ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 7(1) 2008, 59-65*

THE EFFECT OF TEA INFUSIONS ON THE PROLIFERATION OF SELECTED BACTERIA IMPORTANT FOR THE HUMAN INTESTINAL TRACT

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Abstract. Tea infusions are consumed frequently and in large amounts, so they may play a role in modelling intestinal microflora or in preventing bacterial diseases. The present study examined the effect of different kinds of tea (black, green and pu-erh) on *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteritidis*. In order to ascertain the connection between antioxidant and antimicrobial properties, the content of polyphenolic compounds and the reducing power of the teas were assessed. The rate of bacterial proliferation was measured by calculating the generation time between the 2nd and 6th hour of incubation. *Staphylococcus aureus* was the most sensitive to the addition of tea extracts to the media. Among lactobacilli (LAB), *Lactobacillus casei* proved to be sensitive mainly to the extracts of black and pu-erh tea. The value for reducing power does not fully correspond to the content of polyphenols and antimicrobial properties.

Key words: tea, antimicrobial properties, LAB, reducing power

INTRODUCTION

The antibacterial effect of many herbs and other materials of plant origin is widely known [Draughon 2004]. Studies have been conducted on, among other topics, the possibility of using plant extracts in treating diseases caused by microbial strains resistant to antibiotics [Nascimento et al. 2000, West et al. 2001] and also as food biopreservatives [Draughon 2004]. These investigations also include tea extracts. However, unlike foods consumed occasionally or in very small amounts which significantly affect the human body only after the intentional use of an appropriately large dose of active substances, tea extracts consumed frequently and in fairly substantial amounts may play an important role in disease prevention. The possibility of using them as a biopreservative was analysed by, among others, Sakanaka, Raj Juneja and Taniguchi [2000], who showed that polyphenolic compounds present in green tea inhibit the development of

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thermophilic spore-forming bacteria *Bacillus stearothermophilus* and decrease the heat resistance of *Bacillus stearothermophilus* and *Clostridium thermoaceticum* spores. Tang et al. [2001] observed the effect of tea catechins on slowing down the oxidation of fats in raw meat. Diker and Hascelik [1994] reported that extracts of both black and green tea inhibit *in vitro* the growth of *Helicobacter pylori* strains. This was confirmed for Lung Chen tea by Yee and Koo [2000]. Toda et al. [1989] found inhibitive properties of black, green and pu-erh teas in relation to *Staphylococcus aureus*, and *Staphylococcus epidermidis*, and of black and green teas in relation to *Vibrio cholerae*, *Vibrio mimicus*, *Plesiomonas shigelloides*. Sakanaka et al. [1996] report that polyphenolic compounds isolated from green tea inhibit the growth and adherence of *Porphyromonas gingivalis* to human buccal epithelial cells. Furthermore, Hirasawa et al. [2002] in *in vivo* and *in vitro* investigations observed the beneficial effect of green tea catechins in the treatment of periodontal disease. The properties of tea which inhibit bacterial growth are mainly related to their polyphenolic components which are believed to act as antioxidants [Benzie and Szeto 1999, Lai Kwok Leung et al. 2001, Liebert et al. 1999, Roeding--Penman and Gordon 1997, Gow ChinYen and Hui Yin Chen 1995, Yildirim et al. 2000], and in some conditions as prooxidants [Cao et al. 1996, Gow ChinYen et al. 1997]. Polyphenols are believed to help prevent heart disease, mutagenesis, cancers and Parkinson`s disease [Hadi et al. 2000, Olthof et al. 2003, Pan et al. 2003, Yamada and Tomita 1994, Yang et al. 2001, Gow ChinYen and Hui Yin Chen 1995]. However, Othof et al. [2003] proved that colonic microflora convert most of the investigated dietary phenols obtained from black tea into metabolites with lower antioxidant power then their parent compounds, resulting in lesser antioxidant activity of the polyphenols in the human body than *in vitro*. As pointed out by Scalbert and Williamson [2000], the degree of intestinal absorption and character of the metabolites circulating in the plasma is determined by the chemical structure of the polyphenols.

The purpose of the present study was to check if, and to what extent, different kinds of tea (black, green and pu-erh) affect microorganisms significant for human health and whether these properties correlate with their antioxidant potential.

MATERIAL AND METHODS

Material

The following strains were used for investigation: Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum, Leuconostoc mesenteroides subsp. mesenteroides, Escherichia coli (2 strains), Staphyloccocus aureus, Salmonella enteritidis.

