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Microbiological Purity of Tea and its Antimicrobial Activity

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1. Introduction

Tea is produced from leaves and non-developed buds of a tea shrub *Camelia sp. (thea),* which occur in two botanical varieties: *Camelia assamica* (L) and *Camelia sinensis* (L). Tea is cultivated and manufactured in many countries of the world [9], but the best conditions for its cultivation take place on mountain regions or close to tropical jungles, on acid soils at mean day temperature of 19ºC. The leading producers of tea in the world are India (black tea), Japan (green tea) and China (different sorts of tea). Depending on tea manufacturing methods tea is divided mainly on: the green and the black one. The process itself of the receiving of a dried tea material has many similar stages for both tea sorts but the black tea is additionally exposed to a process of fermentation [16].

Consuming of tea, which contains compounds with broad action spectrum, has according to many authors [4] a positive influence on a human organism. The mineral compounds, for example fluorine and manganese are responsible for a good functioning of a bone system. The organic compounds, such as vitamins A, B, F, K, P influence positively a functioning of sight and skin, are helpful in organism immunity and regeneration of nervous system, have an anti-bleeding effect and hold the right functioning of blood vessels [13]. Among alkaloids, theine has a stimulating action, theobromine – a diuretic action, and theophylline stimulates a heart activity [19]. Polyphenols, and especially flavonoids belonged to catechins (EGCG, ECG, EC and C) strengthen the defensive mechanism of an organism, have an anti-microbial, anti-carcinogenic and antioxidant action [1, 2, 10, 14] as well as reduce a risk of heart diseases [11]. Quercitin and flavonome reduce a lipids number in a blood [12]. The black tea contains moreover L-teanin, from which in a liver an ethylamine is formed. This compound plays in an organism a role of antigen, a recognition of which prepare the organism to defence against infections [15].

The aim of the research was an evaluation of microbial purity as well as a bacteria- and fungistatical activity of several sorts of tea.

2. Material and methods

The following material was investigated:

- vacuum-packed tea, containing 25 g of material in PE foil. There were a green and black tea of two manufacturers (“X” and “Y”);
- selected bacteria species inhabited or seasonally colonized alimentary canal, a namely: *Escherichia coli* (Escherich) – coliform bacteria (G-) isolated from water and *Bacillus subtilis* (Prażmowski) and *Bacillus cereus* (Fischer) – rod shaped bacteria (G+) isolated from food material, as well as commonly occurred fungus from genus *Penicillium sp.*

Evaluation of the tea microbial purity was made with a method of 10-fold dilution and using for a transfer a Koch’s method with a dilution of 10^{-2}. As a substratum for bacteria an agar and for fungi a Czapek’s nutrient medium was used. As an evaluation criterion a number of vegetative and sporulative forms of bacteria and fungi was taken. The experiment was arranged in six replications for each sort of tea. An identification of the cultivated bacteria was made under microscope and by help of mini-analyser of the firm bio Merieux and using the tests API 50 CHB and API ID 32 GN. A fungi identification was made on a basis of macro- and microscopic features.

The activity evaluation of tea in relation to *E. coli, B. subtilis, B. cereus* was performed with a disc-diffusion method on an agar nutrition medium and in relation to *Penicillium sp.* on Czapek’s nutrition medium. For this purpose from a dried tea material 5, 10 and 15% – water extracts (macerations and infusions) were prepared which were then used for soaking the filter discs with a diameter of 9 mm. A suspension of bacteria as well as spores and particles of mycelium of fungi with a density of 0,5º McFarland’s in sterile deionised water was prepared. The activity of the extracts in relation to bacteria was read out after 24 hours and in relation to *Penicillium sp.* after 5-th day culture taking as a criterion a size of an inhibition zone in mm. These values were expressed on diagrams as a deviation from an absolute control (H_2O = 0%). The experiment was established in six replications for each bacteria species, tea sort and the method of an extract preparation. The results obtained were statistically analysed with a single analysis of variance. When compared the obtained results, a linear correlation was applied.

