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THE EFFECT OF ANTIOXIDANTS ON *LACTOBACILLUS CASEI* CULTURES

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Abstract. The growing popularity of functional foods causes increasing interest in raw materials, which can raise the pro-health value of food when supplemented. The aim of the study was to assess the effect of compounds with antioxidant properties on *Lactobacillus casei*, as a representative of probiotic microorganisms. In the experiments the pure antioxidants and plant extracts were used. The results showed that catechin at concentration of 100-400 μM and chlorogenic acid (400 μM) had a stimulatory effect on *L. casei* growth. Quercetin at concentrations of 25-50 μM showed an inhibitory effect when incubation time was ≥ 6 h. The lingonberry leaves extract caused a slight increase in the dry substance of biomass in comparison with control. Extracts of coffee, chokeberry, and dog rose should not be used as additives for probiotic food as they significantly inhibited *L. casei*. We conclude that: 1) antioxidants at concentrations higher than 100 μM may stimulate growth of *L. casei*; 2) except the antioxidants, some other compounds which are present in plants extracts e.g. tannins and alkaloids may exert an antibacterial influence; 3) the probiotic yoghurts supplementation with fruit and other plant materials should be preceded by careful studies about their influence on the bacteria.

Key words: bio-yoghurts, probiotics, *Lactobacillus casei*, functional food, antioxidants, food additives

INTRODUCTION

In the consequence of the high incidence of civilization diseases, science and industry direct their interest toward production of food products, which beyond the "normal" nutritional function deliver health benefits, i.e. reduce risk of diseases. These products are called functional foods, and the probiotics are an example [Fuller 1991, Roberfroid 2000]. The consumption of living or lyophilized cultures of probiotic bacteria improves the immune system action, prevents cancer, atherosclerosis and coronary diseases. The beneficial effect of probiotics on diarrhea, gastroenteritis, irritable bowel syndrome, inflammatory bowel disease, lactose digestion, infant allergies, hyperlipidemia, hepatic

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disease, *Helicobacter pylori* infections was proved [Brown and Valiere 2004, Marteau et al. 2001].

The most frequently consumed probiotic products are bio-yoghurts. They contain, besides the typical yoghurts strains *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus*, cultures of live probiotic bacteria, such as *Lactobacillus casei Defensis*, *L. acidophilus LA-5* or *Bifidobaterium BB-12* [Holzapfel et al. 2001, Reid and Hammond 2005]. The growing consumer interest in bio-yoghurts, as well as commercial competition, caused that their producers competed in diversifying assortment of goods. Usually it consists in a new flavor introduction by adding rare fruit. The producers endeavor also to enhance the marketability by supplementation with other components that act positively on health, mainly vitamins and antioxidants. The plant raw materials rich in antioxidants, coupling both the attributes, constitute a valuable material for bio-yoghurts production.

On the other hand, the interest in antioxidants is growing because of their antimicrobial activity. Despite advanced food production and preservation techniques, the spoilage and poisoning of foods by microorganisms is still the problem. The consumers' acceptance for preservatives with chemical origin is decreasing; therefore, the producers are looking for natural compounds which can be an alternative and supplemented to food products will help to prolong their shelf-life and microbial safety.

Plant extracts abound in antioxidant compounds which exert antibacterial [Rauha et al. 2000], antiviral [Droebner et al. 2007, Gabbay et al. 2007], antitoxin [Friedman 2007] or antifungal activity [Newton et al. 2002]. It was proved that they can inhibit the growth of food-associated pathogens and microorganisms responsible for food spoiling, as well as intestinal microflora, both pathogenic and physiological [Kim et al. 2004, Mabe et al. 1999, Medina et al. 2006, Nagayama et al. 2002]. Some researches indicate that antioxidants may have also negative effect on bacteria which are desirable for human organism. Ligstroside, one of the polyphenolic compounds present in virgin olive oil, showed the strong bactericidal activity against a broad spectrum of microorganisms, both gram-positive and gram-negative. The olive oil was effective towards foodborne pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia* sp., *Shigella sonnei*), intestinal microflora (*Clostridium perfringens*, *Escherichia coli*), as well as positively acting microorganisms, like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* [Medina et al. 2006].

