

## Резюмета на научните публикации на доц. д-р Георги Георгиев Беев

Представени за участие в конкурс за заемане на академичната длъжност „доцент“ по научна специалност „Екология и опазване на екосистемите“, област на Висше образование

4. Природни науки, математика и информатика, професионално направление

4.3. Биологически науки

1. Vachkova, E., V. Petrova, N. Grigorova, Z. Ivanova, **G. Beev**. 2023. "Evaluation of the Anticancer and Probiotic Potential of Autochthonous (Wild) *Lacticaseibacillus paracasei* Strains from New Ecological Niches as a Possible Additive for Functional Dairy Foods" *Foods* 12, no. 1: 185. <https://doi.org/10.3390/foods12010185>. ISSN: 23048158, (IF2021 = 5.561) (Q1)

### Abstract

Probiotics such as *Lactobacillus* spp. could modulate the intestinal microbiota composition, supporting gastrointestinal tract barrier function and benefiting human health. To evaluate the anticancer and probiotic properties of potentially active autochthonous *Lacticaseibacillus paracasei* strains on proliferating and differentiated enterocytes, human colon adenocarcinoma cell line HT29 was used as a model. The lactic acid bacteria (LAB) were isolated from new ecological niches—mountain anthills populated by redwood ants (*Formica rufa* L.). Human colorectal adenocarcinoma cells (HT29, ATCC, HTB-38™) were treated for twenty-four hours with supernatants (SNs) derived from four strains of *Lacticaseibacillus paracasei*: P4, C8, C15 and M2.1. An MTT assay, alkaline phosphatase activity, IAP, Bax and Bcl-2 gene expression analysis (RT-qPCR) and the Bax/Bcl-2 ratio were evaluated. The MTT assay revealed that the observed effects varied among groups. However, 10% neutralized supernatants from P4, C8, C15 and M2.1 strains did not show cytotoxic effects. In contrast to non-differentiated cells, a significant ( $p < 0.001$ ) rise in ALP activity in all treatments, with an average of 18%, was established in differentiated cells. The IAP expression was remarkably downregulated in the differentiated M2.1 group ( $p < 0.05$ ) and upregulated in the non-differentiated P4 ( $p < 0.05$ ) and M2.1 ( $p < 0.05$ ) groups. The Bax/Bcl-2 quantity expression ratio in P4 was significantly ( $p < 0.05$ ) upregulated in proliferating cancer cells, but in P4- and M2.1-differentiated cells these values were downregulated ( $p < 0.05$ ). The obtained results indicate that the isolated *L. paracasei* strains possess anticancer and probiotic properties and could be used as additives for functional dairy foods and thus benefit human health.

**Keywords:** symbiotic microbial products; non-differentiated and differentiated HT29 cell line; alkaline phosphatase activity; IAP gene expression; Bax/Bcl-2 ratio

2. Borisova, V., G. Kostadinova, G. Petkov, D. Dermendzhieva, **G. Beev** (2022). An Assessment of Two Types of Industrially Produced Municipal Green Waste Compost by Quality Control Indices. *Applied Sciences*, 12, (20): 10668. <https://doi.org/10.3390/app122010668>. ISSN: 20763417, (IF2021 = 2.838) (Q2).

#### **Abstract**

Municipal green waste (MGW) has significantly increased with the development of urban green areas, and its utilization by composting is a good alternative to solve the problem. This paper presents the results from the quality assessment of two industrial composts (from the composting facility of a regional nonhazardous waste landfill) based on their physicochemical properties, hygienic safety (microbiological parameters), fertilizing potential (by fertilizing index, FI) and heavy metal polluting potential (by clean index, CI). Compost 1 (C1) was made from MGW (100%) and Compost 2 (C2) was made from MGW (75%) and discarded green peppers (25%). The evaluation of physicochemical parameters was conducted according to Bulgarian Standards (BDS) methods and microbiological analysis using selective, chromogenic detection systems. It was found that the EC, P, K, Mg, Cu, Cr and Ni were lower for C1 ( $p < 0.05$ – $0.001$ ). On the other hand, Pb concentration was higher compared to C2 ( $p < 0.001$ ); the concentrations of Cd, Hg and the *E. coli* were very low for both composts; presence of *Salmonella* was not detected. The estimated quality indexes (FI and CI) classified C1 as Class B compost (very-good-quality compost with medium fertilizing potential) and C2 as Class A compost (best-quality compost with high soil fertility potential and low heavy metal content). The C1 and C2 composts meet the requirements of EU and Bulgarian legislation and can be used as soil fertilizers.

**Keywords:** green waste; industrial composting; compost; clean index; fertilizing index; quality

3. Yordanova, R., Z. Yaneva, D. Gencheva, **G. Beev** (2022). Antimicrobial Resistance Distribution and Quorum-Sensing Regulation of Enterococcal Strains, Isolated from Hospitalized Patients. *Applied Sciences*, 12, (17): 8735. <https://doi.org/10.3390/app12178735>. ISSN: 20763417, (IF2021 = 2.838) (Q2).