3. Results

The researches have shown that the investigated tea sorts were contaminated with vegetative and sporulative forms of *Bacillus subtilis* and *B. pumilis* as well as of *Pantoea spp.* (table 1). Occurrence of *Rhizopus sp* was also observed. The largest number of vegetative forms of bacteria and their endospores were isolated from tea manufactured by "X". The general bacteria number in black tea amounted about $2,8 \times 10^3$, and in the green one 3×10^3 / 1g. The number of endospores in black tea amounted $5,0 \times 10^2$, and in the green one $1,0 \times 10^2$ /1g. The number of bacteria reared on tea manufactured by "Y" amounted respectively: for black tea 3×10^2, and for green one 2×10^2 /1 g.

Table 1. Microbiological contamination of tea
Tabela 1. Zanieczyszczenie mikrobiologiczne herbat

Tea sort and manufacturer	Number / 1g		Species / genus
	bacteria	endospores	
Black tea "X"	$2,8 \times 10^3$	$5,0 \times 10^2$	*Bacillus pumilis, B.subtilis, Pantoea sp.*
Green tea "X"	$3,0 \times 10^3$	$1,0 \times 10^2$	*Bacillus pumilis, Pantoea sp., Rhizopus sp.*
Black tea "Y"	$3,0 \times 10^2$	$1,0 \times 10^2$	*Bacillus pumilis, Rhizopus sp.*
Green tea "Y"	$2,0 \times 10^2$	$2,0 \times 10^2$	*Bacillus pumilis, Rhizopus sp.*

The analysis of biological activity of water extracts made from tea being under examination has shown that it depended on a sort of tea, mode of an extract preparation and its concentration as well as on the tested microorganism.

The mean activity of the tested tea sorts was greatest in relation to *Escherichia coli* and amounted about +29,7% in relation to control. Regardless of the remaining factors a greater efficacy have shown:

- extracts from green sorts of tea (+30,8%) in relation to the black ones (+28,3%);
- infusions (+27,1%) in relation to macerations (+20,4%);
- extracts in a highest concentration (+32,9%) than in a lowest one (+25,8%).

Taking into account all the tested factors (Fig. 1) it was proved the strongest bacteriostatic activity on *E. coli* of 15% infusions from black tea "Y" (+47,0%) and of 10% infusions from green tea "X" (+41,6%). The lowest efficacy in relation to *E. coli* proved 10% of macerations and 5% of infusions from black tea "Y" (respectively: +14,8% and +19,4%).

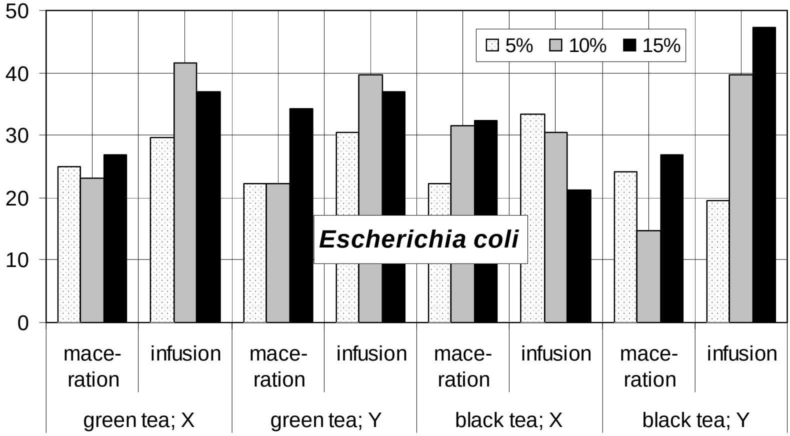

Fig. 1. Activity of several sorts of tea in relation to *Escherichia coli* depending on an extract preparation mode and its concentration (deviation from control)
Rys. 1. Aktywność herbat w stosunku *do Escherichia coli* w zależności od sposobu przygotowania wyciągu i koncentracji (odchylenie od kontroli)

The response variability (V%) of *Escherichia coli* on the tested tea extracts amounted 27,1% and was lower from those of *Bacillus subtilis* (V = 62,6%). No response similarity for these bacteria species on the factors investigated (an origin, sort of tea and an extract preparation mode) has been stated ($r=0,376 < r_{crit.}$.). Also the mean activity of the tested tea in relation to *Bacillus subtilis* was lower than in the case of *Escherichia coli* and amounted about +20,6%.

