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## PROCEEDINGS

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# CONTENT

## Innovative and Applied Research in Biology

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# EVALUATION OF PROTECTIVE PROPERTIES OF BIOTEXTILE WITH INCORPORATED AMBER NANO/MICRO PARTICLES AGAINST THE LOW-FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF)

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**Abstract**: The impact of extremely low frequency electromagnetic field (ELF-EMF) on living organisms and the identification of their threshold levels remain uncertain. Amber nanoparticles and microparticles are being explored as potential raw materials used for production of innovative biotextiles. This study aimed to develop methods for assessing the protective properties of biotextiles against LF-EMF including testing the influence of a novel biotextile with incorporated amber particles on growth parameters of model bacteria and fungi species.

The assessment of biotextile properties is based on comparison of mycelial growth in Petri dishes of fungi *Chaetomium globosum* Kunze: Fries (ATCC® 6205™), and the growth rates of bacteria *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 (NCTC 12697) and *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (AL) (NCTC 12241), grown in flasks wrapped and unwrapped in fabrics with incorporated amber particles under LF-EMF exposure. While no significant differences were observed in the growth rate of *E. faecalis* under LF-EMF exposure, regardless of fabric wrapping, the biotextile with amber particles demonstrated a statistically significant protective effect ($P < 0.01$) inducing growth rate changes in *E. coli*.

**Key words**: succinate, mycelial growth, *Chaetomium globosum,* bacterial growth, optical density, flow cytometry, *Escherichia coli* and *Enterococcus faecalis*

## Introduction

The general aim of the research projects "Innovative multifunctional bio-textile, integrated with silica dioxide and succinate development, and its impact on biosystems" and ''3D Biotextile with Technological Composition of nano particles to enhance

the protecting properties" was to determine protective properties of newly developed innovative biotextile with incorporated amber nano and micro particles.

Modern cities present a growing concern: a complex of environmental factors cause negative impact on human health. Increasing ultraviolet radiation, amplified by contemporary architecture, enhansment of electromagnetic radiation including low-frequency electromagnetic fields, and a multitude of chemical pollutants all contribute to this global social problem (Duhaini, 2016). Ubiquitous electrical devices, from power lines to everyday appliances, create electromagnetic fields documented to potentially influence biological processes (Strasak et al., 1998; Panagopoulos et al., 2002; Grassi et al., 2004). Public anxieties have risen around low-frequency electromagnetic fields (LF-EMF), becoming the most common reasons increasing magnetic fields density in our environment (caused by alternating current below 300 Hz), due to their potential health risks (Feyyaz & Kargi, 2011). Research on the effects of these fields on living organisms, including bacteria (Strašák et al., 2002; Strašák, 2005; Cellini et al., 2008; Masood et al., 2020; Salmen et al., 2018), has seen a recent surge.

One promising solution to mitigate these negative urban environmental impacts lies in the development of innovative biotextiles with protective properties (Grauda, et al., 2023). Researchers are exploring the potential of textiles to shield against extremely low frequency electromagnetic field (ELF-EMF), thereby contributing to improved human health (Ziaja et al., 2008). Baltic Amber (*succinite*, CAS 9000-02-6), a natural polymer resin material with potential protective properties, is under investigation as a raw material that could be applied for designation of novel biotextiles. This study focuses on the assessment of protective properties of such biotextiles incorporating amber nano and microparticles aiming to establish convenient methods for evaluation of shielding effects against LF-EMF. The research employs microscopic cellulose degrading fungi (*Chaetomium globosum*) and bacteria (*Escherichia coli* and *Enterococcus faecalis*) as test organisms.

The aim of this study was to adapt methods and techniques for determination of protective properties of biotextiles against electromagnetic field looking for the ways to make evaluation of the protective properties of innovative biotextiles most relevant and cost-effective. Following these considerations microscopic cellulose degrading fungi *Chametomium globosum* and bacteria *Esherichia coli* and *Enterococcus faecalis* were chosen as test objects.

## Materials and methods

### Designation of the System for Assessment of the Effects of Magnetic Field on Test Objects Exposured to Low Frequency Electromagnetic Field

A Helmholtz coil was employed to generate a constant 50 Hz LF-EMF (Figure 1, 2). The coil's parameters were R = 25 cm, distance between coils = 25 cm, number of turns = 189. This configuration ensures uniform magnetic field (MF) intensity at the coil's center. The regulation of magnetic field intensity (0-725 μT) generated by LF-EMF was

controlled by adjusting the electric current and mesaured using. Narda model 8532-60 device. For this study, a moderately high MF intensity of 518 μT was selected, considering existing MF intensity standards and potential health impacts.

## Description of textiles

Two fabric types were used in the experiments:

1. **Control:** Twill Code T564, warp Linen yarn 28 Tex, weft Cotton yarn 20 Tex (11.02.20), 320±60 g/m$^2$; weaving textile material was developed by A Grupe, JSC (Jonava, LT).
2. **Biotextile:** Twill Code T561, warp Linen yarn 28 Tex, weft Cotton yarn 20 Tex, Amber fiber 7.8 Tex (11.02.20), 330±60 g/m$^2$; weaving textile material was developed by A Grupe, JSC (Jonava, LT).

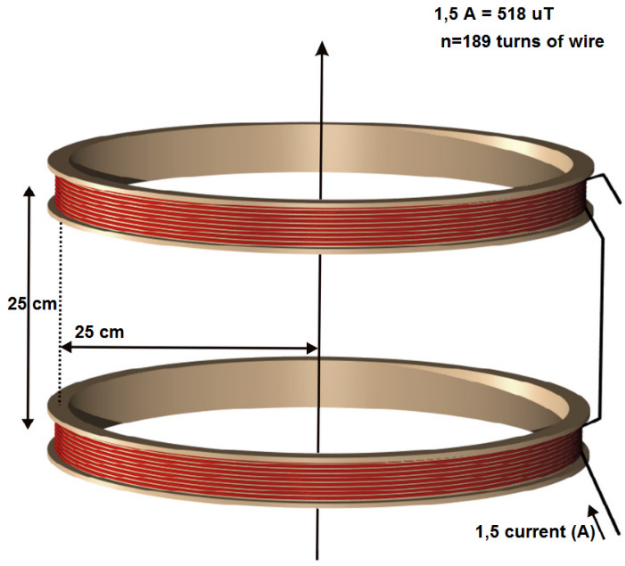



*Figure 1.* Helmholtz coil scheme.

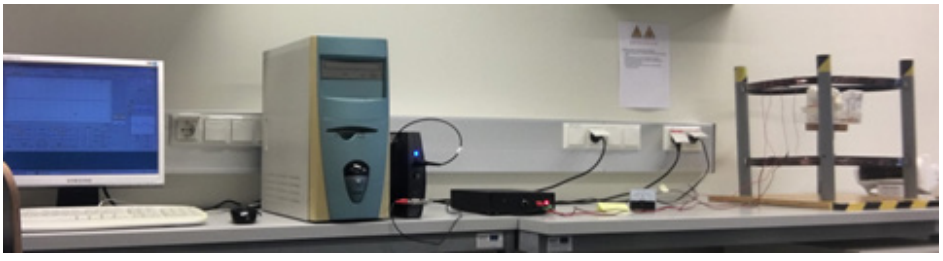


*Figure 2.* Helmholtz coil and MF intensity measuring equipment – Narda, model 8532-60.

## Microorganism cultures used in expirements

The strain *Chaetomium globosum* Kunze: Fries (ATCC® 6205™), obtained from the company Microbiologics (US) in form of Kwik Stick, stored at 5 ± 3 °C were used in experiments. The *C. globosum* strain ATCC® 6205™ is the licensed derivative of original stock cultures obtained exclusively from the American Type Culture Collection (ATCC®). The *C. globosum* was originally isolated from cotton fabric in 1933 and is therefore suitable for testing biotextiles as representative of a common strain environment (Government of Canada 2018). Selected fungal strain were maintained on potato dextrose agar (PDA) medium (SIFIN, Berlin) and were grown on PDA for ten days before being used in the study.

Bacterial strains gram – negative *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (AL) (NCTC 12241) and gram – positive *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 (NCTC 12697), were obtained from TCS Biosciences Ltd, in form of culture discs. These cultures are the first-generation derivative of original stock cultures obtained exclusively from the National Collection of Type Cultures (NCTC®). Culture discs were stored at 5 ± 3 °C. Before use in experiments, culture discs were removed from vials by sterile forceps, then culture disc inoculate on the Tryptic soy agar (TSA) and incubate under optimum conditions for 3 h.

## Determination of fabric's ability to retain electromagnetic field impact on mycelial growth

The radial growth method was used to assess the impact of EMF on fungal mycelial growth. Agar plugs (5 × 5 mm) from pure fungal cultures were placed in the center of 90 mm Petri dishes with PDA.

Six experimental variants were set up:

- **Control:** Uncovered, wrapped in linen/cotton fabric, or wrapped in biotextile No CL561, all non-exposed to EMF.
- **EMF-Treated:** Uncovered, wrapped in linen fabric, or wrapped in biotextile No CL561, all exposed to EMF growing test organisms in Petri dishes placed in the center of the Helmholtz coil.

Each variant was replicated six times. Inoculated Petri dishes were incubated at 24 ± 2 °C. Mycelial growth was measured daily for ten days or until the fungus reached the dish edge. Mycelial growth was compared between control and EMF-treated groups and expressed as a percentage in comparison to control growth.

## Bacterial Growth Assay

Bacterial cultures were re-inoculated onto fresh TSA and incubated at 37 °C for 18 hours. A single colony was sub-cultured in 100 ml of TSB and incubated at 37 °C for 2 hours. The optical density was adjusted to 0.1 MFa at 565 nm to obtain 1 × 10^6 CFU/ml.

Two sets of experiments were conducted, each with three variants: uncovered tubes, tubes wrapped in linen/cotton fabric, and tubes wrapped in biotextile encompassing amber particles. Each variant was replicated six times. One set was exposed to the magnetic field, while the other served as a control. Experiments were performed at room temperature (24 ± 2 °C).

Bacterial growth rate was determined by measuring optical density at 565 nm after 16, 24, and 48 hours of EMF exposure. Morphological changes of bacteria have been studied using light microscopy; the smear method and stained with MGG Quick Stain kit (Bio-Optica Milano s.p.a).

### Flow Cytometry

Flow cytometry was employed to assess cell count and reaction changes. Bacterial suspensions were filtered and stained with propidium iodide. A BD FACSJazz® cell sorter was used to measure relative fluorescence.

Following the methods outlined by Grauda et al. (2015), 2 mL of lysis buffer was added to cytometer tubes. After 16, 24, and 48 hours of incubation, bacterial cultures were homogenized by vortexing. 100 µL of the bacterial suspension was filtered through a 40 µm flow cytometry filter and added to the lysis buffer. To induce cell fluorescence, 10 µL of propidium iodide was added.

A BD FACSJazz® cell sorter (BD Biosciences, USA) was used to measure the relative fluorescence of bacterial cells. The device was equipped with a 100 µm nozzle and used phosphate-buffered saline (PBS) as a sheath fluid. Cell counting events were triggered by forward-scattered light. A 488 nm blue laser excited cell fluorescence, and emission was measured at 585 nm (bandwidth 29 nm). Before measurements, the flow cytometer was calibrated using Sphero™ rainbow calibration particles (3.0 µm, BD Biosciences, USA) in PBS. A coefficient of variation (CV) below 3 % was considered successful calibration.

The intensity of bacterial relative fluorescence was expressed in arbitrary logarithmic units. For each subsample, events were recorded over a fixed 5-minute period.