Although there is a great number of articles dealing with the antioxidants inhibitory effect on different species of bacteria, only few of them refer to the effect of antioxidants on probiotic bacteria [Välimaa et al. 2007]. The knowledge of interaction between particular microorganisms and antioxidants is indispensable for appropriate utilization of those compounds. The doubts appear especially in the situation when food containing probiotic bacteria is supplemented with plant raw material rich in antioxidants. On the basis of experiments with other microorganisms one can expect that antioxidants would significantly diminish the amount of bacteria or even kill them.

The objective of this study was to evaluate the influence of compounds with antioxidant activity on *Lactobacillus casei*, which is a representative of probiotic microorganisms. In our experiments the pure antioxidant substances and plant extracts were used.

MATERIAL AND METHODS

Chemicals

Diammonium salt of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS diammonium salt); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); a phosphate buffer (PBS): 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride; pH 7.4 at a temperature of 25°C; (+)catechin hydrate, and chlorogenic acid were purchased from the SIGMA-Aldrich Company. Quercetin dihydrate was obtained from Fluka. The chemicals: potassium persulfate ($K_2S_2O_8$) and methanol (analytically pure) were purchased from the POCh Company, and MRS from the Biocorp.

Bacterial strain and culture condition

The strain of *Lactobacillus casei* (DSM 20011) was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Deutschland). The *Lactobacillus casei* were grown in a sterile, liquid MRS medium, at a temperature of 30°C, in glass tubes (10 cm^3) under anaerobic condition.

Preparation of the pure antioxidants solutions

The 20 mM methanolic solutions of pure antioxidants were prepared by dissolving catechin hydrate, quercetin dihydrate and chlorogenic acid (0.5806, 0.6765, and 0.7086 g, respectively) in 100 cm^3 of methanol. A 0.2-cm^3 amount of such solutions added to 9.8 cm^3 of bacterial culture resulted in concentration of 400 µM of each antioxidant. The serial dilutions of antioxidants in methanol were also performed. The final concentrations of particular antioxidant in the bacteria culture (always 0.2 cm^3 of solution was added into 9.8 cm^3 of bacteria culture) were: 200, 100, 50, 25, and 10 µM.

Preparation of methanolic plant extracts

Plant extracts were prepared from fruit of chokeberry (*Aronia melanocarpa*), dog rose (*Rosa canina*) or hawthorn (*Crataegus oxyacantha*), leaves of lingonberry (*Vaccinium vitis-idaea*) and roasted coffee. A portion of the lyophilized (chokeberry, dog rose and hawthorn) or dried (coffee, lingonberry) sample was placed in a container of the laboratory mill and grounded (2 × 12 seconds). An amount of 40 cm^3 of methanol was poured over a 0.500 g ground sample and mixed for 2 h with a magnetic stirrer (500 rpm). The whole mixture was seeped and centrifuged for 15 minutes (1400 × g, 20°C), and the supernatants obtained were collected into twisted probes. Those methanol extracts were then stored in a freezer (–20°C) until the analysis.

Preparation of bacteria suspension

The 24 h-old broth culture of bacteria was used. Bacteria were centrifuged (8600 × g, 1 min) and supernatant was removed. 1 cm^3 of sterile broth was added and the optical density (OD) of the resuspended pellet was determined at 550 nm by nephelometry

(Beckman Spectrophotometr DU 650). The OD_{550} of the sample was adjusted to 0.2 with the addition of sterile broth. The resulting bacteria suspension (OD_{550} = 0.2) was used for testing the effect of antioxidants on bacteria.

Analysis of the antioxidants' effect on *L. casei* cultures

For each antioxidant compound 8 glass tubes with 9.3 cm^3 of sterile MRS broth were prepared. 200 mm^3 of appropriate antioxidant dilution were added aseptically to consecutive tubes; the solutions were vortexed, and then each test sample was inoculated with 500 mm^3 of the bacteria suspension (OD_{550} = 0.2). The final concentrations of antioxidants in bacteria culture were: 10, 25, 50, 100, 200, and 400 µM.