#### **Abstract**

Background: Enterococci are intrinsically resistant/tolerant to various antimicrobial agents and can also acquire and combine different mechanisms of resistance, including quorum-sensing regulation, to most active compounds, which makes enterococcal infection treatment even more challenging. The aim of this study was to evaluate the pattern of antimicrobial resistance and to analyze the frequency of quorum-sensing *asa1* and *esp* genes in clinical isolates representing the genus *Enterococcus*. Methods: Multiplex PCR assays were performed for the identification of 110 enterococcal isolates and the determination of their antibiotic susceptibility and the presence of *asa1/esp* genes. Additionally, the antibiotic resistance of the isolates was tested by the Kirby–Bauer disk diffusion method. Results: 90% of the isolates were identified as *Enterococcus faecalis*

and 10% as *Enterococcus faecium*. Quorum-sensing regulation genes were present in 109 isolates. Aminoglycoside (aac(6′)/aph(2′′)-, quinolone (emeA)-, β-lactams (TEM)-, and vancomycin (vanA)-resistance genes were detected in 108 isolates. All of the isolates tested were vanB negative. According to the Kirby–Bauer method, 39% of the isolates expressed multidrug resistance (MDR) and 33% of the MDR *E. faecium* were vancomycin-resistant. Conclusion: The large percentage of MDR enterococci possessing *asa1/esp* genes indicated a possible connection between quorum-sensing regulation and drug resistance. Therefore, the regular monitoring of the antimicrobial resistance of *Enterococcus* spp., and the identification of virulence factors are needed. It is also important to prevent host colonization through the elimination of factors leading to the expression of quorum-sensing genes.

**Keywords:** antimicrobial resistance; quorum-sensing; regulation genes; *Enterococcus* spp.; nosocomial infections

4. Yaneva, Z., G. Beev, N. Rusenova, D. Ivanova, M. Tzanova, D. Stoeva, & M. Toneva (2022). Antimicrobial Potential of Conjugated Lignin/Morin/Chitosan Combinations as a Function of System Complexity. *Antibiotics*, 11(5), 650. <https://doi.org/10.3390/antibiotics11050650>. ISSN: 01266039, (IF2021 = 5.222) (Q1).

### Abstract

As natural biopolymers, chitosan and lignin are characterized by their good biocompatibility, high biodegradability and satisfactory biosafety. The active polymers' functional groups are responsible for the potential of these biomaterials for use as carrier matrices in the construction of polymer–drug conjugates with prospective applicability in the fields of medicine, food and agriculture—subjects that have attracted attention in recent years. Hence, the aim of this research was to place substantial emphasis on the antimicrobial potential of flavonoid–biopolymer complex systems by assessment of the probable synergetic, additive or antagonistic effects arising as a function of systemic complexity. The joint implementation of morin, chitosan and lignin in conjugated two- and three-component systems provoked species-dependent antimicrobial synergistic and/or potentiation effects against the activity of the tested bacterial strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and the clinical isolate *Bacillus cereus*. The double combinations of morin–chitosan and morin–lignin resulted in a 100% increase in their inhibitory activity against *S. aureus* as compared to the pure biocompounds. The inhibitory effects of the three-component system, in decreasing order, were: *S. aureus* (IZ = 15.7 mm) > *P. aeruginosa* (IZ = 15 mm) > *B. cereus* and *E. coli* (IZ = 14 mm). All tested morin-containing two- and three-component systems exhibited clear and significant potentiation effects, especially against *S. aureus* and *B. cereus*. The results obtained are a prerequisite for the potential use of the studied conjugated lignin–morin–chitosan combinations in the construction of novel drug-carrier formulations with improved bioactivities.

**Keywords:** morin; chitosan; lignin; antibacterial activity; combined systems

5. Yaneva, Z., E. Simeonov, N. Rusenova, D. Ivanova, G. Nikolova, Y. Karamalakova, C. Chilev, and **G. Beev** (2022). Flavonoids Extraction Kinetics, Antimicrobial Activity and Radical Scavenging Potential of Bulgarian Woundwort (*Solidago virgaurea* L.). *Separations*, 9(2):27. <https://doi.org/10.3390/separations9020027>. ISSN: 22978739 (IF2021 = 3.344) (Q3).

#### **Abstract**

The medicinal plant woundwort (*Solidago virgaurea* L.) characterizes by diuretic, antimutagenic, anti-inflammatory activity and it has been applied for urinary tract, nephrolithiasis and prostate disorders treatment. The aim of the present study was to analyze the extraction kinetics of catechin, epigallocatechin and quercetin from Bulgarian woundwort extracts, to assess the antibacterial potential of the medicinal plant extracts against four bacterial strains (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus cereus*), their antioxidant capacity and radical scavenging potential. The concentrations of the flavonoids in the extracts obtained at different extraction conditions (solvent, temperature, extraction time) were determined by newly-developed by the scientific team RP-HPLC-PDA methodologies. The agar well diffusion method was applied to evaluate the antibacterial activity of the plant extracts. The 70% EtOH extracts at 20 °C displayed significantly higher antibacterial activity against the foodborne pathogenic bacteria *S. aureus* and *P. aeruginosa* as compared to the 70% and 98% EtOH extracts at 30 °C and 20 °C, respectively. The medicinal plant exhibited satisfactory antioxidant potential and radical-scavenging activity.