The investigations of tea activity in relation to ***Bacillus subtilis*** have shown that:

- the extracts made from green tea were more active (+27,3%) than from the black one (+14,1%);
- the mean efficacy of macerations and infusions has been established on the same level and amounted 16,5%,
- water extracts in a concentration of 15% were more active (+27,9%) than those in lower concentrations (10% – +20,9%; 5% – +13,1%).

Taking into account all the analysed factors it turned out that the most active in relation to *B. subtilis* were the highest concentrations of infusion and maceration from the green tea “X” (+53,7%; +38,5%) and 15% of maceration from black tea “X” (+37%). The lowest activity have proved however: 5% of maceration from green tea “Y” and 10% of maceration from black tea “Y” (+6,4%).

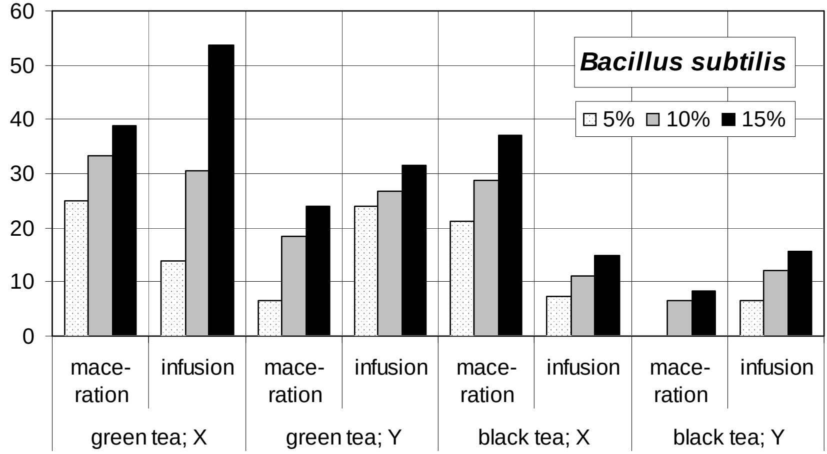

Fig. 2. Activity of several tea sorts in relation to *Bacillus subtilis* depending on an extract preparation mode and its concentration (deviation from control)
Rys. 2. Aktywność herbat w stosunku do *Bacillus subtilis* w zależności od sposobu przygotowania wyciągu i koncentracji (odchylenie od kontroli)

Although the response of *Bacillus cereus* was significantly consistent with those of *B. subtilis* (r = 0,573** > $r_{crit.}$, nevertheless the mean activity of the sorts of tea in relation to this species was the lowest (+11,3%). This species has shown the highest response variability to the tested factors (V = 148,4%). Independently of remaining factors the highest efficacy have shown:

- extracts from green tea (+22,1%), while the green ones have shown no activity in relation to this species;
- infusions (+12,1%) were more active than macerations (+5,9%);
- activity of extracts increased along with a growth of concentration.

Taking into account all the analysed factors the most strong action on *B. cereus* have shown the 15% and 10% infusions from green tea „Y" (+48,1% and +39,0%) and 10% and 15% infusions of green tea „X" (both +33,3%). The black tea has shown no activity in relation to *B. cereus.*

The response of *Penicillium sp.* has shown no correlation with the response of the tested bacteria species. The values of correlation coefficients, varied between r = 0,325 and 0,400 < r_{crit}. point out on merely a tendency to a positive dependence. The variability of response of *Penicillium sp.* (V= 41,3%) was lower than those of both species of *Bacillus* an lower than the response variability of *E. coli*. The mean activity of the tested tea sorts in relation to *Penicillium* amounted 22% and was similar to its activity on *B. subtilis.*

