antimicrobial activity of extracts and phenolic compounds from several Nordic berries against probiotic bacteria and intestinal bacteria, including pathogenic species *S. Typhimurium* and *E. coli* [14]. Blackcurrant, blueberry, cranberry and raspberry were common berries in their study. Gram-positive Lactobacillus spices were found as the least sensitive bacteria to the berry extracts compared to gram-negative pathogens including *S. Typhimurium* and *E. coli*. Raspberry was found to have the highest inhibitory activity and total phenolic content. However, the inhibitory power of other berries was not related to total phenolic content. This lack of correlation might be due to a variation in the content of organic acids. For example [7] has mentioned the growth inhibition of Salmonella seemed caused by other compounds, such as organic acids. Perhaps uncertainty about the antimicrobial activity of berries due to the effect of low pH leads to the neutralization of berry extracts in later studies. [13] and [26] fractionalized the fruit extracts into sugars and organic acids, phenolica and anthocyanins. Both studies reported the antimicrobial effect of water-soluble fraction (sugars and organic acids) and neutralized phenolic and neutralized anthocyanins fractions. However, it was confirmed that the antimicrobial effect of sugars plus organic acids fraction is dependent on pH since a loss of the antimicrobial effect happened at pH 7 [13].

The dependence of the bactericidal activity of berry extracts on the power of organic acids and low pH can be assessed by different methods. For example, by using the neutralized berry extracts for the antimicrobial experiments and by studying the effect of organic acid and pH on the viability of bacteria. It is known that weak organic acids, such as lactic and citric acid can increase the permeability of the outer membrane of gram-negative bacteria. That is why perhaps in the earlier studies where the extracts have not been neutralized gram-negative bacteria were found more sensitive. Accordingly, Lacombe showed the effect of the sugars plus organic acids fraction of cranberry on *E. coli* cells, which caused cytoplasm coagulation, outer membrane damage and cells malformation. This function of organic acids may increase the sensitivity of the Gram-negative bacteria to other antimicrobial substances [10]. So, regarding this information, it can be speculated that the antimicrobial activity of berry extracts can be greater in lower pH which is often the case in food products.

In the present study, neutralized berry extracts of *aronia*, blackcurrant, blueberry, cranberry and raspberry had antimicrobial activity on *S. aureus* NM, *L. monocytogenes*, *E. coli* 0157 and *Salmonella Typhimurium*. The stronger antimicrobial activity was observed for blueberry and *aronia* followed by blackcurrant and cranberry, while the least activity was observed for raspberry. In addition, in-contrast to the few other previously mentioned studies, the Gram-positive *S. aureus* NM and *L. monocytogenes* were found to be more sensitive to the berry extracts than the Gram-Negative *E. coli* and S.Typhimurium. Since in present study all the antimicrobial examinations took place at pH 7 and yet the antimicrobial effect had observed it can be suggested that the berry extracts have other active compounds in addition to organic acids. Furthermore, it can be said that the observed variation between results of different studies is due to the presence of organic acids and deviation on pH. Considering the present study and others, the end note would be that at the neutral pH Gram-positive bacteria are more sensitive and at the natural pH Gram-negative bacteria are more susceptible to the berries compounds.

Further experiment that took place in this study was addition of food constitutes to the medium of bacteria. The aim of this addition was to make a condition which represents a simple food model. In other word to see if the berries can be used in the food as novel food preservative ingredients and if “yes” which type of food would be a better choice. For this part of the experiment starch, meat extract, casein, vegetable oil and milk were separately added to the bacterial medium growth. Overall, starch had little to no effect while meat extract had the greatest influence on the antibacterial activity of *aronia*, blackcurrant and blueberry extracts, while the effect of other food components varied between extracts and between the two tested bacteria. In general, the food constituents (casein, milk and especially, the meat) which are known to contain more protein had a higher inhibitory effect on the antimicrobial activity of berry extracts. Therefore, it

was hypothesized that the presence the proteins caused this inhibitory effect. This hypothesis was tested by correlating relative protein concentrations of different media with the antimicrobial activity of berry extracts (see Figure 4). From the figure 4, it can be seen that there is a negative correlation between the measured amounts of protein and the antibacterial activity, particularly for *S. aureus* NM. This notion is in accordance with some other researches demonstrating food protein-mediated (e.g. milk protein) inhibitory effects on properties such as bioavailability, antioxidant activity and antibacterial activity of phenolic and other flavonoids [27-31]. In these studies the masking of the antibacterial activity was found to be dependent both on species of protein and types of flavonoids [28]. However this transformation on the activity of berry extracts may not be limited only to the protein. Many other factors such as pH, temperature and the amount of phenolic compounds (see Table 5) of the original plants can affect the results. It is worth mentioning that the implemented environment in this research was consisted of 37°C of temperature and acidity of close to pH 7 which is far more different from the optimal storage condition of many food products.