The effect of antioxidants originated from plant extracts on bacteria was evaluated analogically. To tubes containing 9.3 cm^3 of sterile MRS broth, 200 mm^3 of methanolic plant extract was added followed by the inoculation with 500 mm^3 of bacteria suspension. The extract volume used was chosen on the basis of preliminary determinations of polyphenol content. In this way, the plant extract of the highest antioxidant activity introduce into a culture the same amount of polyphenols that catechin at concentration of 400 µM. Bacteria cultures without antioxidants or extracts addition constituted the controls (K). The addition of 200 mm^3 of pure methanol effect on bacteria was evaluated as well. The experiment described above was performed five times for each antioxidant supplement.

The effect of antioxidant compounds on bacteria growth was evaluated on the basis of the determination of dry matter of biomass obtained from cultures at 3, 6, 10, 24 and 48 h after inoculation. An assumption was made that the number of bacteria is proportional to dry substance content of bacterial biomass. So an increase in bacteria number causes an adequate rise of the dry substance content of biomass. The cultures after appropriate incubation time were centrifuged (8600 × g); the pellet of bacteria was washed twice in PBS, and quantitatively transferred into weighing bottle dried formerly to constant weight. Then the sample in the weighing bottle was dried at 105°C till the constant weight was obtained, cooled in dessicator and weighed on the analytical balance. The dry matter of bacteria biomass was calculated with formula (1):

$$\%\,\text{dry matter} = \frac{c-a}{b-a} \times 100\% \tag{1}$$

where:

a – the weight of empty weighing bottle (g),
b – the weight of weighing bottle with fresh sample (g),
c – the weight of weighing bottle with dried sample (g).

For results analysis the dry substance content in the biomasses obtained from the control samples (cultures without the extract addition) was assumed as 100%, and the others were recalculated and expressed as percentage of control.

Assessment of the antioxidant activity by ABTS method

Using this method, it is possible to quantitatively assess the capacity of elements to scavenge a stable ABTS radical. The antioxidant activity of pure antioxidant solutions

or plant extracts was assayed on the basis of a protocol represented by Re et al. [1999] with some modifications incorporated. The ABTS radical was generated during a chemical reaction between the 7 mM aqueous solution of diammonium salt of the 2,2'-azino--bis(3-ethylbenzothiazoline-6-sulfonic) acid and the 2.45 mM potassium persulfate. The solution was kept at a room temperature in darkness throughout the night, in order to complete the reaction and to stabilize the ABTS cation-radical. Then, a concentrated solution of the radical was diluted with a phosphate buffer (PBS, pH 7.4) to obtain a final absorbance of the solution, measured at 734 nm, of A = 0.70 ±0.02 ($ABTS_{0.7}$).

An amount of 100 mm^3 of the properly diluted sample investigated or of Trolox solutions (their concentration rates ranging from 0 to 10 mg × 100 cm^{-3}) was added to 1 cm^3 $ABTS_{0.7}$; next, the absorbance was measured in the 6th minute upon the completed mixing. The antioxidant capacity of samples under study was calculated using a standard curve drawn up for solutions of the synthetic vitamin E (Trolox) and expressed as mg Trolox × 100 cm^{-3}. All determinations were performed in triplicate.

Statistical analysis

The results were shown as an arithmetic mean (± standard deviation) of five independent determinations. A single-factor Analysis of Variance test (ANOVA) with a *post hoc* Tukey-Kramer test was applied to perform a statistical analysis. A Kołmogorov-Smirnov test was applied to examine the normality of distribution. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

There is an increasing interest in probiotics and antioxidants for the sake of their positive impact on human organism. Moreover, the growing demand for natural antioxidants is observed in food and cosmetic industries, because antioxidants can be used as natural supplements prolonging the stability and storage life of food products. The probiotics yoghurts containing fruit are the example of functional food products that include both mentioned components.

The pertinent literature references available [Almeida et al. 2006, Kim et al. 2004] proved that antioxidants exhibit antibacterial activity against different bacteria species, but the majority of the papers focused on pathogen or spoilage microorganisms. The objective of our investigation was to explain the doubts concerning the interactions between probiotic microorganisms and antioxidants, which can take place in some types of functional food.