**Keywords: woundwort; catechin; epigallocatechin; quercetin; antimicrobial activity; antioxidant potential**

6. Dinev T., N. Rusenova, K. Velichkova, **G. Beev** (2022). Antimicrobial potential of eleven *Lacticaseibacillus paracasei* strains isolated from mountain anthills. *Bulgarian Journal of Agricultural Science*, 28(5), 949-955. ISSN 2534-983X – online, (**SJR 2021 = 0.25**) (**Q3**).

#### **Abstract**

Probiotics with antimicrobial activity are important alternative to antibiotics, which are ever more restricted because of the developing microbial resistance and some adverse effects following frequent application. The aim of the present study is to determine the antibacterial and antifungal activity of supernatants of eleven *Lacticaseibacillus paracasei* strains (FR1-11) isolated from mountain anthills and identified by Amplified Ribosomal DNA Restriction Analysis (ARDRA). Antimicrobial activity was determined against reference strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Penicillium chrysogenum*, *Aspergillus niger*, *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Fusarium oxysporum*, *Fusarium graminearum* and clinical isolates of *Bacillus cereus*, *Listeria monocytogenes* and

*Salmonella enteritidis* using agar well diffusion method. All strains of *Lacticaseibacillus paracasei* inhibited the growth of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteritidis* and *Penicillium chrysogenum*, 10 strains – *Pseudomonas aeruginosa*, 9 strains – *Aspergillus carbonarius*, 6 strains – *Fusarium oxysporum*, 5 strains – *Escherichia coli*, and 3 strains – *Bacillus cereus*. There are no active strains against *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus parasiticus* and *Fusarium graminearum*. The highest antibacterial activity was measured against *Pseudomonas aeruginosa* with 14.7 mm inhibition zone of FR3 strain. The largest zones of inhibition against fungal strains were 12 mm, determined by the activity of FR2 and FR4 strains against *Penicillium chrysogenum*. As a whole, the supernatants of *Lacticaseibacillus paracasei* strains showed higher activity against bacterial strains compared to fungal strains.

**Keywords: antibacterial; antifungal; ARDRA; *Lacticaseibacillus paracasei***

7. Dermendzhieva, D., T. Dinev, G. Kostadinova, G. Petkov, **G. Beev** (2021). Agro-ecological characterization of vermicomposted sewage sludge from municipal and poultry enterprise wastewater treatment plants. *Sains Malaysiana*, 50(8), 2167-2178. ISSN: 01266039, (IF2021 = 1.006) (Q2).

### **Abstract**

The purpose of this study was to make an agro-ecological characterization of vermicompost (VC) produced from sewage sludge (SS). As a substrate, SS from municipal and poultry meat processing enterprise wastewater treatment plants (MTP and PTP, respectively) was utilized. The substrates were vermicomposted by Red Californian earthworm (*Lumbricus rubellus*) for 120 days. For VC quality assessment, 19 physicochemical and 6 microbiological parameters were used. The evaluation of physicochemical parameters was done according to ISO standard methods and microbiological analysis-by plating 1 mL of sample dilutions on selective, chromogenic culture medium sheets. It was found that the vermicompost from MTP (VC-M) had higher levels of EC, mineral elements (N, P and K compounds in forms available to plants), heavy metals (Mn, Cu, Zn, Cr, Ni, Pb, Cd) and coliforms, and lower levels of pH, TOC, C/N ratio, Fe, total plate count (TPC), Enterobacteriaceae and Salmonella spp. counts compared to VC from PTP (VC-P). During the vermicomposting process, the substrates from both wastewater treatment plants (TPs) showed similar trends towards decrease in pH, TOC, N-NH<sub>4</sub><sup>+</sup>, C/N ratio, TPC, coliforms, *E. coli*, Enterobacteriaceae and Salmonella spp. counts, whereas the opposite trends were established for EC, TKN, N-NO<sub>3</sub><sup>-</sup>, TP, P<sub>2</sub>O<sub>5</sub>, TK, and K<sub>2</sub>O values. The vermicomposting had a negligible effect on heavy metal concentrations. In the final substrates *E. coli* were not detected, while the bacterial spore forms (*Clostridium perfringens*) were not eliminated. The final substrates cannot be used as fertilizers or soil amendments because of the presence of Salmonella spp. and *C. perfringens* over the permissible limits according to EU and Bulgarian regulations.

8. Dinev, T., M. Tzanova, N. Rusenova, N. Grozeva, M. Gerdzhikova, **G. Beev** (2021). Antimicrobial and antioxidant potential of methanolic extracts from different parts of *Stevia rebaudiana* Bertoni cultivated in Bulgaria. *Sains Malaysiana*, 50(9), 2641-2651. ISSN: 01266039, **(IF2021 = 1.006) (Q2)**.