Extracts of green (2 brands), black and pu-erh tea, obtained from the local supermarket, were prepared by adding 100 ml of boiling distilled water to 2 g of tea and leaving them for 10 minutes to infuse. The infusion was then strained through a microfilter with a pore size of 0.20 μm (Nalge Nunc Internatinal, Canada).

Methods

Cultures of lactic acid bacteria strains were grown on liquid and solid MRS media (BTL, Poland), and those of other bacteria in nutrient broth and PCA (BTL, Poland). 5 ml of liquid, sterile, double concentration media was prepared in test tubes, to which

was added 5 ml of extract of each tea tested. The control sample contained an additional 5 ml of distilled water. The thus prepared media were inoculated with 0.5 ml 10^4 dilution of 20 hr culture of the tested strain and incubated at 30°C (*L. mesenteroides*, *L. plantarum*) and 37°C (the remaining strains). The number of bacteria in cultures was determined directly after preparation and at the 2nd and 6th hours of incubation by transferring respective dilutes to a double row of plates with adequate medium (PCA or MRS). The rate of bacteria proliferation in the analysed environment was assessed by calculating the generation time between the second and the sixth hour of incubation [Zalewski 1985].

The polyphenol content in the individual extracts was determined using the Folin-Ciocalteus reagent, based on the method described by Singleton and Rossi [1965]. The calibration curve was established for gallic acid. The results were expressed as gallic acid equivalents in mg/100 ml infusion.

Antioxidant properties were assessed by measuring the reducing power value, showing the ability of the extract to reduce Fe^{2+} to Fe^{3+} [Gow ChinYen and Hui Yin Chen 1995]. The reducing powers of diluted extracts (0.5%) were expressed as absorbance at 700 nm measured on spectrophotometer Cecil CE 9500 (Cecil Instruments, Cambridge England).

RESULTS AND DISCUSSION

The generation times for individual microorganisms between the second and sixth hour of incubation in the control test and in tests with tea extracts are presented in Table 1.

Table 1. Generation times of studied microorganisms on media with the additions of tea extracts and in the control sample, min

Tabela 1. Czasy generacji badanych szczepów drobnoustrojów na podłożu kontrolnym oraz z dodatkiem ekstraktów herbat, min

Microorganism tested Badany mikroorganizm	Generation time on control medium and with tea extract added Czas generacji na podłożu kontrolnym oraz z dodatkiem naparów				
	control kontrola	black czarna	pu-erh pu-erh	green I zielona I	green II zielona II
Lactobacillus rhamnosus	97	103	92	103	102
Lactobacillus casei	60	78	74	63	71
Lactobacillus plantarum	29	31	31	29	32
Leuconostoc mesenteroides	37	38	38	39	36
Escherichia coli	20	28	21	33	33
Escherichia coli	21	33	21	28	25
Salmonella enteritidis	23	43	26	29	30
Staphylococcus aureus	25	*	*	*	*

*Dying out of bacteria were found.
*Stwierdzono wymieranie bakterii.

The strongest inhibition of bacterial growth was shown in black tea and the weakest in pu-erh tea (Table 1). Both types are fully fermented [Jen-Kun Lin et al. 1998]. Similar results, not much lower than for black tea, were obtained for both green teas. Cheng--Chun Chou et al. [1999] report that the higher the degree of fermentation, the weaker the antimicrobial properties. The analysis carried out in this study does not confirm this conclusion. However, our choice of samples was random from among teas available on the market. Among the pathogenic microorganisms, *Staphylococcus aureus* proved to be very sensitive to the addition of extracts to the media. Black and green tea also slowed down the growth of *Escherichia coli* and *Salmonella enteritidis*. The latter was inhibited to a larger extent by black than by green tea. The information provided in the literature on these microorganisms is not entirely consistent. Cheng-Chun Chou et al. [1999] reported inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. by black tea, Paochung tea, green tea and dry tea flush. Yam et al. [1997] showed sensitivity to Japanese Sencha green tea in all 33 analysed strains of *Staphylococcus aureus* (including Meticillin resistant), 3 strains of *Salmonella spp*. and 8 out of the 20 analysed strains of *Escherichia coli.* Toda et al. [1989] report that none of the *E. coli* (enteroinvasive, enterohaemorrhagic, entheropathogenic, enterotoxic) tested by them was inhibited by any of the teas examined (black, green, pu-erh), while *Salmonella enteritidis* was inhibited by black tea but not by green or pu-erh. *Staphylococcus aureus* was sensitive to all the teas tested by these authors. Also West et al. [2001] showed the inhibiting effect of green tea on *Staphylococcus aureus*, whereas Yildirim et al. [2000] found no inhibitive effect of black tea extract on *Staphylococcus aureus* and *Escherichia coli.*

The effect of the tested extracts on LAB varied. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were not inhibited. Inhibition of *Lactobacillus rhamnosus* was negligible, while *Lactobacillus casei* proved to be more sensitive to the extracts of black and pu-erh tea and, to a lesser extent, to one of the green teas tested. The available literature does not provide many data on the effect of tea extracts on lactic acid bacteria; the data obtained are therefore difficult to compare.