Our results showed very interesting differences among antioxidant compound used in experiments. First of all, there was a great diversity in their antioxidant activity. Among the 20 mM solutions of pure antioxidants analysed, the highest antioxidant activity was demonstrated for quercetin (2898.0 ±2.3 mg of Trolox × 100 cm^{-3}), then for catechin (2793.5 ±75.5 mg of Trolox × 100 cm^{-3}). The chlorogenic acid showed much lower antioxidant capacity (Table 1). Among all the plant methanolic extracts, the extract from lingonberry leaves had the highest antioxidant activity (1132.1 ±71.4) and the value was almost 3-fold higher than the antioxidant capacity of hawthorn (455.0 ±60.0) and chokeberry (406.6 ±0.1).

Table 1. Antioxidant activity of compounds' solution used in experiment, results presented as arithmetic mean ±SD (n = 3)

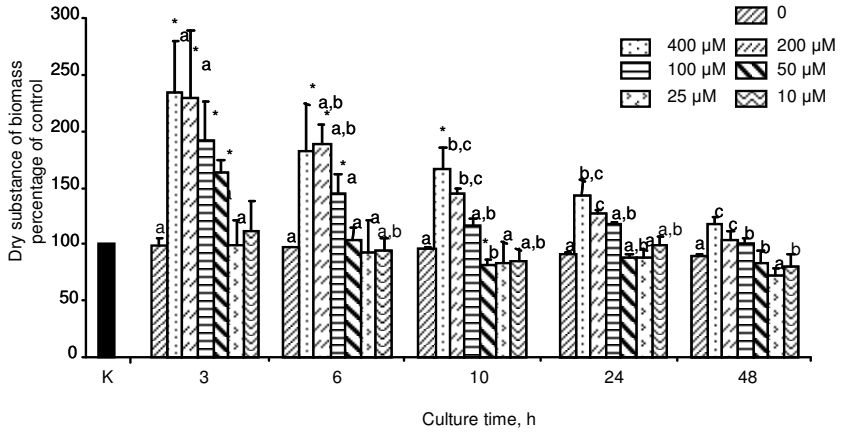
Compound	Antioxidant activity, mg of Trolox × 100 cm^{-3}
Catechin[a]	2 793.5 ±75.5
Quercetin[a]	2 898.0 ±2.3
Chlorogenic acid[a]	697.6 ±0.3
Lingonberry[b]	1 132.1 ±71.4
Chokeberry[b]	406.6 ±0.1‡
Hawthorn[b]	455.0 ±69.0‡
Dog rose[b]	170.9 ±0.1
Coffee[b]	42.4 ±0.1

[a]The 20 mM methanolic solutions of pure antioxidants were analysed; 200 µL of solution added to 9.8 cm^3 of bacterial culture resulted in 400 µM concentration of antioxidant.
[b]Plant raw material were analysed as methanolic extracts (0.500 g d.w. in 40 cm^3).
‡The symbol denotes the lack of statistically significant differences ($p < 0.05$) between the values.

The influence of pure antioxidants' solutions on *Lactobacillus casei* is presented in Figure 1-3. It was demonstrated that solvent itself (methanol) added to culture exerted no significant influence on bacteria growth. It was also shown, that all differences as compared with control diminished with time. The significant increase in dry substance content of biomasses was revealed at 3 h in all *Lactobacillus casei* cultures, which had been supplemented with antioxidants at concentrations of 100-400 µM. However, in subsequent culture stages as well as in lower concentrations the antioxidants influence on the bacteria was diversified.

Catechin, in concentrations ranging from 100 to 400 µM, stimulated the growth of bacteria during the whole time of the experiment (Fig. 1). An increase in dry matter of biomass obtained from 3 h culture ranged from 191% of control to 234% for 100 µM and 400 µM catechin, respectively. The lower were catechin concentrations in *L. casei* culture, the weaker and shorter influence on the bacteria growth was demonstrated. Quercetin, in the concentrations of 100-400 µM stimulated bacteria growth only in 3-h cultures, and there were no significant differences in dry matter of biomass obtained from longer incubation times when compared to control (Fig. 2). However, the 25-50 µM quercetin solutions showed an inhibitory effect on bacteria growth when incubation time was ≥ 6 h. Chlorogenic acid, when added to the culture in 400 µM concentration stimulated *Lactobacillus casei* growth (Fig. 3); however, its lower concentrations exerted an influence only at 3-h cultures.