#### **Abstract**

*Stevia rebaudiana* Bertoni is a plant species, which is frequently used not only as a sweetener, but also for its antibacterial and antioxidant properties. Nowadays, there are a large number of studies on the antimicrobial and antioxidant activity of *S. rebaudiana* leaves, but there are almost no data about the antimicrobial and antioxidant potential of extracts from the other parts of *S. rebaudiana*. The aim of the present study is to provide data of the antibacterial and antioxidant potential of methanolic extracts from different parts of *S. rebaudiana* (flowers, leaves, stems, rhizomes, and tubers) cultivated in Bulgaria. Antibacterial activity of the extracts against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* was evaluated by agar well diffusion method, rutin content - by HPLC method, total phenolic content and radical scavenging potential - by UV-Vis analysis. *S. rebaudiana* extracts demonstrated antibacterial activity mainly against *S. aureus* - flower extracts expressed the highest activity, followed by the leaf and stem extracts. Only flower and leaf extracts demonstrated very low antibacterial activity against *B. cereus*. *S. rebaudiana* extracts did not show any antibacterial activity against *E. coli*. Methanolic extracts of this plant are rich in antioxidants. The highest concentrations of rutin and total phenols were found in the rhizomes of the plants, followed by the leaves, tubers, flowers, and stems, which corresponded to the radical scavenging potential of the same plant part. Comparisons between Trolox equivalents and gallic acid equivalents in different parts of *S. rebaudiana* on one hand, and Trolox equivalents and rutin concentration on the other hand showed a positive dependence and high values of the Pearson correlation- 0.9612 and 0.9707, respectively. The most important part of *S. rebaudiana* with medicinal significance (the leaves) has both comparatively good antibacterial activity and high antioxidant content, although the flowers and rhizomes expressed higher antibacterial and antioxidant activity, respectively. The experimental results imply that the cultivation area and climatic conditions of Bulgaria are very suitable for cultivation of *S. rebaudiana* plants with high content of antioxidants.

**Keywords:** Antimicrobial; antioxidant; methanolic extracts; plant parts; *Stevia rebaudiana*

9. Dinev T., M. Tzanova, K. Velichkova, D. Dermendzhieva, **G. Beev** (2021). Antifungal and antioxidant potential of methanolic extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kützing. *Applied Sciences*, 11(11), 4745. ISSN: 20763417, **(IF2021 = 2.838) (Q2)**.

**Abstract.** Plant extracts are an important alternative to antibiotics, which are ever more restricted because of their developing microbial resistance and some adverse effects that have been observed following frequent application. The aim of the present study was to determine the antifungal and antioxidant activity of the methanolic extracts of *Acorus calamus*, *Chlorella vulgaris*, *Lemna minuta* and *Scenedesmus dimorphus*. The antifungal activity of the extracts against strains of *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus carbonarius*, *Fusarium graminearum*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Alternaria alternata* was evaluated via the agar well diffusion method. The antioxidant activity of the extracts was measured through the determination of three parameters—total phenolic content, total flavonoid content and radical scavenging potential (determined through UV/Vis analysis). *A. calamus* extracts had the highest antimicrobial activity against eight fungal strains, followed by the *C. vulgaris*, *L. minuta* and *S. dimorphus* extracts, which were inhibitory against two to three strains. Among the extracts from the species studied, the extract from *S. dimorphus* showed the highest antioxidant potential, as determined via the DPPH (1,1'-diphenyl-2-picrylhydrazil-radical) method. This correlated to its high total phenolic and flavonoid content. From *A. calamus* and *L. minuta*, methanolic extracts were obtained that exhibited similar values of the aforementioned parameters, followed by *C. vulgaris* extracts, which showed the lowest antioxidant activity. Based on the Pearson correlation coefficients, the impacts of the total phenolic content and the total flavonoid content on radical scavenging capacity are similar, and flavonoids were a significant part of the total phenolic compounds extracted from the plant materials studied.

10. **Beev, G.**, M. Michaylova, T. Dinev, N. Naydenova, M. Tzanova, Z. Urshev (2021). ARDRA analysis on biodiversity of lactobacilli isolated from Bulgarian raw buffalo milk. *Acta Microbiologica Bulgarica*, 37(1), 22-26. ISSN:02048809 (**SJR 2021 = 0.12**) (**Q4**).

**Abstract.**

Lactic acid bacteria are widespread in nature and occur naturally as indigenous microflora in raw milk. Considering that buffalo milk is an excellent medium for the growth of a large variety of lactic acid bacteria, the aim of this study was the isolation of *Lactobacillus* spp. strains from raw buffalo milk originating from different areas and their species identification, using Amplified Ribosomal DNA Restriction Analysis (ARDRA) and a set of five reference strains of the most frequently isolated *Lactobacillus* species.

From the analysis of the patterns generated after treatment with *Hae*III it was found that 24 (88.8%) of the isolates had profiles that matched the reference 16S rDNA of *Lactobacillus casei*. The restriction profiles of the remaining three isolates (12.2%) did not match any of the reference strains and they were identified by API 50 CHL as *Lactobacillus fermentum*. This indicates that *Lactobacillus casei* is highly adaptive and dominates in raw buffalo milk regardless of the climatic conditions and the method of raising animals.