### Statistical methods

The means of colony diameters were separated using Least Significance Different Test, at $P < 0.05$. Analytical statistical methods were performed with the R program version 4.2.0.

## Results and discussion

### In vitro mycelial growth assay

We assessed the impact of ELF-EMF exposure on the mycelial growth of *C. globosum* by comparing growth between control (non-exposed) and exposed variants. Two days after ELF-EMF exposure, a significant ($P < 0.01$) stimulation of mycelial growth was observed in the unwrapped variant, with a 119.51 % increase compared to the control (Table 1). In all variants where Petri dishes with *C. globosum* were wrapped in linen fabric or biotextile with amber particles, mycelial growth stimulation was limited to 9.72 % and 7.81 %, respectively.

As shown in the unwrapped variant, the growth stimulating effect of ELF-EMF is most pronounced two days post-exposure, followed by a decline on the effect at third day. Five days after ELF-EMF exposure, a slight growth increase (103.52 % to 104.92 %) was observed in unwrapped group, but this was not significantly different ($P < 0.05$) from

the fabric-wrapped variants. After seven days, mycelial growth was indistinguishable from the control. These findings align with those of Gao et al. (2011), who reported that EMF initially stimulates metabolite production.

*Table 1.* Evolution of protective properties of linen/cotton fabric and biotextile with amber particles on *Chaetomium globosum* mycelial growth after inoculation on PDA, fabric wrapping, and 2–7-day ELF-EMF Exposure.

| Variant | Colony diameter in variants exposed to ELF- EMF expressed in percentage comparing to control (%) (mean ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | Days of exposition in ELF-EMF | | | | | |
| | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| Unwrapped | **119.51** ± 0.00 a | **114.06** ± 0.37 a | **110.04** ± 1.08 a | 104.70 ± 1.63 a | 104.92 ± 2.27 a | 103.52 ± 0.90 a |
| Wrapped in linen/cotton fabric | 106.83 ± 1.08 b | 107.50 ± 0.30 b | **109.72** ± 0.77 a | 105.13 ± 0.70 a | 104.04 ± 1.98 a | 104.32 ± 1.70 a |
| Wrapped in biotextile with amber particles | 101.72 ± 1.22 c | 106.81 ± 1.28 b | 104.26 ± 1.03 b | 106.32 ± 2.06 a | 105.78 ± 0.88 a | 103.96 ± 0.60 a |
| Means with the same letters within columns are not significantly different at $P < 0.01$. | | | | | | |

## Bacterial Growth Assay Results

Bacteria have coexisted with electric and geomagnetic fields throughout their evolutionary history. Numerous studies have investigated the effects of extremely low-frequency electromagnetic field (ELF-EMF) exposure on various bacterial strains (Strašák, 2005; El-Sayed et al., 2006; Cellini et al., 2008; Aslanimehr et al., 2013; Ibraheim et al., 2013; Martirosyan et al., 2013; Bayır et al., 2015). These studies have shown that EMFs can exert either negative (Strašák et al., 2002; Fojt et al., 2004; Justo et al., 2006) or positive (Gaafar et al., 2006; Inhan-Garip et al., 2011) effects on cell growth and viability, depending on factors such as frequency, exposure duration, magnetic field intensity, and the specific bacterial strain. The results of our experiment indicate that ELF-EMF exposure has a variable impact on the growth of different bacterial cultures and depends on the duration of exposure. We observed that 16-hour exposure to EMF stimulated the growth of *E. coli* compared to the control (Figure 3A). The optical density of the unwrapped, exposed variant after 16 hours was 8.86 McF, which is significantly different from the control (unwrapped, non-exposed) with an optical density of 5.33 McF ($P < 0.01$). However, after 24 and 48 hours of exposure, bacterial growth reached a stationary phase, and no significant differences were observed between the control and exposed variants.

## Properties of Biotextile Affecting Bacterial Growth

In the variant where *E. coli* was wrapped in biotextile and exposed to ELF-EMF for 16 hours, the growth rate, measured as optical density, differed significantly from the unwrapped, exposed variant but was not significantly different from the unwrapped, non-exposed control. This indicates that the biotextile with amber particles can protect

*E. coli* from the detrimental effects of EMF, preventing both growth inhibition and stimulation. After 48 hours, bacterial growth reached a stationary phase, and no significant differences were observed between control and exposed variants.

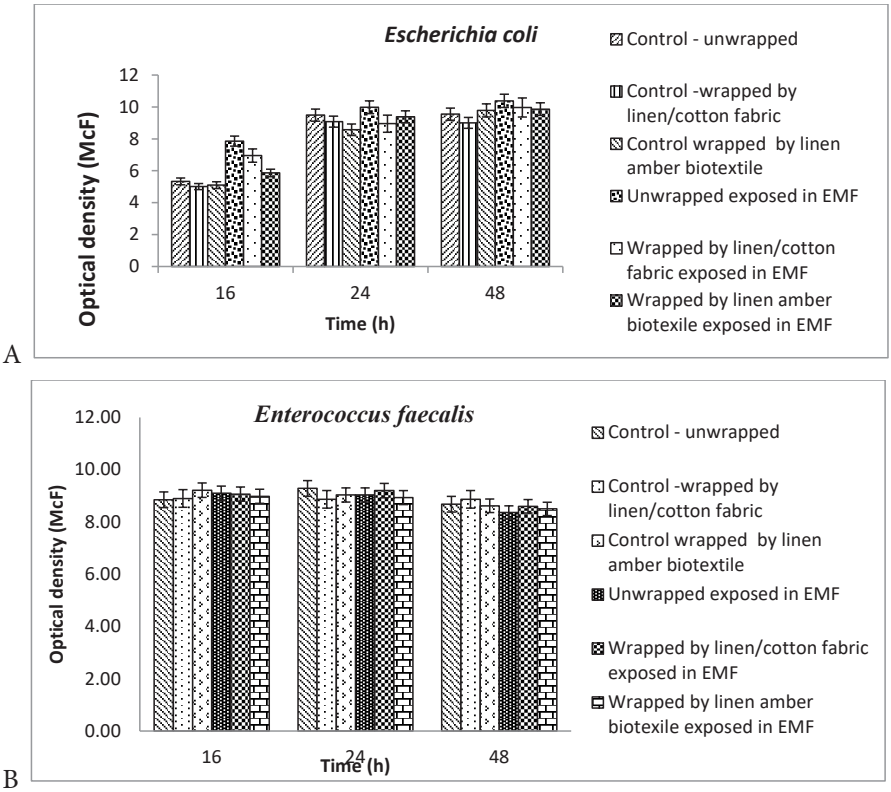



*Figure 3.* Impact of ELF EMF (exposing time 16, 24 and 48h) on bacteria growth (optical density in McFarland units) dependent on presence/absence of used protective wrapping. A – *Escherichia coli*; B – *Enterococcus faecalis*.

In contrast to *E. coli*, no significant differences were found in the growth of exposed *E. faecalis* (Figure 3B), regardless of whether it was wrapped in fabric or not.

While we observed a growth stimulating effect of EMF on *E. coli* after 16 hours of exposure, the optical density measurement method does not provide information about changes in bacterial viability. Therefore, we conducted additional morphological examinations and fluorescence studies.

Light microscopy of bacterial smears after 48 hours of incubation revealed no visible differences between non-treated controls and ELF-EMF-treated samples (Figure 4 A, B, C, D).

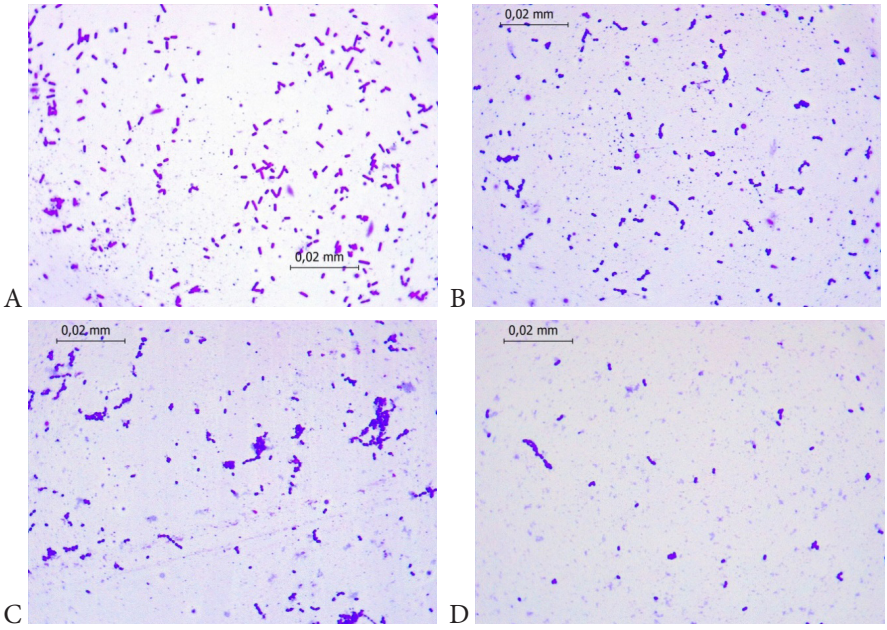
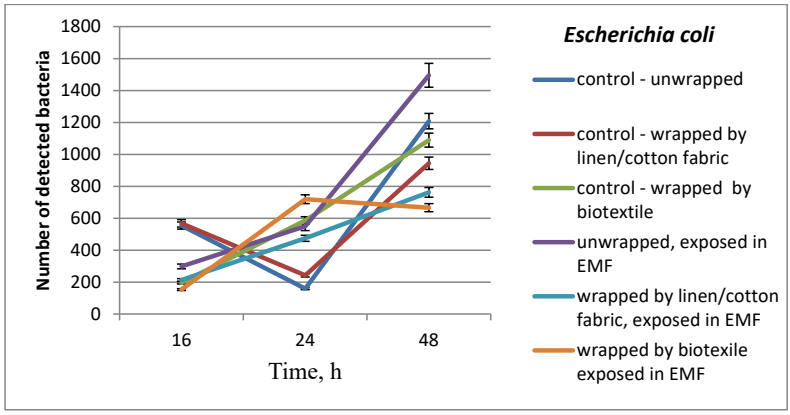


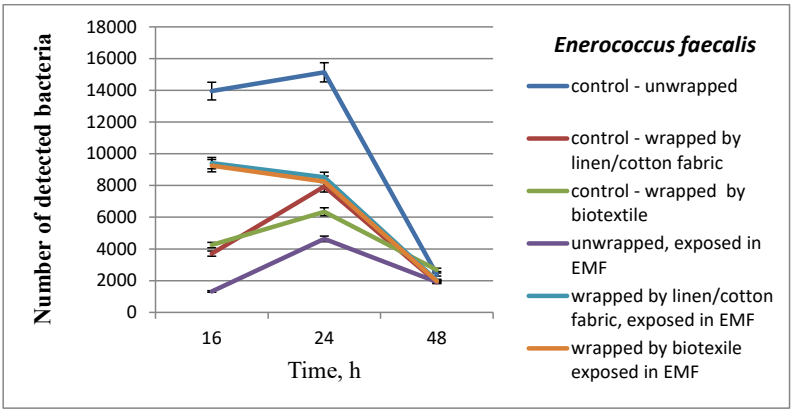

*Figure 4.* Light microscopy of bacterial smears of bacterial cultures wrapped in biotextile, both non exposed and exposed to ELF-EML 50 Hz (518 μT) 48 h. A – *E. coli* wrapped in biotextile and non-exposed, B – *E. coli* wrapped in biotextile and exposed to ELF-EML; C – *E. faecalis* wrapped in biotextile and non-exposed; D – *E. faecalis* wrapped in biotextile and exposed to ELF-EML.