The differences achieved for those substances can not be explained by their different antioxidant activity only. Chlorogenic acid had several-times lower antioxidant capacity than catechin, and it could explain its lesser effect on bacteria. Nevertheless quercetin caused inhibition of bacteria growth in spite of its higher antioxidant activity than catechin. It seems that the chemical structure of those three substances is more responsible for results achieved than their antioxidant activity. Chlorogenic acid is a derivative of phenolic acids, while quercetin and catechin are flavonoids composed of 3 aromatic



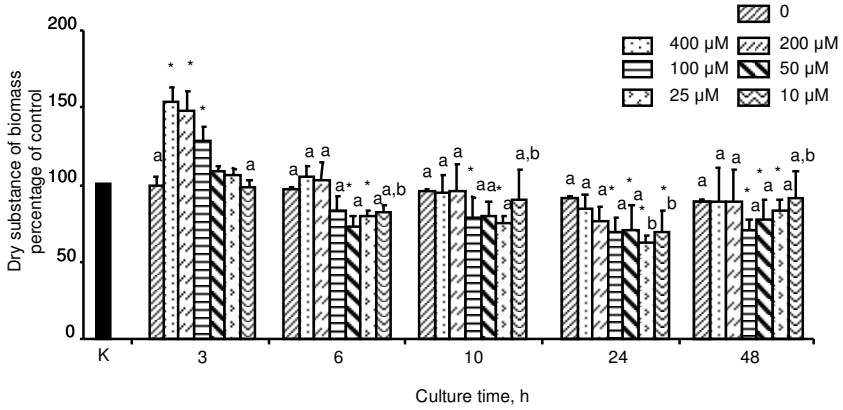
*Means statistically significant differences when compared to control ($p < 0.05$).
a, b, c – identical letters by the columns representing the same concentration denote the lack of statistically significant differences ($p < 0.05$) between particular time points of experiment.

Fig. 1. The influence of catechin solutions on *Lactobacillus casei* culture. In the experiment catechin was used in the concentrations ranging from 10 µM to 400 µM (different design of bars). The dry substance content in the biomasses obtained from the control samples was assumed as 100%, and the results from other samples were recalculated and expressed as percentage of control. Bars represent an arithmetic mean ±SD (n = 5); K – control (cultures without the antioxidant addition), 0 – cultures with pure solvent (methanol)

rings [Rice-Evans et al. 1996]. A few investigations confirm that polyphenols effect on intestinal bacteria depends on bacteria type and the chemical structure of compound. Caffeic acid (3,4-dihydroxycinnamic acid) showed a strong inhibitory impact on the growth of intestinal bacteria, specially *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Clostridium* and *Bacteroides*, with no limiting effect on autochthonic and probiotic microflora. Catechin had no influence on growth of *Clostridium* sp., but stimulated *Lactobacillus* and *Bifidobacterium* [Lee et al. 2006]. Catechin and gallic acid at concentrations normally present in wine stimulated the growth rate of *Lactobacillus hilgardii* [Alberto et al. 2001]. In our study it was revealed that catechin at appropriate concentrations could significantly activate the growth of *Lactobacillus casei*. These results are in agreement with those observations.