**Key words:** *Lactobacillus casei*, *Lactobacillus fermentum*, identification, ARDRA, API.

11. Gencheva, D. and **G.Beev** (2021). Molecular Identification of *Fusarium* spp. Isolated from Wheat Grain Based on Sequencing of Internal Transcribed Spacer (ITS) Region. *Acta Microbiologica Bulgarica*, 37(1), 27-33. ISSN:0204-8809 (**SJR 2021 = 0.12**) (**Q4**).

**Abstract.**

Molecular identification via Internal Transcribed Spacer region (nrDNA-ITS) sequencing of *Fusarium* spp. isolates from wheat originated from Stara Zagora region, were performed for the first time in Bulgaria. A total of 60 wheat samples (*Triticum aestivum*) were morphologically identified at the genus level as *Fusarium* spp. in advance. The rDNA-ITS region of all isolates was successfully amplified and the PCR products obtained were directly sequenced. After a comparison of detected sequences with NCBI database, members of three different fungal genera (*Fusarium*, *Chaetomium*, and *Alternaria*) were identified. Among *Fusarium* isolates, the *F. tricinctum* was prevailing, followed by *F. poae*. A total of three isolate *F. proliferatum*, *F. graminearum* and *F. equiseti* were presented with a single probe. The lowest genetic distance (0.004) was detected between *F. tricinctum* isolates. On the base of genetic distances, fungal isolates were grouped in two main clusters – one comprising *F. tricinctum* isolates and *F. proliferatum*, and second including *F. equiseti*, *F. graminearum* and *F. poae*. It could be concluded that the rDNA-ITS genome region of the genus *Fusarium* may be used as a suitable marker of early detection, accurate and reliable identification of *Fusarium* spp. contamination of wheat. The timely and accurate information would assist in the selection of appropriate approaches for control of fusarium infections and possible mycotoxins contamination of agricultural production.

12. Dinev, T., N. Rusenova, M. Tzanova, N. Grozeva, M. Gerdzhikova, P. Stoyanov, R. Mladenova, **G. Beev** (2020). Antimicrobial Potential of Methanolic Extracts from *Betonica bulgarica* Degen et Neič. (Lamiaceae). *Ecologia Balkanica*, 12(2): 165-174. ISSN:13140213 (**SJR 2020 = 0.144**) (**Q4**).

**Abstract:**

*Betonica bulgarica* Degen et Neič. (syn. *Stachys bulgarica* Hayek) is a Bulgarian endemic plant included in Red Data Book of Bulgaria under the category "endangered". The aim of the present study is to provide data about the antimicrobial activity of *B. bulgarica* leaf, flower, seed, stem and root methanolic extracts against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus cereus*, *Aspergillus ochraceus* 2002 IM-BAS, *Fusarium moniliforme* 394 FN-9, *Fusarium graminearum* 2294 IMI 155426 and *Penicillium verrucosum* 2003 NRRL F-143. Antimicrobial activity of the extracts was evaluated by agar well diffusion method. Root extracts of *B. bulgarica* exhibited the highest antibacterial activity against *S. aureus* and *B. cereus* with large zones of inhibition. All extracts demonstrated either low and statistically insignificant activity against *E. coli* or a lack thereof. As a whole, extracts of Ablanovo area (in Sinite kamani National Park) exerted the highest activity against *S. aureus*, *B. cereus* and *E. coli*. Leaf, flower, stem and root extracts of *B. bulgarica* showed either a lack of antifungal activity or low and statistically insignificant one.

13. Z. Yaneva, D. Ivanova, **G. Beev**, K. Besheva, 2020. Quantification of catechin in Acacia catechu extract by non-derivative, first Derivative UV/Vis spectrophotometry and FT-IR spectroscopy, *Bulgarian Chemical Communications*, 52 (D):41 ISSN:0861-9808 (**SJR 2019 = 0.142**) (**Q4**).

**Abstract:**

The aim of the present study was to quantify catechin in spray-dried extract of Acacia catechu by applying nonderivative (ND), first derivative (FD) UV/Vis spectrophotometry and FT-IR spectroscopy. The ND methodology at pH= 7.9 demonstrated to be the most sensitive, linear, precise, simple and accurate among all applied methods. Catechin content in two series of 12 Acacia catechu extract solutions (70% EtOH) at pH= 4.0 and pH= 7.9, respectively, was determined by the developed UV/Vis ND methods. The statistical analyses between the experimental data sets obtained by both techniques proved to be statistically significant. The highest catechin content in the non-diluted ethanol Acacia catechu extract was quantified as 169.88 mg/L at pH= 7.9 and 171.52 mg/L at pH= 4.0. The comparative analyses of the FT-IR spectra of pure catechin and Acacia catechu extract in powdered form and the insignificant bands width and intensity deviations proved undoubtedly the high content of the natural antioxidant in the plant extract. The latter conclusion was sustained by the established significant average percent recovery (97.17%) of catechin in the raw plant extract

14. Dermendzhieva D., G. Zhelyazkov, **G. Beev**, G. Kostadinova, T. Dinev, G. Petkov, 2019. Eco-Agricultural Assessment of Ovcharitsa Dam (Bulgaria) Water Used For Cooling of Thermal Power Plant. *Ecologia Balkanica*, 11(2):167 – 180. ISSN номер: 13140213. **SJR – 0.123** (2018), **Q4**.