In order to arrive at a more accurate characterization of the extracts tested and establish a link between their antioxidant and antimicrobial properties, the content of polyphenolic compounds and the reducing power of teas were analysed and presented in Table 2.

Table 2. Content of polyphenolic compounds and reducing power of tea extracts
Tabela 2. Zawartość polifenoli i siła redukująca ekstraktów herbacianych

Tea type Typ herbaty	Content of polyphenolic compounds mg/100 ml Zawartość polifenoli ogółem mg/100 ml	Reducing power (absorbance at 700 nm) Siła redukująca (absorbancja przy 700 nm)
Black – Czarna	124.2 ± 1.53*	0.905 ± 0.0138
Pu-erh – Pu-erh	98.4 ± 2.58	0.896 ± 0.0011
Green I – Zielona I	111.3 ± 1.53	0.920 ± 0.0435
Green II – Zielona II	135.3 ± 0.98	0.944 ± 0.0330

*Mean value ± standard deviation.
*Wartości średnie ± odchylenie standardowe.

The relative content of polyphenolic compounds was, in decreasing order, green II, black, green I, pu-erh. Differences in polyphenol content between both the green teas did not fully correspond to differences in antimicrobial properties. However, Muroi and Kubo [1993] in their studies on *Streptococcus mutans* found a more significant role was played by the flavour compounds in green tea than the polyphenols. Our findings possibly support this hypothesis to some degree.

The value for reducing power as an indicator of antioxidant properties does not fully correspond to the content of polyphenolic compounds or antimicrobial properties. Extracts of green tea, in particular II, proved to be the strongest antioxidants, while black tea and pu-erh were weaker. Lai Kwok Leung et al. [2001] believe that the flavins in black tea posses at least the same antioxidant potential as catechins present in green tea. On the other hand, Gow Chin Yen and Hui Yin Chen [1995] report that the strongest antioxidant properties are shown in semifermented tea and the weakest in fermented tea, with green teas in the middle. Benzie and Szeto [1999] found a strong correlation ($r = 0.956$) between total phenolic content and antioxidant capacity in tea. They also showed that of the teas they examined, the strongest antioxidant properties were found in green tea and the weakest in black tea.

CONCLUSIONS

Black and green tea may exert a modelling effect on the balance of the intestinal microflora, while pu-erh tea rather does not exhibit such properties. However, further studies are required to confirm this, particularly with regard to the lactic acid bacteria group. No significant relationship was found between antioxidant activity and antimicrobial properties in the tea extracts examined.

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WPŁYW NAPARÓW HERBAT NA WZROST WYBRANYCH BAKTERII ISTOTNYCH DLA UKŁADU POKARMOWEGO

Streszczenie. Napary herbaciane konsumowane regularnie i w dużych ilościach mogą odgrywać rolę w modelowaniu mikroflory jelit lub zapobieganiu chorobom bakteryjnym. W pracy badano wpływ różnych rodzajów herbat (czarnej, zielonej i pu-erh) na wzrost *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Escherichia coli*, *Staphylococcus aureus* i *Salmonella enteritidis.* Zależność pomiędzy właściwościami antyoksydacyjnymi i antybakteryjnymi określano, oznaczając zawartość związków polifenolowych i siłę redukującą naparów, natomiast szybkość wzrostu bakterii mierzono, obliczając czas generacji pomiędzy 2 i 6 godziną inkubacji. *Staphylococcu aureus* był najbardziej wrażliwy na dodatek herbat do podłoży. Pośród bakterii kwasu mlekowego *Lactobacillus casei* wykazywał największą wrażliwość głównie w stosunku do ekstraktów herbaty czarnej i pu-erh. Wartość siły redukującej nie w pełni korespondowała z zawartością polifenoli i właściwościami antymikrobiologicznymi ekstraktów.

Słowa kluczowe: herbata, właściwości antybakteryjne, LAB, siła redukująca

Accepted for print – Zaakceptowano do druku: 10.01.2008

For citation – Do cytowania: Michalczyk M., Zawiślak A., 2008. The effect of tea infusions on the proliferation of selected bacteria important for the human intestinal tract. Acta Sci. Pol., Technol. Aliment. 7(1), 59-65.