We employed flow cytometry to assess cell reaction changes in test bacterial cultures wrapped in textiles, both non-exposed and exposed to ELF-EMF (518 μT) after 16, 24, and 48 hours of incubation (Figures 5 and 6). Propidium iodide, a stain that penetrates only bacteria with damaged membranes (Cellini et al., 2008), was used.

Our results showed that the number of *E. coli* bacteria with damaged membranes (fluorescent) after 16 hours of incubation ranged from 153 ± 6 to 568 ± 23 (Figure 5A). Notably, the number of damaged *E. coli* bacteria was significantly higher in control variants than in exposed variants. After 24 and 48 hours of exposure to ELF-EMF (518 μT), the number of fluorescent *E. coli* (damaged bacteria) increased in exposed variants compared to controls.

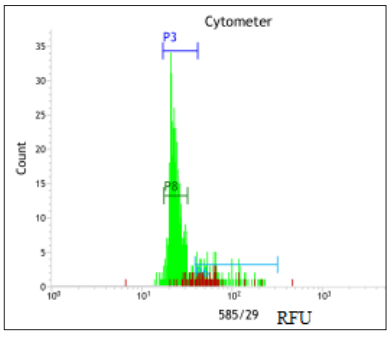



A

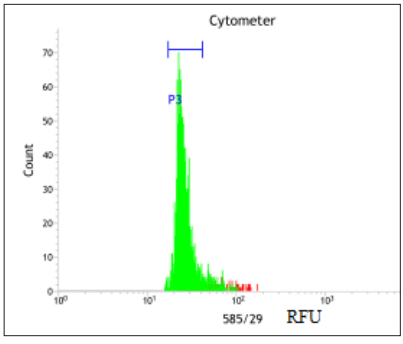



B

*Figure 5.* Number of fluorescent bacteria counted by FaxJaz flow cell sorter, depending on used protective wrapping, incubation time (16, 24 and 48 h) and presence of ELF – EML treatment. A – *Escherichia coli*; B – *Enterococcus faecalis*. The error bars indicate the confidence interval, at $P < 0.05$.



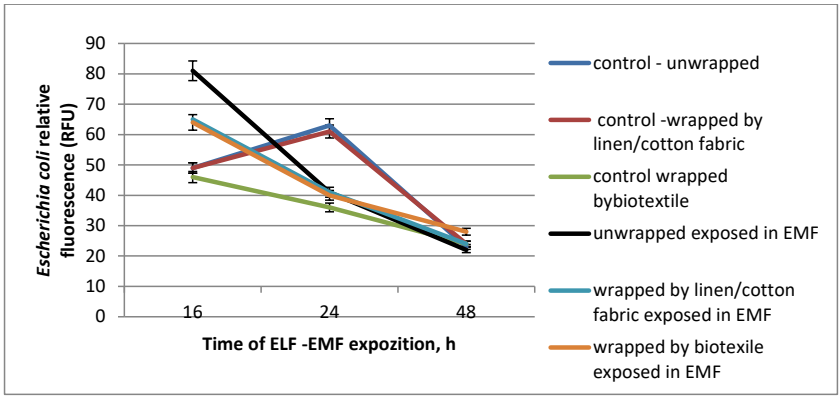

A1



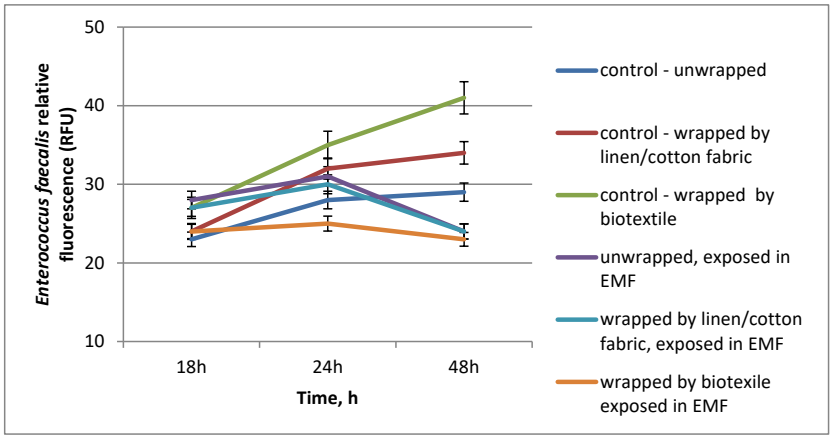

A2

*Figure 6.* Count of *Escherichia coli* wrapped by linen/cotton amber biotextile depending on exposition to ELF-EML (518 μT). A1 – non exposed, after 24 h; A2 – exposed to ELF-EML (518 μT) for 24 h.

The relative fluorescence of *E. coli* cells after 16 h ranges from 46 ± 2 to 80 ± 3 RFU and in control variants are significantly lower ($P < 0.01$). than in exposed variants (Fig. 7A). In unwrapped variant exposed to ELF-EML (518 μT) for 16 h *E. coli* relative fluorescence is significantly higher ($P < 0.01$), than in wrapped exposed variants. After 48 h relative fluorescence decreases in both control and exposed variants.




A




B

*Figure 7.* Impact of ELF-EML (exposing time 16, 24 and 48h) on bacteria relative fluorescence depending on used protective wrapping. A – *Escherichia coli*; B – *Enterococcus faecalis*.

The relative fluorescence of *Enterococcus faecalis* cells after 48 h ranges from 23 ± 3 to 41 ± 4 RFU and in control variants are significantly higher ($P < 0.01$), than in exposed variants (Fig. 7B). In unwrapped variant exposed to ELF-EML (518 μT) for 48 h *Enterococcus faecalis* cells relative fluorescence are significantly higher ($P < 0.01$), than in wrapped exposed variants. After 48 h relative fluorescence decreases in all exposed variants.

## Conclusions

This study successfully adapted methods to determine the protective properties of biotextiles against extremely low-frequency electromagnetic fields (ELF-EMF). The research employed two bacterial strains (*Escherichia coli* and *Enterococcus faecalis*) and a microscopic cellulose degrading fungus (*Chaetomium globosum*) as test organisms. Here are the key findings:

Unwrapped *C. globosum* exhibited stimulated mycelial growth after exposure to LF-EMF compared to the control. However, wrapping the fungus in either linen fabric or the biotextile with amber particles significantly reduced this stimulation.

Short-term (16 hours) exposure to ELF-EMF increased *E. coli* growth compared to the control. The biotextile with amber particles appeared to protect *E. coli* from the detrimental effects of prolonged exposure, maintaining growth similar to the non-exposed control.

Bacterial Growth (*Enterococcus faecalis*): No significant differences in *E. coli* growth were observed under LF-EMF exposure, regardless of fabric wrapping.

The Flow Cytometry analysis of the biotextile with amber particles seemed to offer some protection for *E. coli* by reducing the number of bacteria with damaged membranes after short-term exposure (16 hours) compared to the control.

These findings suggest that the biotextile with amber particles holds promise for mitigating the potential negative effects of LF-EMF on *E. coli*. Further research is needed to explore the long-term effects of LF-EMF exposure on various bacterial strains and to optimize the protective properties of the biotextile.

### Acknowledgements

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# MIXOTROPHIC CULTIVATION OF MICROALGAE IN WHEY MEDIUM FOR POULTRY DIET SUPPLEMENTATION WITH MICROALGAL BIOMASS

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**Abstract:** During this research microalgal strains of *Graesiella emersonii* KM01 and *Tetradesmus obliquus* OM02 were isolated from various freshwater bodies, also microalgal cultures *Chlorella vulgaris* CCAP 211/111, *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14 were from Culture Collection of Algae and Protozoa (CCAP, United Kingdom) and *Galdieria sulphuraria* UTEX 2919 from Culture Collection of Algae of the University of Texas at Austin (UTEX, USA). It has been shown that to increase microalgae biomass yield, it is possible to cultivate microalgae mixotrophically in whey medium. By comparing growth performance of microalgal cultures in lactose containing media and whey, a promising approach for achieving high biomass yield, thus also for decreasing environmental impact of improper whey disposal, was demonstrated for *T. obliquus* OM02. Growth of both *G. emersonii* KM01 and *C. zofiengiensis* CCAP 211/14 on lactose containing media and whey was found to be relatively low, not exceeding biomass productivity of isolate *T. obliquus* OM02. Cultivation of *T. obliquus* OM02 in whey demonstrated that it is possible to achieve the highest biomass in 50 % diluted whey, which resulted in 2.83 ± 0.11 g/L and is the highest yield between microalgal isolates in this research. Results confirm that mixotrophic cultivation of certain lactose assimilating microalgal isolates in whey compared to photoautotrophic cultivation enables to obtain a comparable biomass yield, which can be attributed to whey medium composition.

**Key words:** microalgae, *Graesiella emersonii, Tetradesmus obliquus,* whey, mixotrophic cultivation

## Introduction

As the global human population is increasing every day, also the demand for goods follows. To be able to deliver enough goods for human consumption, scientists need to expand research not only on land, but also on freshwater and saltwater bodies. There is a group of aquatic microorganisms that is drawing favorable attention by scientists – microalgae (Cheirsilp et al., 2023).

Poultry farming has a great impact on delivering human diet with eggs and meat. To obtain eggs and meat of high quality, an adequate diet should be provided for poultry

being rich in lipids, polyunsaturated fatty acids, proteins, vitamins, complex carbohydrates, and other functional compounds (Uguz & Sozcu, 2023).

Microalgae are microorganisms that can be used to fulfil those requirements. It has been demonstrated that, to increase microalgae biomass yield, it is possible to cultivate microalgae mixotrophically in a media supplemented with industrial and agricultural by products (Abril Bonett et al., 2020; Chong et al., 2022; Khanra et al., 2021; Mat Aron et al., 2021; Vidya et al., 2023). By use of whey for microalgal cultivation media, it is attainable to decrease ecological footprint of its improper disposals, as it is known that whey considerably composes most important part of dairy processing by-products, as well as reduce production cost beside increased biomass production (Pescuma et al., 2015). However, only certain microalgal cultures are able to consume lactose, which is the main sugar component in whey. So, search of microalgal strains which are able to consume lactose as carbon source during mixotrophic cultivation is required (Doebbe et al., 2007).

This study aimed to evaluate the possibility for use of lactose assimilating freshwater microalgal isolates in whey mixotrophic bioconversion as industrial by-product and potential source for efficient microalgal biomass production. In respect of that, the tasks of the present research were as follows:

- to evaluate heterotrophic growth of microalgal cultures in carbohydrates (lactose, glucose, galactose) containing media;
- to evaluate biomass production of chosen microalgal isolates in whey or whey media.

## Material and methods

### Microalgal cultures

Freshwater microalgal strains of *Graesiella emersonii* KM01, *Tetradesmus obliquus* OM02 and unidentified strain X1 isolated from different water bodies in Riga (Latvia), were obtained from Microalgae Culture Collection of the Institute of Biology (University of Latvia). Microalgal strains of *Chlorella vulgaris* CCAP 211/111, *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14 were obtained from Culture Collection of Algae and Protozoa (CCAP, United Kingdom); *Galdieria sulphuraria* UTEX 2919 culture was obtained from The Culture Collection of Algae of the University of Texas at Austin (UTEX, USA).