**Abstract:**

Ecological (as a natural source) and agricultural (as a resource for fish farming and irrigation of crops) assessment of Ovcharitsa Dam water, used for thermal power plant (TPP) cooling was carried out in one monitoring point by measurement of 12 physicochemical parameters (temperature, transparency, pH, EC, DO, COD, BOD<sub>5</sub>, unionized NH<sub>3</sub>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, total N and P-PO<sub>4</sub>), one biological parameter (chlorophyll-a), 9 pesticides and volatile organic compounds /VOC/ (atrazine, simazine, diuron, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, tetrachloroethane, trichloromethane, hexachlorobutadiene) and 6 microbiological parameters (aerobic mesophilic microorganisms, coliforms, total coli titer, *Escherichia coli* counts, *E. coli* titer, *Salmonella* spp. counts), stipulated in Bulgarian legislation in 2016-2017 (REGULATIONS: No. 4, 2000; No. 18, 2009; On EQS for priority substances and certain other pollutants, 2010 and No. H-4, 2012). The water samples were taken periodically during a two-year period: for physicochemical and biological analysis – in February, April, June, August and November; for microbiological observation – in June, August and November; for pesticides and VOC – in April. Water sampling, sample preparation and analysis were performed according international ISO and BSS standards. It was found that: a) the dam water ecological status was determined as “poor” by chlorophyll-a content and “very poor” by orthophosphates content based on the lowest estimates for the monitored parameters; b) with regard to the content of pesticides and VOC, the dam water was defined as water “in good chemical status”; c) the values of all

monitored parameters were within the ranges (recommended and mandatory) for carp fish water with exception of temperature and unionized NH<sub>3</sub>, which exceeded the norms during some months of the year; d) according to measured water transparency, the trophic state of dam water was determined as hypereutrophic; e) the microbial status of the analyzed water demonstrated that it was not suitable for irrigation because it exceeded norms for total coli-titer and *E. coli*-titer, and due to the presence of intestinal pathogens (*Salmonella* spp.), which are not allowed in the water for irrigation.

15. **Beev G.**, T. Kolev, N. Naydenova, T. Dinev, M. Tzanova, G. Mihaylova (2019). Physicochemical, sanitary and safety indicators changes during the ripening of Bulgarian white brined cheese from local farms. *Bulgarian Journal of Agricultural Science*, 25 (Suppl. 3): 109-115. (SJR 2019 = 0.19) (Q3).

**Abstract:**

The present study aims to determine the physicochemical and microbiological changes of white-brined cheese from local farms during manufacturing and ripening. Milk pasteurization for white-brined cheese production leads to a severe reduction of microorganisms in milk. Thus, after pasteurization the total number of microorganisms decreases from 480 000 to 810 cfu/ cm<sup>3</sup>, *Salmonella* spp. from 800 to 2 cfu/cm<sup>3</sup> and *E. coli* from 4000 to 0 cfu/cm<sup>3</sup>. Ripening processes lead to a drastic reduction of cheese microflora with prevalence of specific lactic microflora (lactobacilli and lactococci) on the 45th day and complete annihilation of *E. coli* and *Salmonella* spp. These changes in the cheese microflora made the final product safe for consumption. On the other hand, the experimental data shows a strong multiplication of *Salmonella* spp. on the 7th day (10 cfu/cm<sup>3</sup> at the 24th hour reached 0 cfu/cm<sup>3</sup> on the 7th day) and insufficient decrease of the number of other microorganisms, making fresh white-brined cheese at its early ripening stages unsafe for consumption. Ripening of the cheese brings about an increase of the dry matter percentage (from 33.5% at 24th hour to 38.5% at 45th day), the fat content (from 13.3% to 16.4%), salt content (from 4.1% to 5.8%) and total protein content (from 13.7% to 16.7%) and reduction of moisture in non-fat substance (from 76.7% to 73.8%) of the final product. These changes are in accordance with the accepted standards for white-brined cheese production.

**Keywords:** white-brined cheese; physicochemical properties; microbiology; safety

16. **Beev G.**, D. Stratev, I. Vashin, D. Pavlov, D. Dinkov, 2018. Quality Assessment of Bee Pollen: A Cross Sectional Survey in Bulgaria. *J. Food Qual. Hazards Control*, 5 (1) :11-16. DOI: 10.29252/jfqhc.5.1.11. ISSN: 2345685X, (SJR 2018 = 0.27) (Q3).

**Abstract:**

**Background:** Due to its nutrient content, a variety of spoilage microorganisms can grow in bee pollen, especially when handling practices are not appropriate. So, this survey was designed to assess the physicochemical and microbiological properties of bee pollen collected from Bulgaria.