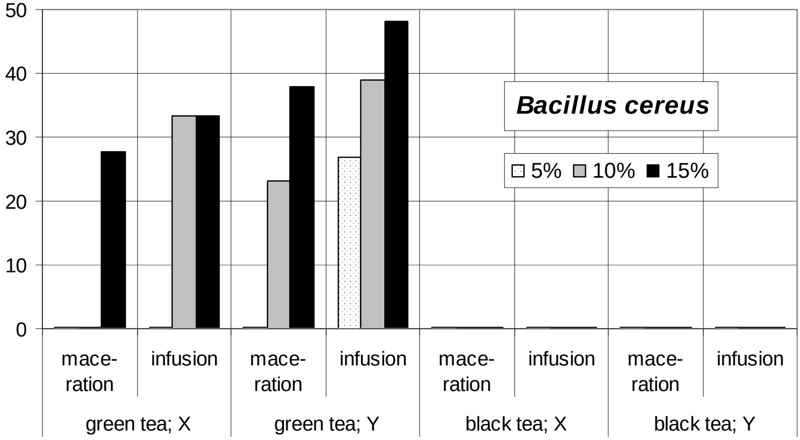

Fig. 3. Activity of several sorts of tea in relation to *Bacillus cereus* depending on an extract preparation mode and its concentration (deviation from control)
Rys. 3. Aktywność herbat w stosunku do *Bacillus cereus* w zależności od sposobu przygotowania wyciągu i koncentracji (odchylenie od kontroli)

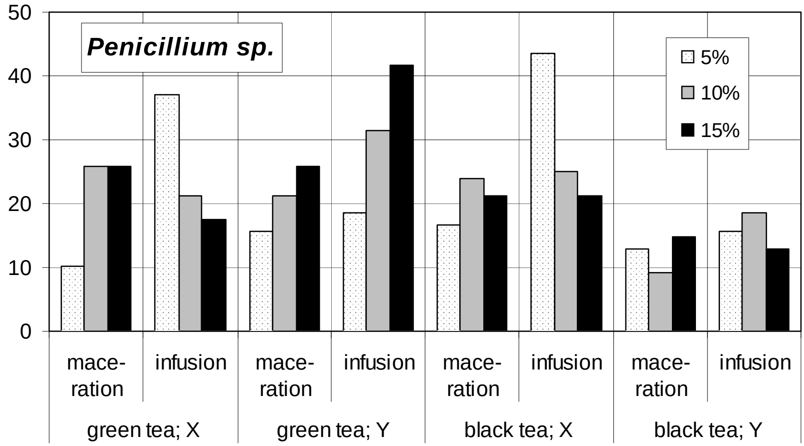

Fig. 4. Activity of several sorts of tea in relation to *Penicillium sp.* depending on an extract preparation mode and its concentration (deviation from control)
Rys. 4. Aktywność herbat w stosunku do *Penicillium sp.* w zależności od sposobu przygotowania wyciągu i koncentracji (odchylenie od kontroli)

The experiments have shown independently of other factors that:

- green tea (+24,3%) were more active than the black one (+19,6 %);

- infusions (+20,2%) demonstrated a higher efficacy than the macerations (+14,8%);
- extracts used in various concentrations have shown a similar action (+21,2 – +22,6%).

Taking into account the tested factors the highest activity in relation to *Penicillium* has shown an 5% infusion from a black tea „X" (+43,4%) and an 15% infusion from a green tea „Y" (+41,6%). The lowest activity has shown a 10% maceration from a black tea „Y" and a 5% maceration from green tea „X"(10,2%).

4. Discussion

A tea is a medium where different microorganisms can exist. Number and kind of the microorganisms in a final product depend to a large degree on a way of a raw material receiving, its processing and maintain of hygiene in manufacturing. A rich microflora is found on a surface and in plant tissues, what depends mainly on the environmental factors [5].

A soil is a primary factor that influences the microflora composition on a plant. Numerous microorganisms determining a soil fertility and its phytosanitary potential as well as a proper plant growth are found in a rhisosphere. It is estimated that a 15 cm deep soil layer on an area of 1 ha comprises about 4,5 tons of microorganisms biomass, and their number varies from several thousands to a several billions per 1g of a dry matter. To the soil dominants which can contaminate a plant raw material may be mentioned following representatives of the families: *Enterobacteriaceae*, *Flavobacteriaceae* and *Micrococcacea* and bacteria from genera: *Bacillus, Pseudomonas*, yeasts *Rhodotorula sp., Torulopsis sp.* and mould fungi *Aspergillus sp., Penicillium sp., Fusarium sp., Rhizopus sp.* [8].