Until now it has been observed that how much the pH and presence of organic acids can determine the type bacteria which are affected by berries. Later it has been observed that how much the medium composition can be determinant on the efficacy of the berries against different bacteria. This later observation raised an idea about the difference caused by variation on standard growth media used for different food pathogens accordingly. The question would be that how much the standard media that are used in all of these experimented as control can affect the results? This question initiated two more experiments. The first experiment was measurement of the total protein content of different standard media used for growth of *S. aureus* NM and *L. monocytogenes*. The second experiment exposed *S. aureus* NM and *L. monocytogenes* to berry extract in saline solution (FKP). In this way the probable masking effect of protein content of standard growth media could be avoided. Interestingly enough at the first experiment it has been observed that the protein content of the standard growth medium used for *L. monocytogenes* had a higher content of protein; nearly as twice more as what was in the standard growth medium for *S. aureus* NM (see Table 6). The results of the second experiment showed *L. monocytogenes* more sensitive to berry extract then *S. aureus* NM. Here the difference between *L. monocytogenes* sensitivity to berry extracts in the standard growth medium compared to FKP it must be emphasized. Despite of being interesting, these results warns that the used standard media can have a masking effect on the antimicrobial activity of the tested compounds. This masking effect can lead to a false conclusion.

All in all, *aronia*, blackcurrant and blueberry showed bactericidal activity and significant influence even at neutral pH and in the presence of food constituents on *S. aureus* NM and *L. monocytogenes*. However, the addition of food compositions, in general, caused an increase on the MBC and the MIC of berry extracts. The addition of the meat-extract had the biggest negative influence on the inhibitory activity of berries. Therefore the protein reach foods might need more amount of berries to be preserved. Finally *L. monocytogenes* can be reported as the most sensitive to the berry extract in the saline solution and can be disinfected easily in the absence of protein. This influence may be caused by an interaction of polyphenols and protein. In general, it can be said that for similar berry ingredients to be used in food, products with lower content of protein would be more suitable. However, more molecular studies on the mechanism of interaction between berries' active compounds and different types of proteins are required.

The inhibitory activity of *aronia* and blueberry was confirmed against *S. aureus* NM and *L. monocytogenes* in milk and also presence of casein at neutral pH. This may offer the use of these berries in dairy as an inhibitor against *S. aureus* and *L. monocytogenes*. However, before this implementation, a pilot experiment for determining the best concentration of berries needs to be considered; due to the fact that a stimulating effect on growth of *L. monocytogenes* was observed at sub-MIC concentrations of blackcurrant in milk (see Table 2 & 4).

Due to the toxicity of methanol, aqueous ethanol can be used for extraction of berries' active compounds for antimicrobial applications or studies, whereof the results confirmed no significant difference between the uses of these solvents (data not shown).

Berries are found as more active against gram-positives bacteria. Therefore, the influence of berries on growth of *S. aureus*, B. cereus and Clostridium can be considered for future studies.

This study as a semi-preliminary study warrants further evaluation of the antibacterial compound/s driven from extracts of these berries, and perhaps, especially those of *aronia*, blackcurrant and blueberry. Therefore, purification and isolation of the active compound/s would potentially shine a light for a better understanding of the mechanisms and usage of these possible food additives and provide more flexible option for food producers. The effect of standard growth media on the antibacterial activities of berries have shown that more careful planning is required during the assembly of similar experiment. Because for example the used standard growth media can have masking effects on the antimicrobial activities of testing compounds and therefore cause false interpretation.

Overall, this study provides information about the antibacterial properties of berries in the presences of food constituents, which can be used as a source of information or inspiration by relevant industry and research groups.

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Contributions description

Massoud Attarianshandiz developed the idea, designed research, conducted experiments analyzed data and wrote the manuscript. Under the supervision of Professor Hanne Ingmer and Dr. Jette Kjeldgaard. The only author of this manuscript (Massoud Attarianshandiz) read and approved the manuscript.