**Methods:** In June 2014, 13 fresh and 19 dried bee pollen samples were collected from Bulgaria. Water activity (aw), pH, Total Titratable Acidity (TTA), and bacteriological and fungal counts were evaluated. Data were analyzed using Statistica ver. 10.

**Results:** Mean aw value for fresh and dried bee pollen samples were 0.717 and 0.359, respectively showing significant ( $p < 0.01$ ) difference. The mean pH values of fresh bee pollen samples (4.23) was significantly ( $p < 0.01$ ) lower than that of dried ones (5.21). Also, there is statistically significant difference ( $p < 0.01$ ) between mean TTA value of fresh bee pollen (3.69 g/100 g) and mean TTA value of dried samples (2.09 g/100 g). The mean total viable count in fresh bee pollen samples was 182153.8 Colony Forming Unit (CFU)/g which was significantly ( $p < 0.01$ ) higher than that of dried samples (30352.6 CFU/g); whereas statistical analysis demonstrated that there were no significant differences ( $p > 0.01$ ) among the fresh and dried pollen for Enterobacteriaceae, lactic acid bacteria, and Staphylococcus spp. counts. The fungal colony count in the fresh bee pollen samples varied from 560 to 37000 CFU/g which were significantly ( $p < 0.01$ ) higher than that in the dried ones.

**Conclusion:** The fresh bee pollen analyzed in this survey had poor microbial quality compared to the dried bee pollen. There is also need to adopt appropriate manufacturing practices to prevent possible contamination by equipment or handling of Bulgarian bee pollen.

17. Dinev, T., G. Beev, M. Tzanova, S. Denev, D. Dermendzhieva & A. Stoyanova, 2018. Antimicrobial activity of *Lactobacillus plantarum* against pathogenic and food spoilage microorganisms: A review. *Bulgarian Journal of Veterinary Medicine*, 21(3), 253-268. DOI: 10.15547/bjvm.1084. ISSN: 13111477, (SJR 2018 = 0.167) (Q3).

**Abstract:**

One of the most important properties of probiotic bacteria is their antimicrobial activity against many species of microorganisms which could be useful to prevent food spoilage caused by certain sensitive bacteria and fungi as well as to control the speed of propagation of potentially pathogenic bacteria by probiotic application. *Lactobacillus plantarum* is considered one of the probiotic bacteria with broadest spectrum of antibacterial activity which makes it useful in veterinary, human medicine and food industry. According to a number of studies *Lactobacillus plantarum* exerts inhibitory activity against many Gram-positive and Gram-negative bacteria – *Escherichia coli* (including *E. coli* 0157:H7), *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Klebsiella*, *Salmonella*, *Shigella*, *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus* spp., etc. as well as a number of moulds and yeasts – *Aspergillus*, *Fusarium*, *Mucor*, *Candida* spp., etc. The main antibacterial compounds of *Lactobacillus plantarum* are the bacteriocins and organic acids whereas the antifungal compounds are the organic acids, hydroxy fatty acids and cyclic dipeptides. Because of the high antifungal activity of some *L. plantarum* strains against food spoilage microorganisms they can be used as effective biopreservatives in food industry. Also, some *L. plantarum* strains could be applied as supporting therapeutic agents in treatment of infections caused by the corresponding susceptible microorganisms.

**Key words:** antimicrobial activity, *Lactobacillus plantarum*, food spoilage microorganisms, pathogens

18. Kostadinova, G., D. Dermendzhieva, G. Petkov, **G. Beev**, K. Koev. 2018. Evaluation of wastewater quality at the inlet-outlet of the most modern wastewater treatment plant in Bulgaria. *Fresenius Environmental Bulletin*, 27(12): 9723–9738. ISSN номер: 10184619, **IF 2018=0.673 (Q3)**.

**Abstract:**

The study was carried out during the period 2015-2016 based on 24 physicochemical and 5 microbiological wastewater (WW) parameters. WW samples were collected twice per month from both monitoring points (MPs) of the Municipal Wastewater Treatment Plant (MWWTP) - MP-1 (inlet) and MP-2 (outlet) and screened parameters were analyzed according to Bulgarian standards complied with ISO standards. For the estimation of total and specific microbial load, selective chromogenic culture medium sheets were used. Multivariate statistical technique was applied to analyze the data for different parameters. It was found the ranges of variability and trends of inlet-outlet WW values changes. The MWWTP demonstrated different removal efficiency (8.31-97.8%) referring to different WW parameters. 127 strong positive and negative correlations exist between controlled WW parameters. The parameters involved in the most numerous statistically significant correlations were T°C and Cl. EC at inlet/outlet affected at a great extent Factor 1 of Rotated factor loading matrix. The factor analysis determined MP as a factor influencing the largest number of parameters (14), followed by factors Month (7) and Year (2). PCA revealed different WW parameters at inlet-outlet that were affected by F1 and F2. The treated WW did not meet the requirements for discharge in the receiving water body (with respect to the total P content) and for irrigation (as fats content and the number of *E. coli*, *Enterobacteriaceae* and *Salmonella* spp. was concerned).