A second factor influencing the microflora composition on plants are aerotransmitted micro-organisms as well as permanent inhabitants of a plant phyllosphera. Among these micro-organisms may be mentioned following genera: *Bacillus, Micrococcacus, Staphylococus,* yeast from the genera: *Rhodotorula, Candida, Torulopsis,* and spores of fungi *Penicillium, Aspergillus, Rhizopus, Mucor, Alternaria.* Their number is unstable and depends on a year season, place of dwelling and a plant development phase [21].

The third extremely important factor influencing the microflora composition of plant material are man-made pollutions occurring in a processing stage. A processing of tea leaves comprises several stages: withering (at a temperature about 50ºC, 3÷12 hours), rolling (34÷36ºC, 3÷5 times, 30 minutes each time), drying (85÷88ºC, 20÷30 min), sorting, packaging. The green tea leaves after a withering stage are roasted in high temperature and only then they are rolled. The black tea leaves are after withering stage subjected to a process of fermentation at a temperature about 100ºC during 4 hours [16]. The number of contaminating microflora on a tea should be due to this processing reduced. The only danger may be resulted from bacteria spores. No observing of a production hygiene in particular processing stages is a most often reason of a secondary product contamination [21].

The contaminating microflora inhabiting the tested dried tea material belong mainly to a mesophilous organisms, which have an optimal temperature range between 25 and 45ºC. In a process of drying and fermentation of tea leaves what occur in elevated temperatures, the number of microflora should be reduced. However the identified in the researches bacteria are mostly representatives of an organisms which form endospores (*Bacillus sp.*) and thus sustain many hours of heating [17]. The residues of this microflora in a tested material, can testify to a contamination of the plants already in a stage of harvesting. In the contrary to these organisms, bacteria from a genus *Pantoea ssp.* (syn. *Enterobacter agglomerans*) are rod-bacteria G(-) which do not form an endospores. Their presence in the tested material shows a secondary contamination, what may be occurred in a final stage of production. The contamination of tea with *Rhizopus sp.* can have also a secondary character especially when a storing of tea was not proper.

On the other hand it should be underlined that active substances occurred in tea may reduce growth and development of microorganisms. The researches of other authors have shown that the highest antimicrobial activity reveal catechins of a green tea: EGCG and ECG as well as theaflavin in a black tea [20]. These substances were active in relation to *E. coli, Staphylococcus aureus*, bacteria from genus *Salmonella, Shigella, Clostridium* and *Bordetella*. In the same time it was proved [3, 18] that a higher resistance on polyphenols damaging cell-membranes show bacteria G(-) than G(+).

The conducted own research has shown that the activity of tested tea sorts depended on their kind, mode of extract preparation, its concentration and used species tested. The effect of active substances occurred in dried tea material is revealed only after preparing of extracts. It may be explained with a presence of some antimicrobial organisms in a tea material. The tea contaminating bacteria were probably in significant degree reduced by polyphenols already in a stage of an extract preparation. The changes of their number and especially of *E. coli* may be also resulted from a presence of other competitive microorganisms producing antibiotic substances (*Bacillus* and *Penicillium*). As a consequence of this effect a modification of a microorganisms response on these substance may occur.

The green sorts of tea have shown in the research a higher antimicrobial activity than the black ones. These differences result probably from a different content of active substances in these tea sorts. For example polyphenols contained in green tea make up 15÷35% of a dry matter, while their content is reduced during a process of fermentation in a case of black tea (as result of chemical changes leading to an arise of theaflavin) and amounts 3÷10% [7].

The research also shown that activity of extracts and included substances in relation to microorganisms depends on a mode of its preparation. The infusions were more effective than macerations and this effectiveness increased in general along with a concentration increase. Similarly a prophylactic effect of tea and efficacy of active substances depend on a boiling mode. The recommended methods of a tea preparation mention about a water temperature what varying about 100°C positively influence the taste and healthy virtues of this beverage [20].

The results of the research point out that some organic compounds (for example catechins) contained in tea could be utilized as an alternative in relation to a chemical preservation of food [6]. The conducted investigations are concentrated on an evaluation of an effect of different plant extracts with antioxidizing properties as additives for meat, potatoes and poultry in order to reduce the negative chemical and biological reaction occurred during a food storing [10]. Of course there are long-term investigations since an utilizing of these substances is connected with an assessment of effective doses and their impact on organoleptic and healthy properties of the preserved food.