### Media and cultivation conditions

In this study microalgae were cultivated in Erlenmeyer flasks. Media for heterotrophic cultivation (3N-BBM-V) were supplemented with sugars (lactose, glucose, or galactose) in 5 g/L concentration. For mixotrophic experiments whey from AS "Rankas piens" was used, diluted with distilled water to obtain needed concentrations. For control in all experiments the 3N-BBM-V medium without added sugars was used. The mixotrophic cultivation was carried out in presence of LED light source with day: night cycle 16 : 8 h and light intensity of 80 μmol/$m^2$s. Heterotrophic cultivation was carried out in dark. Both cultivations were performed statically in an incubator at 25 °C for 14 days.

### Biomass acquisition

After cultivation, samples of 50 mL culture liquids were collected, centrifuged at 8000 rpm for 5 minutes, then supernatant was discarded, and biomass resuspended in distilled water to eliminate media residues. Further, rinsed microalgal biomass has been transferred into pre-weighted weighting bottles and dried at 80 °C for 24 h.

### Statistical analysis

All experiments performed in four replications (n = 4). One-way analysis of variance (ANOVA) was performed using SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) to compare means at a significance level p = 0.05.

## Results and discussion

In a course of this research obtained environmental isolates and microalgal cultures from culture collections were used for screening of lactose assimilation. Obtained isolates have been identified as *Graesiella emersonii* KM01 and *Tetradesmus obliquus* OM02, as well as unidentified isolate X1. Growth of isolates has been compared to that of *Chlorella vulgaris* CCAP 211/11, already being commercially widely used, and other cultures – *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14, *Galdieria sulphuraria* UTEX 2919. Heterotrophic growth pattern demonstrates (Figure 1), that all the used microalgal cultures prefer glucose, as it is monosaccharide and is more accessible to microalgae metabolism. Heterotrophic growth of *G. emersonii* KM01 and *T. obliquus* OM02 and *C. zofingiensis* CCAP 211/14 on lactose showed high biomass yield, compared to other cultures, respectively 0.20 ± 0.03 g/L, 0.08 ± 0,01 g/L and 0.29 ± 0,01 g/L, respectively, which could be related to higher *lacZ* gene activity. This gene is responsible for β – galactosidase enzyme production in microalgae and further lactose hydrolysis into monomers. Other microalgal cultures are less active in using lactose, which could be attributed to a lower β – galactosidase production by the strains (Bentahar et al., 2019). By comparing results of *G. emersonii* KM01, *T. obliquus* OM02 and *C. zofingiensis* CCAP 211/14 to other strains, all of them demonstrated significantly ($p < 0,05$) higher biomass yield compared to other as well as significant differences between the strains. Overall, there are not sufficient research made to investigate β – galactosidase activity in microalgae strains. In research carried out by (Zanette et al., 2019), eight microalgae strains were cultivated, from which only three demonstrated β – galactosidase activity in growth media supplemented with lactose at concentration 5 g/L.

By performing further mixotrophic growth experiments in whey, it has been assessed that both environmental isolates and *C. zofingiensis* CCAP 211/14 demonstrated higher biomass productivity than in photoautotrophic control, which can be attributed to the fact that during mixotrophic growth both light and organic carbon sources for microalgae are available for biomass synthesis and metabolic reactions. It means, that more overall energy is available to be diverted to biomass and metabolites production, than just for cell maintenance processes (Smith et al., 2015).

Results demonstrate that for *G. emersonii* KM01 biomass production the optimal whey concentration is 20 % (Figure 2). At this whey concentration it is possible to obtain 1.93 ± 0.06 g/L of dry biomass, which is by 89.12 % more than in control group grown in the 3N-BBM-V medium without whey supplement. Lowest results were obtained from 100 % and 5 % whey concentrations in the medium, 1.09 ± 0.05 g/L and 1.07 ± 0.04 g/L, respectively. It is about 56 % less, than it has been possibly to acquire from medium supplemented with 20 % whey.

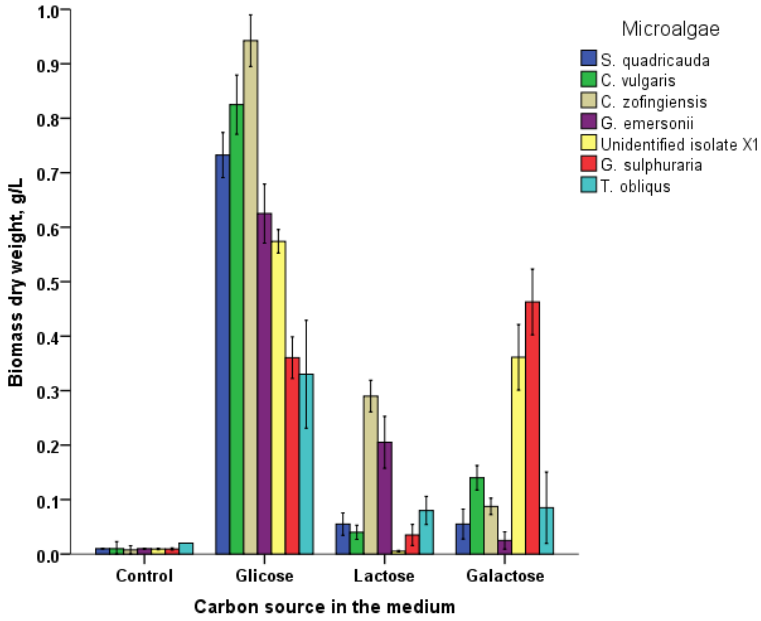


*Figure 1.* Heterotrophic growth of *Scenedesmus quadricauda* CCAP 276/16, *Chlorella vulgaris* CCAP 211/11, *Chromochloris zofingiensis* CCAP 211/14, *Graesiella emersonii* KM01, unidentified isolate X1, *Galdieria sulphuraria* UTEX 2919 and *Tetradesmus obliquus* OM02 in 3N-BBM-V media with added glucose, lactose or galactose compared to 3N-BBM-V control without any added sugars.

Results of *T. obliquus* OM02 cultivation in whey medium (Figure 3) demonstrate that whey concentration wherein the highest biomass is achievable is 50 %, which resulted in 2.83 ± 0.11 g/L of biomass and is the highest yield between three microalgal isolates.

By comparison to the control group (0.09 ± 0.01 g/L), it is by 96.82 % greater dry biomass weight. Notably, that *T. obliquus* OM02 demonstrated its ability to grow in high concentrations of whey, reaching dry biomass weight of 1.88 ± 0.25 g/L in the 100 % (undiluted) whey medium. This means, that it has great adaptability to produce β – galactosidase in presence of lactose in high concentrations, which results in higher presence of lactose monomers in media for use in its metabolic processes. As demonstrated in the Figure 1, microalgae prefer monosaccharides as they can be more easily metabolised (Smith et al., 2015).

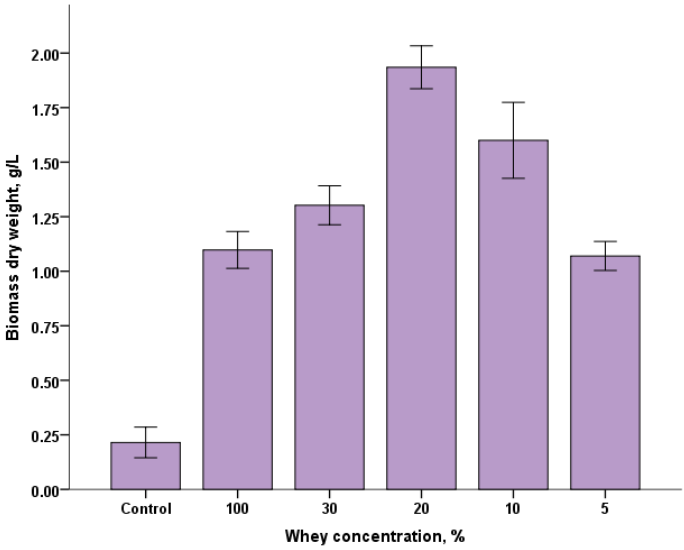


*Figure 2. Graesiella emersonii* KM01 growth in different whey concentrations (diluted with distilled water). Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

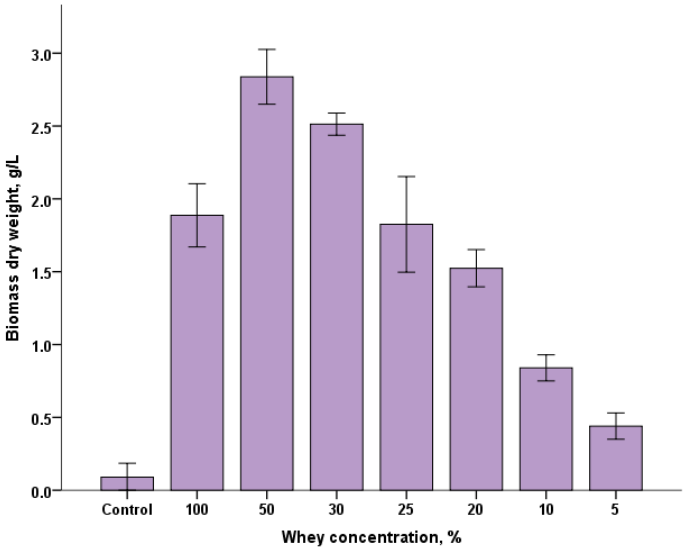


*Figure 3. Tetradesmus obliquus* OM02 growth in whey of different concentrations (diluted with distilled water). Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

Results of *C. zofingiensis* CCAP 211/14 cultivation in whey medium (Figure 4) demonstrate that the optimal whey concentration is 6.5 %. By far this is the lowest optimal whey concentration between reviewed microalgal cultures. Dry biomass weight in the medium supplemented with 6.5 % whey was 0.72 ± 0.08 g/L. However, by comparison with control group (0.05 ± 0.005 g/L) it is by 93.06 % more. Whey concentration above 10 % showed low biomass production for this culture, it can be seen for 10 % diluted whey – 0.14 ± 0.01 g/L, for 20 % – 0.12 ± 0.01 g/L and for 100 % – 0.06 ± 0.01 g/L. Here clearly can be seen that high concentrations of lactose can cause inhibitory effect on biomass production (Fu et al., 2019).

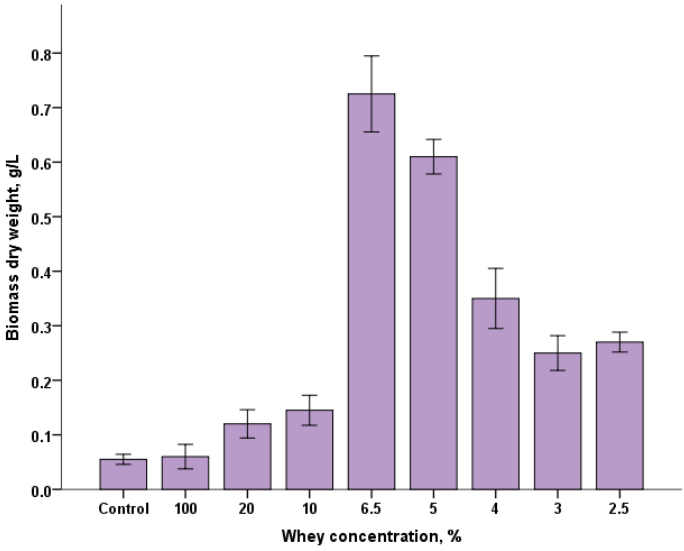


*Figure 4. Chromochloris zofingiensis* CCAP 211/14 growth in different whey concentrations diluted with distilled water. Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

By comparing results of the three microalgae strains, the most promising one for achieving high biomass yield, thus also for decreasing environmental impact of improper whey disposal, was demonstrated to be *T. obliquus* OM02. Both *G. emersonii* KM01 and *C. zofiengiensis* CCAP 211/14 can be considered as relatively slow growing microalgae on lactose substrates, which do not exceed results of *T. obliquus* OM02. Results confirm that during mixotrophic growth in whey media it is possible to obtain higher biomass yield compared to photoautotrophic cultivation, which can be attributed to whey composition. Whey composition is rich in sugars, mainly lactose, also glucose, galactose, and traces of other sugars are present and can be potentially used by microalgae as an additional carbon source. It is also rich with minerals and other growth promoting factors, which could contribute to higher biomass yield. As well C/N ratio in whey could be more favorable then it is in a synthetic medium (Gao et al., 2019; Nabizadeh et al., 2020).