Compliance with Ethical Standards

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Conflict of Interest

Author (Massoud Attarianshandiz) declares that he has no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by the author.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Amit SK, Uddin M, Rahman R, et al. A review on mechanisms and commercial aspects of food preservation and processing. Agricm Food Secur. 2017 ;6(1):1-22.
2. Lee J, Durst RO, Wrolstad RO. AOAC official method 2005.02: total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method. OMA of AOAC Int. 2005;2.
3. Bruna GL, Thais AC, Lgia AC. Food additives and their health effects: A review on preservative sodium benzoate. AJB. 2018;17(10):306-10.
4. Stohs SJ, Miller MJ. A case study involving allergic reactions to sulfur-containing compounds including, sulfite, taurine, acesulfame potassium and sulfonamides. FCT. 2014;63:240-3.
5. Trasande L, Shaffer RM, Sathyanarayana S, et al. Food additives and child health. Pediatrics. 2018;142(2).
6. Wu X, Gu L, Prior RL, et al. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem. 2004;52(26):7846-56.
7. Puupponen-Pimiä R, Nohynek L, Hartmann-Schmidlin S, et al. Berry phenolics selectively inhibit the growth of intestinal pathogens. Appl Microbiol Biotechnol. 2005;98(4):991-1000.
8. McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. MNF. 2007;51(6):702-13.
9. Kahkonen MP, Heinämäki J, Ollilainen V, et al. Berry anthocyanins: isolation, identification and antioxidant activities. J Sci Food Agric. 2003;83(14):1403-11.
10. Puupponen-Pimiä R, Nohynek L, Alakomi HL, et al. Bioactive berry compounds—novel tools against human pathogens. Appl Microbiol Biotechnol. 2005;67(1):8-18.
11. Nohynek LJ, Alakomi HL, Kähkönen MP, et al. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. Nutr Cancer. 2006;54(1):18-32.
12. James A. Duke with Mary Jo Bogenschutz-Godwin, Judi duCellier, Peggy-Ann K. Duke. Duke, James A., Medicinal Herbs Handbook of Second Edition. 1929.
13. Lacombe A, Wu VC, Tyler S, et al. Antimicrobial action of the American cranberry constituents; phenolics, anthocyanins, and organic acids, against Escherichia coli O157: H7. Int J Food Microbiol. 2010;139(1-2):102-7.
14. Puupponen-Pimiä R, Nohynek L, Meier C, et al. Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol. 2001;90(4):494-507.
15. Sadilova E, Stintzing FC, Carle R. Thermal degradation of acylated and nonacylated anthocyanins. J Food Sci. 2006;71(8):C504-12.
16. Burdulis D, Sarkinas A, Jasutiene I, et al. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (Vaccinium myrtillus L.) and blueberry (Vaccinium corymbosum L.) fruits. Acta Pol Pharm. 2009;66(4):399-408.
17. Cesoniene L, Jasutiene I, Sarkinas A. Phenolics and anthocyanins in berries of European cranberry and their antimicrobial activity. Medicina. 2009;45(12):992.
18. Butkhup L, Samappito S. Analysis of anthocyanin, flavonoids, and phenolic acids in tropical bignay berries. Int J Fruit Sci. 2008;8(1-2):15-34.
19. Li HB, Wong CC, Cheng KW, et al. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT- JFST. 2008;41(3):385-90.
20. Chandrasekhar J, Madhusudhan MC, Raghavarao KS. Extraction of anthocyanins from red cabbage and purification using adsorption. Food Bioprod Process. 2012;90(4):615-23.
21. Samappito S, Butkhup L. Analysis of anthocyanin, flavonoids, and phenolic acid contents of ten fruits and antioxidant activity. Int J Fruit Sci. 2010;10(3):264-80.
22. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. AJEV. 1965;16(3):144-58.

23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248-54.

24. Chen CY, Nace GW, Irwin PL. A 6× 6 drop plate method for simultaneous colony counting and MPN enumeration of Campylobacter jejuni, Listeria monocytogenes, and Escherichia coli. J Microbiol Methods. 2003;55(2):475-9.

25. Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol. 2008;124(1):91-7.

26. Siddiqi R, Naz S, Ahmad S, et al. Antimicrobial activity of the polyphenolic fractions derived from Grewia asiatica, Eugenia jambolana and Carissa carandas. JFST. 2011;46(2):250-6.

27. Arts MJ, Haenen GR, Voss HP, et al. Masking of antioxidant capacity by the interaction of flavonoids with protein. FCT. 2001;39(8):787-91.

28. Arts MJ, Haenen GR, Wilms LC, et al. Interactions between flavonoids and proteins: effect on the total antioxidant capacity. J Agric Food Chem. 2002;50(5):1184-7.

29. Serafini M, Bugianesi R, Maiani G, et al. Plasma antioxidants from chocolate. Nature. 2003;424(6952):1013

30. Wiese S, Gärtner S, Rawel HM, et al. Protein interactions with cyanidin-3-glucoside and its influence on α-amylase activity. J Sci Food Agric. 2009;89(1):33-40.

31. Von Staszewski M, Pilosof AM, Jagus RJ. Antioxidant and antimicrobial performance of different Argentinean green tea varieties as affected by whey proteins. Food Chem. 2011;125(1):186-92.

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