5. Conclusions

- The investigated tea sorts were contaminated with vegetative (from 2×10^2 to 3×10^3 per 1 gram) and sporulative forms of bacteria (from $1{,}0 \times 10^2$ to $5{,}0 \times 10^2$ per 1 gram) *Bacillus subtilis, B. pumilis* and rod-shaped bacteria from genus *Pantoea*. There were identified a presence of the fungus *Rhizopus* as well.
- The activity of tea sorts in relation to microorganisms depended mainly on:
    - a kind of tea and a mode of extract preparation and its concentration. The green sorts of tea were characterized with a higher activity in relation to microorganisms than the black ones; the infusions were more active than macerations and the biological activity increased along with an extract concentration;
    - a microorganism tested – tea extracts reduced most strongly growth and development of *Escherichia coli* but their activity on *B. cereus* was most weak.

References

1. **Clifford M. N., Bruschi L., Copeland E.L.:** *Unexpected hyperchromic interactions during the chromatography of theafulvins and simple flavonoids*. Food Chemistry, Volume: 67, Issue 2, November 1999. 143÷146.
2. **Clifford M. N., Copeland E.L., Williams C.M.:** *Preparation of epigallo-catechin gallate from commercial green tea by caffeine precipitation and solvent partition*. Food Chemistry, Volume: 61, Issue 1-2, January1998. 81÷87.
3. **Chosa H.:** *Antibacterial and microbicidal activities of tea and catechins against.* J. Jpn. Assoc. Inf. Dis., Volume: 66, 1992. 606÷610.
4. **Dreosti I.E.:** *Bioactive ingredients: antioxidants and polyphenols in tea*. Nutrition Rev., Volume 54, November 1996. 51÷58.
5. **Gottlieb M.:** *Czynniki determinujące zdolność bakterii z rodzaju Pseudomonas do kolonizacji systemu korzeniowego roślin*. Postępy Mikrobiologii, 41, 3, 2002. 277÷297.
6. **Horubała A.:** *Nowoczesne metody utrwalania żywności – tendencje rozwojowe.* Materiały Konferencji Naukowej, Warszawa 1997. 7÷11.
7. **Keith, Wang, Huafu, Provan, Gordon:** *Tea flavonoids: their functions, utilisation and analysis*. Trends in Food Science and Technology. Volume 11, Issue 4-5, April 5, 2000. 152÷160.
8. **Kozdrój J.:** *Różnorodność mikroorganizmów glebowych w świetle badań molekularnych*. Postępy Mikrobiologii, 43, 4, 2004. 375÷398.
9. **Lucińska A.:** *Ekstrakt z zielonej herbaty. Lecznicza siła polifenoli.* Wydawnictwo Remedium, Wrocław 2002. 91÷95.
10. **Luo M., Kannar K., Wahlqvist M.L., O'Brien R.C.:** *Inhibition of LDL oxida-tion by green tea extract*. The Lancet, Volume 349, Issue 9048, February 1, 1997. 360÷361.
11. **Mukamal K. Maclure M., Muller J.E., Sherwood J.B., Mittleman M.A.:** *Caffeinated coffee consumption and mortality after acute myocardial infarction*. ACC Current Journal Reviev, Volume 13, Issue 9, September 2004. 10÷11.
12. **Rice-Evans C.A., Catherine A., Miller Nicholas J., Paganga, George:** *Structure-antioxidant activity relationships of flavonoids and phenolic acids*. Free Radical Biology and Medicine, Volume 20, Issue 7, 1996. 933÷956.
13. **Różański H.S.:** *Witaminy i prowitaminy.* Ośrodek Badawczo-Rozwojowy LNB Polska, Poznań – Kiszkowo, http://www.luskiewnik.gower.pl/cit (dostęp 31.03.2005).
14. **Ostrowska J., Stankiewicz A., Skrzydlewska E.:** *Antyoksydacyjne właściwości zielonej herbaty.* Brom. Chem. Toksykol., 34, 2, 2001, 131÷140.
15. **Stanisławska J., Interewicz B., Olszewski W.L.:** *Odpowiedź leukocytów gospodarza na antygeny bakteryjne*, Postępy Mikrobiologii, 42 (3), 2003. 301÷317.
16. **Świderski F.:** *Towaroznawstwo żywności przetworzonej*. SGGW Warszawa 1999.
17. **Schlegel H.:** *Mikrobiologia ogólna*. Wyd. Naukowe PWN Warszawa 2000.
18. **Xu, Yong, Chen, Cao, Jin:** *Chemopreventive Effects of Green and Black Tea on Pulmonary and Hepatic Carcinogenesis*. Fundamental and Applied Toxicology, Volume 29, Issue 2, February 1996. 244÷250.
19. **Wu C., Christine D., Wei, Guo-Xian:** *Tea as a functional food for oral health*. Nutrition, Volume 18, Issue 5, May 2002. 443÷444.