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# FAUNA OF SOIL PREDATORY MESOSTIGMATA MITES (ACARI, PARASITIFORMES) IN THE URBAN GRASSLANDS OF RĪGA, LATVIA

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**Abstract:** Soil invertebrates are an integral part of soils and are important for the formation of soils. Soil invertebrates and among them Mesostigmata mites, play a vital role in the production and maintenance of healthy soils. Unfortunately, the significance of the soil invertebrates is seldom recognized. In collaboration with Latvian Fund for Nature and in the frames of the project "Introducing adaptive community-based biodiversity management in urban areas for improved connectivity and ecosystem health urban LIFE circles" collecting of soil samples in Rīga grasslands was performed. In total collection in 14 urban grassland sampling sites was made. Samples were taken by the soil corer and extracted on modified Berlese-Tullgren funnels. Although there was no intensive trampling in the investigated sites, grassland fragmentation and closeness of intensive traffic made a great impact on soil mite fauna. Mesostigmata mite species composition of investigated urban city sites differed from that in the natural grasslands of Latvia. Totally 20 Mesostigmata mite species in Rīga grasslands were determined. Nine of those species are also known from the natural grassland habitats in Latvia. The most frequent Mesostigmata species in urban grasslands were members of the families Rhodacaridae, Parasitidae, and Laelaptidae. Mesostigmata species, known as eudominant in the territory of Latvia, were not found in the urban grassland soils of Rīga.

**Key words:** mites, urban habitats, predation, soil invertebrates

## Introduction

The soil is among the most complex habitat systems, yet its biological systems are poorly understood (Stork, Eggleton, 2014). Soil provides a living space for at least part of the life cycle of many animals. The connectivity of soil foodwebs means that most, if not all, terrestrial organisms depend directly or indirectly on biological processes in the soil. Understanding urban ecosystems requires information about the response of soil biotic communities to environmental changes within large cities (Smith et al., 2006). Unfortunately, the significance of the soil invertebrates is seldom recognized.

Mites are important in the soil as fungivores, detritivores, bacterivores and predators (Largerlof, Andren, 1988). Among the predatory mites, the well-known are Mesostigmata mites (Coleman et all, 2018, Koehler, 1999, Salmane, Brūmelis, 2011). Most of them are free-living predators and they are known for their wide range of habitats. Mesostigmata mites are dominant acarine predators playing a crucial role in soil food webs and are used to be indicators of the state of soil ecosystems including urban ones (Koehler, 1994, 1999, Manu et al., 2021). Mesostigmata mites are adapted to the respective soil conditions like soil moisture, temperature, structure, and chemical composition and they are highly sensitive to changes in those parameters (Coleman et all, 2018). Their presence or absence in the soil horizons may be a good base for describing changes in environmental conditions and ecosystem perturbations (Koehler, Melecis, 2010). Mesostigmata mites prey on springtails (Collembola), nematodes (Nematoda), other mites (Acari), enchytraeids (Enchytraeoidea), insect eggs and larvae. An indicator of the degree of ecosystem degradation is the number of Mesostigmata mite species and individuals, and species composition.

So far there are few investigations of urban soil invertebrates in Latvia (Grina et al., 2023, Minova et al., 2015, Telnov, Salmane, 2015). The current study aimed to get insight into the fauna of Rīga grassland habitats.

## Material and Methods

In the frames of the project "Introducing adaptive community-based biodiversity management in urban areas for improved connectivity and ecosystem health urbanLIFEcircles," collection of soil samples in Rīga grasslands was made in October 2021. In total sampling was performed in 14 urban grassland sites. Samples were taken by the soil corer of 5 cm diameter. Extraction of soil invertebrates for ten-day period on modified Berlese-Tullgren funnels was performed. Microscopic slides for Mesostigmata mites were made. Mesostigmata species identification (Bregetova, 1977, Kaluž, S., Fenda, P., 2005, Karg, 1993) was made.

## Results and discussion

In total, 20 Mesostigmata mite species in urban grasslands of Rīga were collected. Three of those species were the most frequent.

In 11 sampling sites *Rhodacarellus silesiacus* (Willmann, 1936) (Figure 1), *Dendrolaelaps foveolatus* (Leitner, 1949), and *Hypoaspis nolli* Karg, 1962 were found. Of those, *R. silesiacus* and *D. foveolatus* also had the highest number of individuals, 20 and 32 respectively. From 10 sampling sites *Parasitus beta* Oudemans & Voigts, 1904, 8 sites – *Asca bicornis* (Canestrini & Fanzago, 1887) (Figure 1), and 7 sites – *Hypoaspis aculeifer* (Canestrini, 1884) were sampled.

*Table 1.* Mesostigmata mite species and number of sampling sites they were recorded from urban habitats of Rīga city grasslands, October 2021. Identified species arranged in systematic order by genus.

| Mesostigmata mite species | Number of grassland sites in Rīga |
|---|---|
| *Leioseius minutus* | 1 |
| *Leioseius bicolor* | 2 |
| *Leioseius halophilus* | 2 |
| *Lasioseius youcefi* | 1 |
| *Rhodacarus mandibularis* | 3 |
| *Rhodacarellus silesiacus* | 11 |
| *Dendrolaelaps foveolatus* | 11 |
| *Dendrolaelaspis angulosus* | 1 |
| *Asca bicornis* | 8 |
| *Hypoaspis aculeifer* | 7 |
| *Hypoaspis vacua* | 2 |
| *Hypoaspis nolli* | 11 |
| *Hypoaspis karawaiewi* | 2 |
| *Ololaelaps placentula* | 1 |
| *Laelaspis astronomicus* | 3 |
| *Macrocheles glaber* | 5 |
| *Prozercon traegardhi* | 1 |
| *Pergamasus teutonicus* | 4 |
| *Pergamasus lapponicus* | 3 |
| *Parasitus beta* | 10 |

*Figure 1. Rhodacarellus sileasiacus* (Rhodacaridae) (left) and *Asca bicornis* (Ascidae) (right) from urban grasslands of Rīga city (images from the microscopic slides, courtesy Ineta Salmane).

Of the recorded Mesostigmata species *R. silesiacus* is well known as a pioneer species in disturbed soil habitats (Bregetova, 1977, Karg, 1993). It has Holarctic and Australian distribution and mainly is found in urban, barren, and poor soils. Also, *D. foveolatus* is usually found in anthropogenically impacted soils (Bregetova, 1977, Karg, 1993). *Hypoaspis nolli* is frequently recorded in newly forming soils (Bregetova, 1977, Karg,

1993). *Asca bicornis* is another common species in anthropogenically impacted, poor soils (Bregetova, 1977, Karg, 1993). *Hypoaspis aculeifer* and *A. bicornis* are recorded as dominant species in anthropogenically impacted ecosystems of Romania (Manu, Onete, 2016). *Parasitus beta* occurs in various habitats, including anthropogenic ones, and natural grasslands (Bregetova, 1977, Karg, 1993). *Rhodacarus mandibularis* Berlese, 1921 (Figure 2) was found only in three collection sites in urban grasslands of Rīga. It is known as not numerous, but typical inhabitant of anthropogenically impacted soils (Bregetova, 1977, Karg, 1993). Although there was no intensive trampling in the investigated sites of Rīga, grassland fragmentation and closeness to intensive traffic made a great impact on soil mite fauna. Soil Mesostigmata species composition showed significant variability among natural and urban grassland habitats. *Veigaia nemorensis* (C. L. Koch, 1839) (Figure 2) and *Pergamasus vagabundus* Karg, 1968 are known as eudominant species in the territory of Latvia (Salmane, Brūmelis, 2010). None of those were found during our study in urban grasslands of Rīga. Out of the species recording during the present study, nine Mesostigmata species are common for natural and urban grasslands. *Laelaspis astronomicus* C. L. Koch, 1839, *Dendrolaelaps foveolatus*, *Rhodacarellus sileasiacus, Rhodacarus mandibularis, Leioseius halophilus* (Willmann, 1949), *Leioseius minutus* (Halbert, 1915) and *Macrocheles glaber* (Müller, 1860) are rarely recorded in natural grasslands of Latvia (Salmane, Brūmelis, 2010). *Asca bicornis* and *Leioseius bicolor* (Berlese, 1918) are eudominant species in natural grasslands of Latvia (Salmane, Brūmelis, 2010).

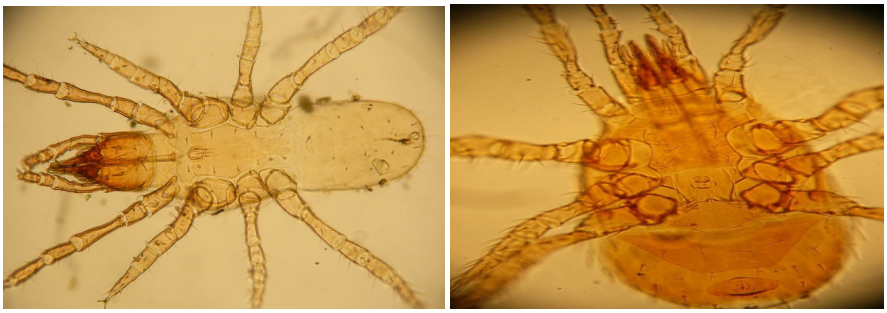

*Figure 2. Rhodacarus mandibularis* (Rhodacaridae) (left) and eudominant in the territory of Latvia *Veigaia nemorensis* (Veigaiaidae) (right) in urban grasslands of Rīga (images from the microscopic slides, courtesy Ineta Salmane).

## Conclusions

Species composition revealed in urban grasslands of Rīga is specific, characterized mainly by small Mesostigmata mite species, commonly found in anthropogenically impacted habitats and bound with poor soils. Few of those are known to be pioneer species, like *Rhodacarellus silesiacus* and *Rhodacarus mandibularis*, typical in the initial stages of disturbed soils succession.

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# APPLICATION OF SPONTANEOUS AND CULTURED FERMENTATION FOR THE SUSTAINABLE PROCESSING OF RASPBERRY POMACE INTO BIOPRODUCTS

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**Abstract:** Raspberry pomace is a by-product of the berry processing industry, which has limited application due to the high water content and risk of microbiological spoilage. Raspberry pomace is rich in phytochemicals that exhibit antimicrobial and antioxidant properties. Phytochemicals found in raspberry pomace can help to reduce the growth of technologically and nutritionally harmful microorganisms during the fermentation process and increase food functionality. For the production of fermented beverages, a symbiotic culture of microorganisms *Medusomyces gisevii* was utilized, composed of bacteria and yeasts. To ensure microbiological stability and safety of final product, lactic acid bacteria cultures (*Lactobacillus reuteri* and *Lactiplantibacillus plantarum*) were used in the secondary fermentation process. Results indicate that raspberry pomace improved the microbiological stability and functionality of fermented beverages.