19. Velichkova K., I. Sirakov, N. Rusenova, **G. Beev**, S. Denev, N. Valcheva, T. Dinev (2018). In vitro antimicrobial activity on *Lemna minuta*, *Chlorella vulgaris* and *Spirulina* sp. extracts. *Fresenius Environmental Bulletin*, 27(8), 5736-5741. ISSN: 10184619, (**IF 2018 = 0.673**) (Q3).

**Abstract.**

Aquatic plants play a major role in sustaining life and are among the most productive ecosystems in the world. The aim of present study was to test different extract from *Spirulina* sp., *Chlorella vulgaris* and *Lemna minuta* for antibacterial, anti-yeast and antifungal activities. The plant extracts (methanol and ethanol) were tested for antimicrobial activity by the Eleven bacterial, yeast and fungal strains were used. The antimicrobial activity was evaluated by measuring zones of inhibition of microbial growth surrounding the plant extracts in the wells. The most effective extract was *L. minuta* ethanol which showed activity against all tested strains of microorganisms with the exception of *B. cereus*. Ethanol extracts of the three studied aquatic plants showed better activity than the methanol extracts.

**KEYWORDS:** Antibacterial, anti-yeast, antifungal, *Lemna*, *Spirulina*, *Chlorella*

20. Tzanova M., V. Atanasov, B. Zaharinov, **G. Beev**, T. Dinev, E. Valkova (2017). Reproduction impact of mancozeb on rainbow trout (*Oncorhynchus mykiss* W.) and accumulation of its carcinogen metabolite, ethylene thiourea in fish products. *Journal of Central European Agriculture*, 18(2), 369-387. (SJR 2017 = 0.161) (Q4).

**Abstract:**

Pesticides can be taken up from the water and accumulated in tissues of hydrobionts, often becoming multiplied thousands of times higher in the organism than in the surrounding water. The dithiocarbamate mancozeb is applied in plant protection as fungicide. In recent years the amount of mancozeb used in Europe significantly increased. It is carcinogen due to its metabolite - ethylene thiourea (ETU), which causes thyroid and pituitary tumors. The purpose of this study is to determinate the quantity of ethylene thiourea in products of rainbow trout (*Oncorhynchus mykiss* W.), reared in environment containing permissible, according to the European law, amount of mancozeb. Seeking an answer to the question: is this concentration limit really safe for the reproduction of rainbow trout and can the more toxic metabolite - ETU, be accumulated in the fish eggs and fillet and afterwards make them harmful to the consumers? The study included 3 stages: feeding, analysis of ethylene thiourea in fish eggs and fillet by a new developed and validated HPLC (high performance liquid chromatography) method and study of the reproductive indicators. The assays of ETU in all analyzed samples (fish and water) were below the limit of quantification of the method, 0.05 mg\*l<sup>-1</sup>, so fish do not accumulate the carcinogen degradation product of mancozeb and the maximum residue level of mancozeb is really safe for the humans as consumers. But these environmental conditions caused reproductive disorders. They can be partly compensated by using sperm activation medium for artificial insemination of trout eggs, but successful fertilization does not guarantee successful hatching, especially of eggs in trout farms with presence of mancozeb in water, even in allowable concentration. The presented results confirm previous investigation, that Salmonidae are very sensitive fish species, react to the lowest deviations in concentration levels of xenobiotics and are used for indicator of non-polluted water.

**Keywords:** ethylene thiourea, HPLC, mancozeb, rainbow trout, reproduction, sperm activation medium

21. Velichkova, K. N., I. N. Sirakov, **G.G. Beev**, S.A. Denev, D.H. Pavlov, 2016. Treatment of Wastewater Originated from Aquaculture and Biomass Production in Laboratory Algae Bioreactor using Different Carbon Sources. *Sains Malaysiana*, 45(4):601-608. ISSN: 01266039, (IF 2016 = 0.47) (Q2).

**Abstract:**

The aim of present study was to explore the effect of different carbon sources on biomass accumulation in microalgae *Nannochloropsis oculata* and *Tetraselmis chuii* and their ability to remove N and P compounds during their cultivation in aquaculture wastewater. Microalgae cultivation was performed in laboratory bioreactor consisted from 500 mL Erlenmeyer flasks, containing 250 mL wastewater from semi closed recirculation aquaculture system. The cultures were maintained at room temperature (25-27°C) on a fluorescent light with a light: dark

photoperiod of 15 h: 9 h. The microalgae species were cultivated in wastewater with different carbon sources: glucose, lactose and saccharose. The growth of strains was checked for 96 h period. In the present study, *N. oculata* and *T. chuii* showed better growth in wastewater from aquaculture with saccharose carbon source during the experiment. The most effective reduce of nitrate and total nitrogen was proved in *N. oculata* cultivated in wastewater with glucose as carbon source. *T. chuii* cultivated in wastewater containing glucose showed 8.27% better cleaning effect in ammonium compared with *N. oculata*. *T. chuii* grew in wastewater with glucose as carbon source showed 19.5% better removal effect in phosphate compared with *N. oculata* strain.

20.06.2023 г.  
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