Another aspect that should be taken into account is the possibility that antioxidant compounds can be transformed by bacteria. The literature references show that intestinal microflora can metabolize some polyphenols compounds. Among other things, the transformations consist in flavonoids glycosides hydrolysis or C-ring cleavage [Winter et al. 1989]. Many polyphenols are metabolized by intestinal microflora to catechol and other simple phenols; and the derivatives formed in these reactions are characterized by different antioxidant activities comparing to the precursors. For example, *Eubacterium ramulus* is a flavonoid-degrading anaerobic bacterium from human gastrointestinal tract

able to cleave the C-ring of genistein and daidzein [Schoefer et al. 2002] and hydrolyze hesperetin dihydrochalcone [Braune et al. 2005]. *Clostridium orbiscindens*, which is an



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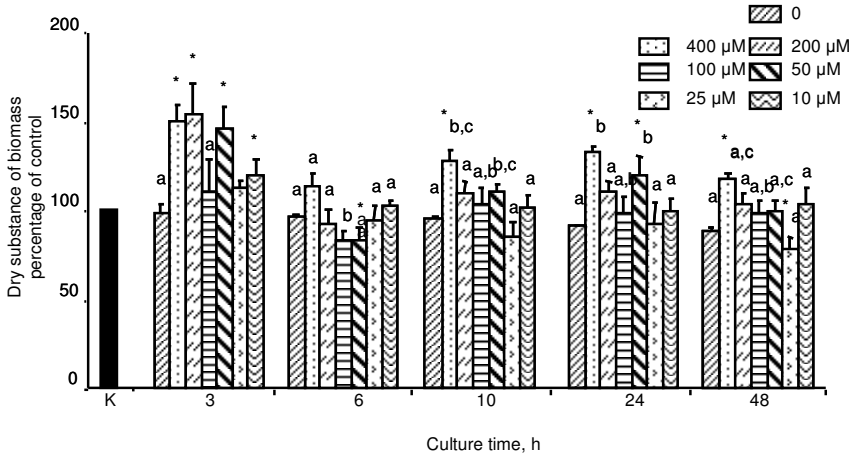
a, b – identical letters by the columns representing the same concentration denote the lack of statistically significant differences ($p < 0.05$) between particular time points of experiment.

Fig. 2. The influence of quercetin solutions on *Lactobacillus casei* culture. In the experiment quercetin was used in the concentrations ranging from 10 µM to 400 µM (different design of bars). The dry substance content in the biomasses obtained from the control samples was assumed as 100%, and the results from other samples were recalculated and expressed as percentage of control. Bars represent an arithmetic mean ±SD (n = 5); K – control (cultures without the antioxidant addition), 0 – cultures with pure solvent (methanol)

obligate anaerobe commonly found in the intestinal tract, is also capable of cleaving the C3-C4 bond of quercetin to give 3,4-dihydroxyphenylacetic acid. It has been also demonstrated that at least three colonic microbiota species (*Bifidobacterium lactis*, *Lactobacillus gasseri*, and *Escherichia coli*) can release hydroxycinnamates from chlorogenic acid [Bingham 2006].

Undoubtedly, further investigations are needed, especially with mass spectrometry and HPLC usage, which allow identifying components in the culture medium. In this way the information on antioxidants' derivatives formed during experiment could be obtained. It is very important because conversion of antioxidants by microorganisms may affect them in two ways. First, the absorption of the derivatives may be enhanced or reduced relative to that of initial compound, so biological activity of antioxidants would change. It was proved that aglycones and glucosidic derivatives are assimilated much better in the intestine than the connections with other sugars, esters, and polymers [Hollman et al. 1995, Manach et al. 2005]. Since some antioxidants in their native form are not able to get through the alimentary tract walls and cannot exert their biological activity, the bacterial conversion before ingestion may improve the biological effectiveness of those food components. Second, the conversion may lead to the deactivation of bioactive compounds or activation of inactive compounds. First of all, glycosides are hydrolysed into their aglycones of higher potential to radicals scavenging. However,

procyanidins are degraded to flavan-3-ols or to low molecular phenolic acids, which are characterized by lower antioxidant activity than their precursors [Okuda 1999, Rice--Evans 1999].



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a, b, c – identical letters by the columns representing the same concentration denote the lack of statistically significant differences ($p < 0.05$) between particular time points of experiment.