**Key words:** plant by-products, fermentation, symbiotic culture of microorganisms, lactic acid bacteria, ultrasound pretreatment

## Introduction

The generation of plant-based waste is a relevant and widespread problem in the biotechnology and food industries. Globally, the largest amount of organic waste is generated in the food industry, especially in the processing of vegetables, fruits and berries (Sarker et al., 2024). As a result, the rational use of plant raw materials, especially their secondary products, has gained considerable attention.

The consumption habits of society are changing, an increasing number of diets aim to balance the ratio of plant and animal food, giving priority to plant-based food (Aschemann-Witzel et al., 2020). Therefore, it is relevant to develop technologies for the processing of plant raw materials and especially their secondary products.

Industrial processing of fruits and berries generates significant amounts of waste. Berry processing yields about 70–80 % of the target product and 20–30 % pomace, which consists of peels, seeds, and pulp (Struck et al., 2016). Raspberries are one of the most widely processed berries, thus large amounts of raspberry pomace is produced. Raspberry pomace has not yet been widely utilized due to the lack of technological solutions and the risk of microbiological contamination (Szymanowska et al., 2021). Pomace, as a by-product of berry processing, is often discarded, resulting in the loss of valuable components such as fibres, sugars, proteins, unsaturated fatty acids, anthocyanins, polyphenols and other secondary metabolites (Sommer et al., 2023). Raspberry pomace is rich in phytochemicals – 100 g of fresh raspberry pomace contains 637,8 mg of polyphenols, 591,7 mg of flavonoids and 65,2 mg of anthocyanins (Vulić et al., 2011). In addition to antimicrobial effect, these phytochemicals have health benefits, such as anti-inflammatory and antioxidant activity in human body (Szymanowska et al., 2021).

Phytochemicals found in raspberry pomace can be used in fermentation processes as antimicrobial agents, thus replacing chemical preservatives or pasteurization (Brodowska et al., 2017). Phenolic compounds found in raspberry pomace exhibit inhibitory effects against *Clostridium*, *Enterococcus*, *Escherichia*, *Mycobacterium*, *Salmonella* and *Staphylococcus* bacteria species (Brodowska et al., 2017).

Symbiotic culture of microorganisms *Medusomyces gisevii* has gained a growing interest in the production of fermented plant-based beverages. *Medusomyces gisevii* is a complex community of microorganisms, composed of bacteria and yeasts (Villarreal-Soto et al., 2018). The primary microorganisms found in *Medusomyces gisevii* include the bacteria *Acetobacter*, *Clostridium*, *Lactobacillus*, *Lactococcus*, and *Gluconacetobacter*, as well as yeasts like *Bretanomyces*, *Candida*, *Saccharomyces*, *Schizosaccharomyces*, *Torulopsis*, *Zygosaccharomyces*, and others (Flyurik et al., 2023). This symbiotic culture of bacteria and yeasts facilitates three types of fermentation: lactic acid, alcoholic, and acetic acid fermentation. Traditionally, *Medusomyces gisevii* is used to ferment sweetened black or green tea. The fermentation process takes place in two stages: during the primary fermentation, the sweetened tea is fermented, and in the secondary fermentation, fruits, berries, or other plant-based ingredients are added. Research has also demonstrated the successful use of alternative substrates instead of sweetened tea in the fermentation process. Several researchers have conducted studies with substrates such as coconut water (Watawana et al., 2015), grape juice (Ayed et al., 2016), and medicinal herbs (Velićanski et al., 2013). These studies suggest that *Medusomyces gisevii* can be successfully adapted for the fermentation of various plant-based raw materials.

Lactic acid bacteria (LAB) can be used in the production of fermented beverages, especially to extend their shelf life and safety. The literature indicates that certain LAB cultures exhibit antimicrobial activity against foodborne pathogenic microorganisms, including bacteria, yeasts and filamentous fungi. In addition, LAB can neutralize mycotoxins released by filamentous fungi (Agriopoulou et al., 2020). LAB are generally considered safe for humans and widely used in the food industry, for example *Lactobacillus reuteri* and *Lactiplantibacillus plantarum*. Also, LAB are naturally found in human gut microbiota (Zapansnik et al., 2022).

The antimicrobial effect of LAB is based on their ability to produce lactic acid and other organic acids, as well as hydroperoxide and bacteriocins, which inhibit the growth of pathogenic microorganisms (Abedi et al., 2020). These properties of LAB are very important and could be used to replace chemical and physical preservation methods (Yadav et al., 2021).

Combination of microorganisms with phytochemicals extracted from plant raw materials can help to ensure the microbiological stability of the product and avoid the use of chemical preservatives. Additionally, the use of biologically active compounds and probiotic bacteria can increase the functionality of the final product.

## Material and methods

### Microorganisms

Cultures of *Medusomyces gisevii*, *L. plantarum* and *L. reuteri* were taken from the collection of the microbiology science laboratory of Food Institute, Kaunas University of Technology.

### Ultrasound pretreatment of raspberry pomace

Ultrasound pretreatment of raspberry pomace was carried out in *Ulsonix Proclean 3.0DSP* 37 kHz (70 W) ultrasonic bath. Fresh raspberry pomace obtained after juice extraction was used for the research. Raspberry pomace was poured into plastic polyethylene bags (8 × 14 cm) with fasteners, 25 grams each. Ultrasound pretreatment was carried out at 35 °C, with ultrasound intensity of 70 % and 45 min pretreatment time.

### Primary and secondary fermentation

*Primary fermentation.* A fermentable solution was prepared under aseptic conditions in the microbiology laboratory. Green tea (3 g/ 1 L) was added into a cylindrical glass container with a capacity of 2.5 L and poured with boiling water. After cooling to 25–27 °C, the tea was strained and unrefined cane sugar (70 g / 1 L) was added. The resulting mixture was homogenized and cooled to a temperature of 20 ± 1 °C. The biological structure of *Medusomyces gisevii* was placed in a 2.5 L container with a cooled mixture of green tea and sugar and fermented for 4 days at a temperature of 20 ± 1 °C.

*Secondary fermentation.* The fermented liquid obtained after primary fermentation was poured into sterile 200 ml bottles. Raspberry pomace (fresh and ultrasonically treated) and LAB suspensions (*L.plantarum* and *L.reuteri*) were added to the bottles containing fermented liquid. Secondary fermentation was carried out for 3 days at a temperature of 20 °C ± 1 °C. After secondary fermentation, fermented beverages were filtered and stored at 3 °C ± 1 °C.

### Microbiological analysis

Microbiological parameters were determined following the LST ISO 21527-1:2008, LST ISO 21527-1:2008, LST ISO 4832:2006, LST ISO 16649-2:2009, LST EN ISO 4833-1:2013 standards.

### Fermented beverage samples composition analysis

The total concentration of phenolic compounds was determined by the Folin–Ciocalteu method (Makkar et al., 2007) and the values were expressed as mg of tannic acid equivalent (TAE)/L of fermented beverage.

The total concentration of monomeric anthocyanins was determined by the pH differential method (Lee et al., 2005) and the values were expressed as mg cyanidin-3-glucoside equivalent (CGE)/L of fermented beverage.

Antioxidant activity was determined by the DPPH method (Brand-Williams et al., 1995) and the values were expressed as mg trolox equivalent (TE)/L of fermented beverage.

### Evaluation of microbial stability over 20-day storage period

Microbiological stability analysis was performed to evaluate the microbiological stability of fermented beverage samples over a 20-day period. Samples were analyzed at four different time points: day 0, day 6, day 13 and day 20. At each time point, the number of yeasts, moulds, mesophilic lactic acid bacteria, anaerobic microorganisms, coliform bacteria and *E. coli* bacteria were determined.

## Results and discussion

### Fermented beverage samples analysis

The results of the study show that ultrasound pretreatment of raspberry pomace resulted in significantly higher concentrations of total phenolic compounds, monomeric anthocyanins and antioxidant activity in the fermented beverages.

It was determined that the use of ultrasound pretreated pomace in the production of fermented beverages (samples US, US-R, US-P) resulted in an average of 32,34 % higher concentrations of phenolic compounds and 17,96 % higher concentrations of monomeric anthocyanins compared to the samples prepared with untreated raspberry pomace (RP, RP-R, RP-P). The results are provided in Figure 1.

Anthocyanins were present in the fermented beverage samples due to the inclusion of raspberry pomace. As a result, the control samples (C, CR, CP) did not contain anthocyanins. Additionally, control samples exhibited lowest concentrations of phenolic compounds.

The research revealed that ultrasound pretreatment of raspberry pomace led to an average increase of 4,02 % in antioxidant activity in fermented beverage samples compared to untreated raspberry pomace samples (RP, RP-R, RP-P). The results are presented in Table 1.

## Microbiological stability of fermented beverage samples

After the preparation of fermented beverage samples (secondary fermentation stage) microbiological stability studies were performed. To assess the impact of raspberry pomace (both fresh and ultrasound-pretreated) and LAB on the microbiological stability of fermented beverages over 20 days, samples were analyzed at four intervals: day 0, day 6, day 13, and day 20. The number of yeasts, mesophilic LAB, aerobic microorganisms and pH values of samples over a period of 20 days is presented in Figure 2.

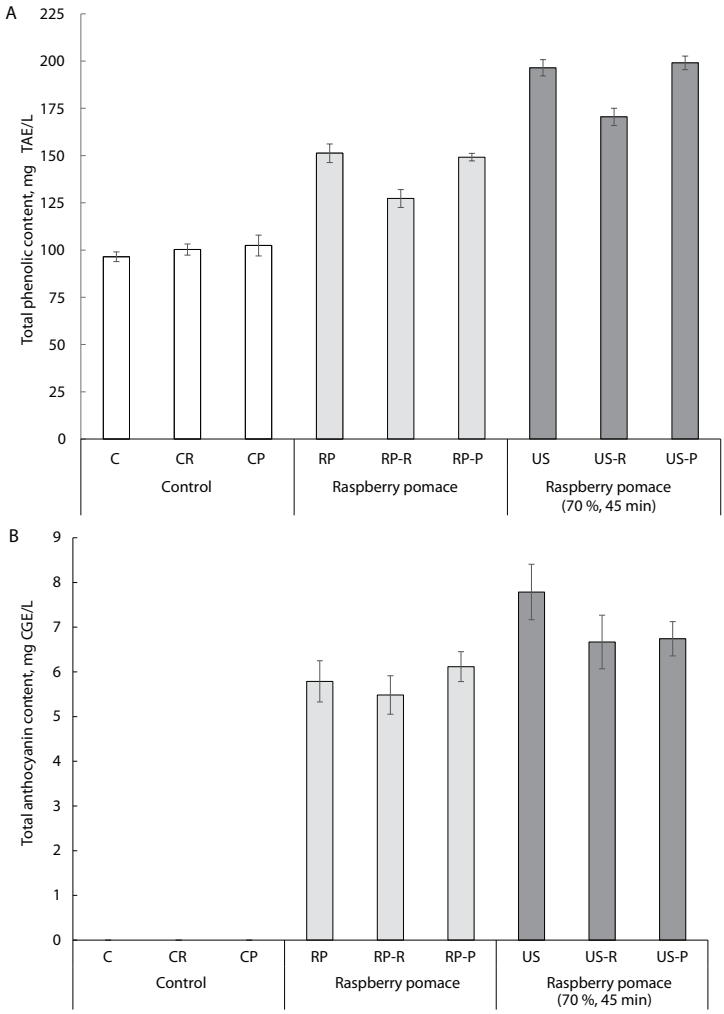


*Figure 1.* The total concentration of phenolic compounds (A) and monomeric anthocyanins (B) in fermented beverages. Control samples (C, CR, CP) – fermented beverages without raspberry pomace, (RP, RP-R, RP-P) – fermented beverages with raspberry pomace, (US, US-R, US-P) – fermented beverages with ultrasound pretreated raspberry pomace. Samples CR, RP-R and US-R were prepared with addition of *L. reuteri*; samples CP, RP-P, US-P were prepared with addition of *L. plantarum*.