20. **Waszkiewicz-Robak B.:** *Porównanie zawartości kofeiny i garbników w herbatach zielonych i czarnych.* Żywienie Człowieka 29, Metabolizm - supl. 451, 2002.
21. **Żakowska Z, Oberman H., Pietkiewicz A.:** *Surowce żywnościowe pochodzenia roślinnego jako źródło zakażeń mikrobiologicznych.* Mat. Konferencji Naukowej, „Bezpieczeństwo mikrobiologiczne produkcji żywności", Warszawa 1997.

Czystość mikrobiologiczna herbat i ich aktywność przeciwdrobnoustrojowa

Streszczenie

W badaniach oceniono czystość mikrobiologiczną suszu wybranych herbat zielonych i czarnych oraz określono ich działanie na bakterie *Escherichia coli, Bacillus subtilis, Bacillus cereus, a także grzyb z rodzaju Penicillium.*

Ocenę czystości mikrobiologicznej herbat wykonano metodą kolejnych 10-krotnych rozcieńczeń i posiewu metodą zalewową Kocha. Jako podłoże dla bakterii użyto agar odżywczy, a dla grzybów podłoże Czapka. Kryterium oceny była ogólna liczba mikroorganizmów oraz liczba endospor. Identyfikację wyhodowanych bakterii wykonano za pomocą analizatora mini API firmy bio MERIEUX stosując testy API 50 CHB i API ID 32 GN, a identyfikację grzybów na podstawie cech makro- i mikroskopowych.

Ocenę aktywności wyciągów wodnych herbat (maceratów i naparów), zastosowanych w koncentracji 5%, 10%, 15%, w stosunku do *E. coli, B. subtilis, B. cereus* i *Penicillium* określano metodą dyfuzyjno – krążkową. Gęstość zawiesiny bakterii wynosiła 0,5° Mc Farlanda. Aktywność wyciągów odczytano po 24 godzinach hodowli (w przypadku bakterii) i 5 dniach (w przypadku grzybów) przyjmując jako kryterium wielkość strefy zahamowania wzrostu. Na wykresach wartości przedstawiono w % skuteczności kombinacji kontrolnej.

Badania wykazały, że herbaty były zanieczyszczone formami wegetatywnymi i endosporami bakterii *Bacillus subtilis, B. pumilis* i przez pałeczki *Pantoea spp.* Zaobserwowano także obecność *Rhizopus* sp. Poziom skażenia herbat dwóch producentów był odmienny. Największą liczbę bakterii izolowano z herbat firmy „X". Ogólna liczba bakterii w herbacie czarnej wynosiła $2{,}8 \times 10^3$, a w herbacie zielonej 3×10^3 w 1 g. Endospory występowały najliczniej w herbacie czarnej ($5{,}0 \times 10^2$ /1 g). Liczba drobnoustrojów w herbacie czarnej firmy "y" wynosiła 3×10^2, a w zielonej - 2×10^2.

Aktywność herbat w stosunku do drobnoustrojów zależała od rodzaju herbaty, sposobu przygotowania wyciągu i jego koncentracji. Wykazano, że: herbaty zielone miały wyższą aktywność w stosunku do drobnoustrojów (26,1%) niż herbaty czarne (15,5%); napary (18,9%) były skuteczniejsze od maceratów (14,4%); wyciągi w koncentracji 15% działały najsilniej (25,5%). Spośród badanych mikroorganizmów najwyższą wrażliwość na zastosowane wyciągi wykazywał gatunek *E.coli* (29,5%), a najniższą *B.cereus* (11,3%).