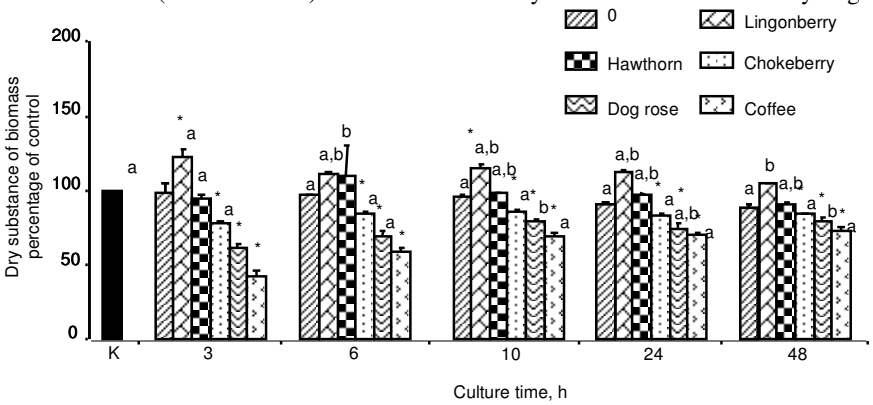
Fig. 3. The influence of chlorogenic acid solutions on *Lactobacillus casei* culture. In the experiment chlorogenic acid was used in the concentrations ranging from 10 µM to 400 µM (different design of bars). The dry substance content in the biomasses obtained from the control samples was assumed as 100%, and the results from other samples were recalculated and expressed as percentage of control. Bars represent an arithmetic mean ±SD (n = 5); K – control (cultures without the antioxidant addition), 0 – cultures with pure solvent (methanol)

It should be highlighted also that in *Lactobacillus* cultures pH decreases, and the changes can cause transformation of the antioxidants present in the raw materials into the forms of lower or higher antioxidant potential. Hence, the additives abundant with antioxidants resistant to low pH should be preferred in food industry. It was suggested by Rios et al. [2002] and Holt et al. [2002] that procyanidins are not broken down in the acidic environment of stomach, so in *L. casei* culture they also should be stable if not metabolized.

Both bacterial and pH-dependent transformation of antioxidants resulted in their decreasing concentration in the culture. Because we observed that the influence of pure antioxidants added to *L. casei* culture had diminished with time, we suggest that derivatives formed during the experiments showed lower antioxidant or antibacterial activity.

When studying results obtained from cultures of *L. casei* supplemented with methanolic plant extracts it was noted that their effect on the bacteria was diversified and depended on plant raw material used (Fig. 4). The lingonberry leaves extract, showing the highest antioxidant activity among extracts, caused a slight increase in the dry substance of biomass in comparison with control. The effect was observed during the whole

experiment duration, but the differences were statistically significant only at 3 and 10 h. One can notice that the extract of lingonberry leaves indicated lower antioxidant capacity than catechin solution (1132 *vs.* 2793 mg Trolox × 100 cm^{-3}), and the biomass gain was also lower (123 *vs.* 234%). Other extracts analysed were characterized by slight



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a, b – identical letters by the columns representing the same concentration denote the lack of statistically significant differences ($p < 0.05$) between particular time points of experiment.

Fig. 4. The influence of methanolic plant extracts on *Lactobacillus casei* culture. The dry substance content in the biomasses obtained from the control samples was assumed as 100%, and the results from other samples were recalculated and expressed as percentage of control. Bars represent an arithmetic mean ±SD (n = 5); K – control (cultures without the extract addition), 0 – cultures with pure solvent (methanol)

antioxidant capacity and exerted completely different effect. The extracts from dog rose, coffee and chokeberry caused statistically significant fall in the dry substance of bacteria biomasses at each time point analysed. The dog rose and coffee extracts' influence was the most evident within the first 10 h after addition to the culture (60% decrease of the dry substance content); then, the inhibitory effect diminished with time, but failed to reach the control level even after 48 h. The extract of hawthorn had no effect on probiotic bacteria.