*Table 1.* Antioxidant activity of fermented beverages expressed as mmol Trolox equivalent (TE/L)

| Sample | | Antioxidant activity, mmol TE/L |
|---|---|---|
| C | Control samples (without raspberry pomace) | 0.87 ± 0.01 |
| CR | | 0.87 ± 0.00 |
| CP | | 0.88 ± 0.00 |
| RP | Samples with raspberry pomace | 0.89 ± 0.00 |
| RP-R | | 0.89 ± 0.00 |
| RP-P | | 0.88 ± 0.00 |
| US | Samples with ultrasound pretreated raspberry pomace | 0.91 ± 0.01 |
| US-R | | 0.93 ± 0.00 |
| US-P | | 0.94 ± 0.00 |

After 6 days of storage, it was found that samples prepared with raspberry pomace inhibited yeast development (Figure 2, B) and reduced the number of aerobic microorganisms (Figure 2, C), compared to control samples C, CR and CP, which were prepared without raspberry pomace.

However, by the 13$^{th}$ day, there was a decline in LAB concentrations in all fermented beverage samples (Figure 2, A) and an increase of aerobic microorganisms in the samples containing raspberry pomace.

By the 20$^{th}$ day, samples with raspberry pomace had stable levels of aerobic microorganisms, yeast and LAB, similar to the concentrations observed on the 13$^{th}$ day. Results indicate that beverages containing raspberry pomace (both fresh and ultrasound pretreated) were more microbiologically stable, compared to control samples (C, CR, CP).

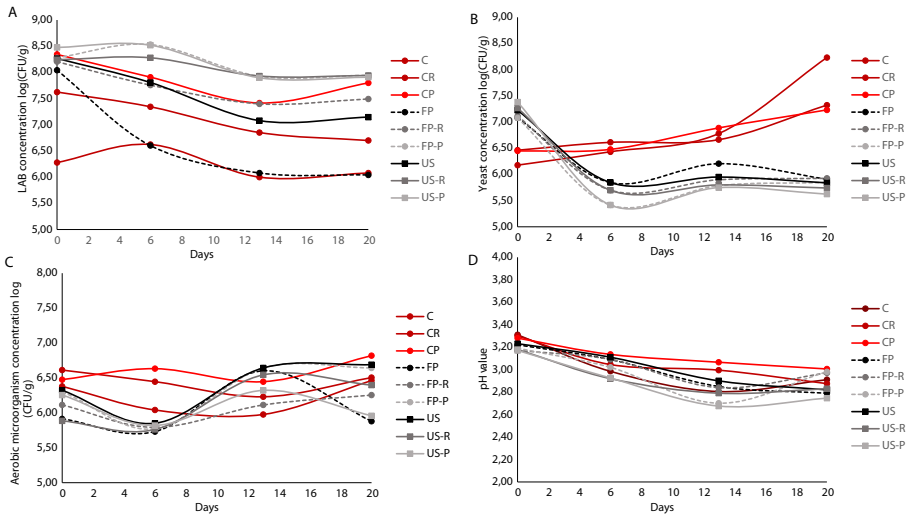


*Figure 2.* The concentration of LAB (A), yeast (B), aerobic microorganisms (C) and pH values (D) of fermented beverage samples. Control samples (C, CR, CP) – fermented beverages without raspbery pomace, (RP, RP-R, RP-P) – fermented beverages with raspberry pomace, (US, US-R, US-P) – fermented beverages with ultrasound pretreated raspbery pomace. Samples CR, RP-R and US-R were prepared with addition of *L. reuteri*; samples CP, RP-P, US-P were prepared with addition of *L. plantarum*.

Concentrations of coliform bacteria, *Escherichia coli* bacteria and mould remained insignificant (< 1 CFU/g) in all fermented beverage samples throughout the 20-day period.

## Conclusions

Research shows that ultrasound pretreated raspberry pomace resulted in higher concentrations of phenolic compounds and anthocyanins in samples of fermented beverages and increased their antioxidant activity. Addition of raspberry pomace resulted in more microbiologically stable fermented beverage samples, compared to control. The results of the study show that the by-product of the berry processing industry can be effectively used for the production of probiotic fermented beverages.

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# MARKING GREYLAG GOOSE *ANSER ANSER* WITH COLOUR NECKBANDS IN LATVIA – THE FIRST RESULTS

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**Abstract:** During the breeding seasons of 2021–2024, 55 greylag geese were marked with leg and neck bands (2 adults, 53 juveniles). Since then, 47 resighting records for 19 birds have been obtained. Before the start of the hunting seasons all resightings came from ringing sites in Latvia. Starting from mid-August birds appear to use Nemunas River delta region in Lithuania, from where resightings came from fish-ponds, a natural lake Kroku Lanka and from arable land. From September till December our geese have been reported from Poland, in October and November – from Germany. The resightings lie in SW direction from Latvia and up to 740 km from the ringing sites.

**Key words:** ringing, migration, staging

## Introduction

Greylag goose is a species with increasing regional population in Latvia. In 2013–2018 its population estimate was 200–500 pairs with 44 national breeding bird atlas squares of possible to prooved breeding (Ķerus et al. 2021). Prior to this study only 8 greylag geese have been ringed in Latvia and no ring recoveries available. Exact wintering, staging and moulting sites of these birds are unknown.

## Material and methods

The first greylag goose broods in Latvia appear in mid- to late April. By late June – early July they have reached the age when leg size is fit enough for banding, but the birds are still flightless. Also, adults may be flightless at that time. Birds were 1) chased and caught with a dip-net or bare hands or 2) net lines were erected in the reedbeds (96 m total length, mesh size 50–70 mm) and checked every 3 hours.

Geese were marked with leg rings and plastic neck bands (height 55 mm, diameter 45 mm, yellow colour). The neck bands carried a 4-digit letter and number combination, colour black. Bird age and in 2021 also sex according to cloaca examination was registered.

The ringing sites were three NATURA sites and a smaller pond in western Latvia (Figure 1).

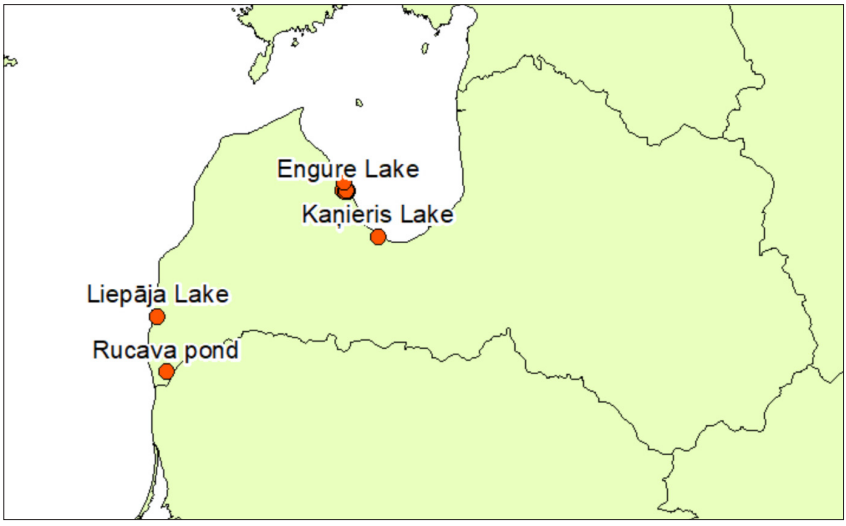


*Figure 1.* Location of the ringing sites in Latvia.

In total during breeding seasons 2021–2024, 55 birds were marked (Table 1).

*Table 1.* Number of geese marked at different sites in 2021–2024.

| Ringing site | Year | | | | Total |
|---|---|---|---|---|---|
| | 2021 | 2022 | 2023 | 2024 | |
| Engure Lake | 14 | 1 | 8 | 9 | 32 |
| Kaņieris Lake | | 1 | 1 | 4 | 6 |
| Liepāja Lake | | | | 7 | 7 |
| Rucava Pauguru pond | | | | 10 | 10 |
| Total | 14 | 2 | 9 | 30 | 55 |

## Results and discussion

### Resighting countries

Since the beginning of the study, 47 resightings of 19 individuals have been accumulated (Table 2). Till early August all resightings came from the ringing sites in Latvia.

Starting from mid-August birds use Nemunas River delta region in Lithuania, from where resightings come from fish-ponds, a natural lake Kroku Lanka and arable land. From September till December the marked geese have been reported from Poland (most records near seacoast but also up to 200 km inland), in October and November – from Germany (the Baltic Sea coastal lagoons and their vicinity). One family group has been recorded in October still in Latvia.

Resightings from the 2nd and following calendar years refer to three birds marked as juveniles in Lake Engure in 2021. A female spent her 1st winter, spring and 2nd summer in Lithuania (8 reports). One male was spotted twice during the 2nd summer in the hatching lake, October the 2nd autumn in Germany, and October the 4th autumn in Poland. Other male has been spotted at the native lake in spring of its 3rd year, and in Poland during autumn of its 4th calendar year (Figure 3).

*Table 2.* Resightings of Greylag Geese ringed in Latvia in 2021–2024. Standard country codes used.

| Year after ringing | Month | LV | LT | PL | DE | Total |
|---|---|---|---|---|---|---|
| 1st | July | 5 | | | | 5 |
| | August | 3 | 1 | | | 4 |
| | September | | 1 | 1 | | 2 |
| | October | 3 | 4 | 6 | 2 | 15 |
| | November | | | | 3 | 3 |
| | December | | | 1 | | 1 |
| | Total 1st cal. year | 11 | 6 | 8 | 5 | 30 |
| 2nd | February | | 2 | | | 2 |
| | April | 1 | 4 | | | 5 |
| | May | | 1 | | | 1 |
| | July | 1 | | | | 1 |
| | August | | 1 | | | 1 |
| | October | | | | 1 | 1 |
| | Total 2nd cal. year | 2 | 8 | | 1 | 11 |
| 3rd | April | 1 | | | | 1 |
| | Total 3rd cal. year | 1 | | | | 1 |
| 4th | October | | | 4 | | 4 |
| | November | | | 1 | | 1 |
| | Total 4th cal. year | | | 5 | | 5 |
| Total | | 14 | 14 | 13 | 6 | 47 |

## Migration distance

The resightings lie in SW direction and up to 740 km from the ringing sites in Latvia (Figure 2).

Studies from the neighbouring countries demonstrate longer travel distances. Birds from Estonia ringed in 1960ies had been found wintering in Austria (1000 km) and even Algeria (ca 3000 km from nesting site) (Kiscinsky 1979). Birds, nesting in Finland

nowadays travel 1500–2000 km to their wintering grounds (Piironen & Laaksonen 2023). Greylag geese nesting in Sweden have recently shortened their migration routes, presumably due to milder winters. Still, many individuals migrate 750–1000 km. Only the subpopulations from southern Sweden travel even shorter distances than our birds (Månsson et al. 2022).