It should be reminded that these plant raw materials contain other active compounds, except polyphenols. Dog rose and chokeberry are abundant with vitamin C, different alkaloids and tannins, which exert antibacterial effects [Gournelis et al. 1997]. Roasted coffee beans contain, except caffeine, a very strong, not identified yet, antibacterial agent [Daglia et al. 1998], which is harmful towards gram-positive and gram-negative bacteria. So, addition of coffee to the food products that include gram-positive *Lactobacillus casei* can cause the fall in the bacteria viability. Lingonberry contains big amounts of tannins (5-8%), and arbutin (5-7%), which in alkaline environment is hydrolysed to hydroquinone, an agent with strong antibacterial activity, especially toward pathogenic bacteria in urinary tract. Blaut et al. [2006] showed that also some intestinal bacteria can transform arbutin to hydroquinone. Our research did not show the bactericidal activity

of lingonberry against *L. casei* (maybe cause of acidic pH of culture). On the contrary, addition of the lingonberry leaves extract stimulated the bacterial reproduction, and it seems that antioxidants are mainly responsible for these properties.

Other extracts exerted inhibitory effect on bacteria. Although the inhibitory effect of dog rose' and hawthorn' extracts diminished with time, dry matter of biomasses obtained from these cultures failed to reach the control level even after 48 h. Because of that one can suppose that antimicrobial components present in the extracts are metabolized (converted into less active forms) by microorganisms and the derivatives do not exhibit so strong inhibitory effect. Hence, bacteria can divide without obstacles but fail to catch up on initial losses and the number of cells was significant lower in such cultures in comparison to control. On the other side, it is possible that antioxidants are transformed into more active derivatives (e.g. aglycones), which attenuate the negative influence of other components. In the cultures with chokeberry extract added, the decrease in bacteria biomass was observed comparing to control, and the effect was unchanged during the experiment. The result suggests that antibacterial and antioxidant compounds of chokeberry are not degraded nor metabolized by bacteria.

It is interesting, that the hawthorn extract, although it showed relatively high antioxidant activity, did not cause statistically significant differences in dry substance content of biomass in relation to control. It suggests that hawthorn extracts contained no components of bactericidal or bacteriostatic activity against *L. casei*.

CONCLUSIONS

Our research revealed that supplementation of probiotic food products containing *L. casei* with catechin at concentration 100-400 μM favors the growth of the bacteria. But the addition of quercetin, a substance with a similar antioxidant activity, may exert an opposite effect and cause fall in number of desirable microorganisms in product. Coffee, dog rose and chokeberry should not be used as additives for probiotic food.

The findings of our investigations clearly indicate that producers should pay attention to the fact, that the probiotic yoghurt supplementation with plant additives rich in antioxidants is not neutral towards the bacterial strains included. The interactions between antioxidants and bacteria have to be taken into account whenever those components are present simultaneously in food products. On the one hand the additives introduced may raise the nutritional and pro-health values (vitamins, antioxidants), but on the other hand they can significantly decrease the number of probiotic bacteria. Some plant-derived additives may contain, besides antioxidants, substances (alkaloids, tannins), which exert a bactericidal or bacteriostatic effect against *Lactobacillus casei*. Moreover, the antioxidant activity of plant antioxidants may be removed by bacterial transformation and the derivatives of different biological activity may be formed as a result.

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WPŁYW PRZECIWUTLENIACZY NA HODOWLE *LACTOBACILLUS CASEI*

Streszczenie. Rosnąca popularność żywności funkcjonalnej wpływa na wzrost zainteresowania surowcami, które dodane do żywności mogłyby podnieść jej wartość prozdrowotną. Celem badań było ocenienie wpływu związków o właściwościach przeciwutleniających na *L. casei* jako przedstawiciela drobnoustrojów probiotycznych. Na podstawie wyników badań stwierdzono, że: 1) przeciwutleniacze w stężeniach wyższych niż 100 μM mogą stymulować wzrost *L. casei*, 2) antybakteryjnie mogą działać nie tylko antyoksydanty, lecz także inne składniki obecne w ekstraktach roślinnych, np. taniny i alkaloidy, 3) suplementacja jogurtów probiotycznych owocami lub innymi surowcami roślinnymi powinna być poprzedzona szczegółowymi badaniami nad ich wpływem na bakterie.

Słowa kluczowe: biojogurty, probiotyki, *Lactobacillus casei*, żywność funkcjonalna, przeciwutleniacze, dodatki do żywności

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