When evaluating the migration distance and direction, not only climate change but also origin of Latvian regional population should be taken into consideration. In 1970ies the greylag goose regional breeding population in Latvia did not exceed 10 pairs (Mednis, 1983). Part of this small number came from reintroduction attempts where birds from Astrakhan, southern Russia (different flyway and a different subspecies – *Anser a. rubrirostris*) were used. Strazds & Ķuze describe history of such reintroduction attempt in Kaņieris Lake – in 1969–1971 goslings had been held captive on one of islands. In 1972 they had been released and in 1972–1974 the first nests of these birds had been found (Strazds & Ķuze 2006). The translocated birds had no parents with them who could teach the migration route. As Sokolovskis team has proven in a similar case for the taiga bean goose *(Anser f. fabalis)*, also unrelated birds can serve as migration mentors (Sokolovskis et al. 2024). We speculate, that the geese introduced at Kaņieris and their descendants could have followed greater white-fronted geese *Anser albifrons* during their first migration.

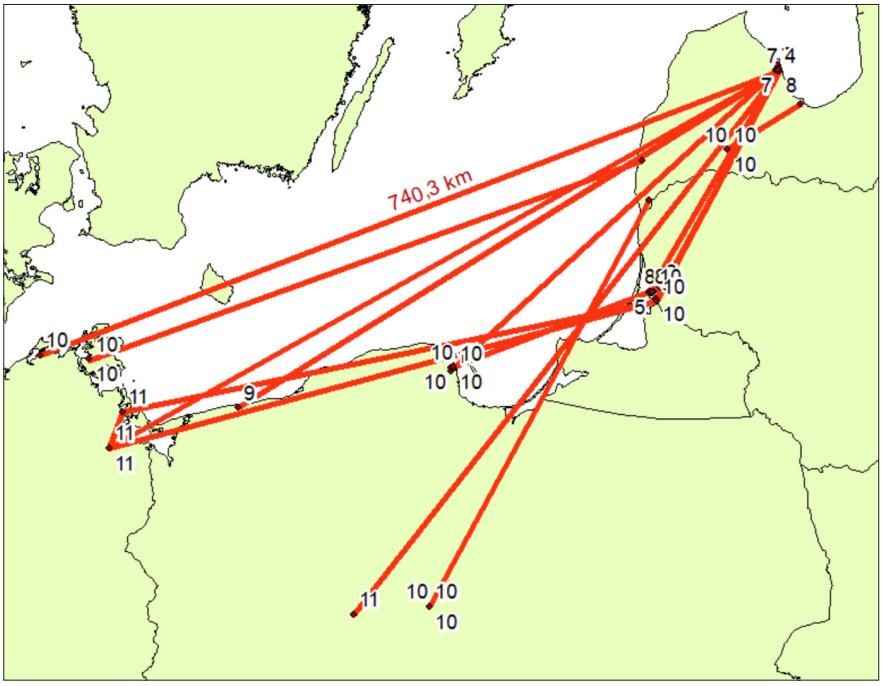


*Figure 2.* Resighting months and connection with the ringing sites of the Latvian individuals of greylag goose.

## Departure time

Two birds were observed already away from Latvia in mid-August. In 2004–2023 waterfowl hunting season in Latvia began on the 2nd Saturday of August. Thus in 2021 hunting began on August 14. Four days before this date one of our geese was present on Lake Engure. Four days later it was observed in Lithuania. The other bird has demonstrated similar migration pattern. Although until 2024 greylag goose season began only in September and there was no direct threat till that time, the hunting disturbance could have influenced the departure. The family group that was observed in Latvia still in October, originates from Lake Kaņieris – the ringing site with a complete hunting ban. The coincidence of the hunting disturbance and bird departure has been well marked in literature (Madsen & Fox 1995, Väänänen 2001, Adam et al. 2016, Kleinhenz & Koening 2018).

*Figure 3.* Greylag Goose LV1C hatched in Engure Lake staging in Poland during its 4th autumn. Image courtesy: Bartosz Krąkowski.

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# OPTIMIZATION OF THE PRODUCTION OF SODIUM ALGINATE DERIVATIVES

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**Abstract:** A natural polysaccharide sodium alginate, a salt of alginic acid, is widely used as a wetting and thickening agent in food, pharmaceuticals, cosmetics and other fields. Interest in the practical use of the derivatives of sodium alginate poses the problem of their convenient and cost-effective production.

Two methods applied for the synthesis of uronic acids from sodium alginate were studied. The previously created method of Prof. A. Mirshafiey (Mirshafiey & Lalander, 2024) has been tested in the laboratory. The method is based on the hydrolysis of sodium alginate with sulfuric acid followed by the separation of D-mannuronic acid (MA) and L-guluronic acid (GA), that included pH regulation and precipitation by centrifugation and quite long-time drying process. However, the method is rather complex, time-consuming and energy-intensive. To simplify and reduce the cost of uronic acids production, it was necessary to optimize the technology. Optimized in the laboratory method kept the previous step of sodium alginate hydrolysis unchanged. Further, the method differed in that the MA and GA mixture was precipitated, washed and filtered on membrane filter without prior separation. The obtained MA + GA gel composition may be used without drying in the creation of new innovative products for human health. The optimization of the method has greatly simplified the synthesis of sodium alginate derivatives and makes it possible to increase the economic efficiency of the process.

**Key words:** mannuronic acid, guluronic acid, production cost-effectiveness

## Introduction

Alginic acid, a widely used natural polysaccharide, is generally derived from brown seaweed. It can also be produced by microbial fermentation using specialized bacteria (Goh et al., 2012). Alginic acid naturally exists in cytoplasm and plays an important role in strengthening the cell wall (Kloareg & Quatrano, 1988).

Alginic acid is a linear polysaccharide consisting of two forms of linked hexuronic acid residues D-mannuronic acid (MA) and L-guluronic acid (GA), Fig. 1.

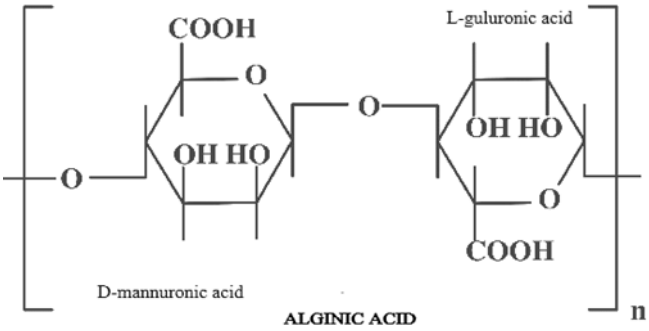


*Figure 1.* Structural formula of alginic acid.

One of the alginic acid forms in cells is sodium alginate, the extract of which is obtained from brown algae. Sodium alginate is a light powder, forming a highly viscous aqueous solution, which has the characteristics of thickening, suspending, emulsifying, stabilizing, forming a gel, forming a film and spinning fibres, and has a long and extensive use in the food, paper and cosmetic industries (Guo et al., 2020). Modern pharmacological studies have shown that alginic acid has antianaphylaxis effect (Jeong et al., 2006), immunomodulatory activities (Caipang et al., 2011; Fattahi, et al., 2015), antioxidant activities (Sarithakumari et al., 2013), and anti-inflammatory effects (Mirshafiey et al., 2007; Sarithakumari & Kurup, 2013). Alginate is a biocompatible and biodegradable natural product. Mannuronic and guluronic acids, as alginate hydrolysate components, are safe and non-toxic ingredients (Sharfi et al., 2024).

The biological activities of alginic acid and its derivatives are of interest for its practical production and further application. Technology for uronic acids production may have some problems including labor-intensive and unhealthy of the process as well as low productivity. Therefore, it is necessary to modify this product, considering the reduction in production costs. In other words, the technological process needs optimization to make it more efficient, productive and cost-effective. Streamlining activities provides improvement of process efficiency, ensures time, resources and financial savings (Miyambu & Seeletse, 2016). Process upgrades will improve technology and keep costs down.

To produce uronic acids from sodium alginate the original method (Mirshafiey & Lalander, 2024) was tested in the laboratory. It was established that the important indicators of the process – duration of the procedure and the cost of the final product – need to be corrected.

The purpose of the present study was to optimize the previously created method for the production of uronic acids and aimed at improving of the technology effectiveness and cost minimization.

## Material and methods

### Chemicals

1. Sodium alginate Type NA4012 (C. E. Roeper, German).
2. Sulfuric acid 95 % solution pure p. a. $H_2SO_4$ (Chempur, Poland).
3. Sodium carbonate, $Na_2CO_3$ (Chempur, Poland).
4. Hydrochloric acid, 35 % HCl (Chempur, Poland).

### Previously created method of sodium alginate derivatives production

The method of Prof. A. Mirshafiey (Mirshafiey & Lalander, 2024) was tested in the laboratory. To produce MA and GA according to this method the following technological steps were used. Hydrolysis of sodium alginate with 20 % $H_2SO_4$ at 85–90 °C during 3–4 h. Hydrolysate (mixture of MA and GA) was produced. Separation of the mixture of uronic acids. This step includes precipitation, centrifugation (4000 g), following re-dissolution by neutralization (using 1M $Na_2CO_3$). After pH regulation till 2.99 and next centrifugation (4000 g), the collected precipitate of L-GA washed with distilled water and spread over a smooth surface following long-term drying at room temperature. The supernatant of GA was adjusted to pH 1.0 by 0.5 M HCl, and MA precipitated by centrifugation (4000 g). The final precipitate (D-MA) washed with distilled water, also spread over the smooth surface, and dried out for 5–6 h. Separated uronic acids GA and MA can be mixed and used as a final product.

### Optimized method of sodium alginate derivatives production

The described above method of alginate - based synthesis of MA and GA was updated in the laboratory. The process of sodium alginate hydrolysis remained unchanged. However, the next step of the procedure was significantly optimized. The produced hydrolysate of sodium alginate (as precipitated MA + GA mixture) was filtrated on membrane filter without prior separation of uronic acids. The received uronic acid mixture washed with distilled water and filtered again. The obtained final gel product of MA + GA composition is a result of technological process optimization and may be used without drying.

## Results and discussion

The laboratory study showed that the previously created method of sodium alginate derivatives (MA and GA) production is rather complex, time-consuming and energy-intensive (Mirshafiey & Lalander, 2024). The activity of these two uronic acids was assessed separately. Having compared the biological effects of MA and GA, only non-essential differences between them were established (Fattahi, et al., 2015). At the same time, MA was the main necessary product, while GA almost was not used (Sharfi et asl., 2024). This suggested the possibility of practical use of a mixture of MA and GA without prior separation. This final composition is used for gel production.

Comparison of two represented methods procedures is offered in Table 1. When using the previously created method, the yield of the product was 53.8 ± 3.1 %, while the optimization of the procedure made it possible to raise this parameter by 21.9 %. Besides it should be noted that the reduction of technological process duration by 5 h is due to the omission of uronic acids separation step. In addition, there was observed a decrease in the consumption of components and the formation of waste requiring special disposal. Thus, it is obvious that the optimized method is simpler and cheaper.

*Table 1.* Manipulation algorithm for preparing a mixture of mannuronic and guluronic acids in two ways.

| **Steps of technological procedure** | |
|---|---|
| **Previously created method** | **Optimized method** |
| Weighing and loading the sodium alginate into the reactor | Weighing and loading the sodium alginate into the reactor |
| Addition of 20 % $H_2SO_4$ | Addition of 20 % $H_2SO_4$ (reduced acid volume by 50 %) |
| Precipitation of mannuronic and guluronic acids mixture | Precipitation of mannuronic and guluronic acids mixture (final product) ready for gel production |
| Dissolution of precipitate in alkaline solution (1M $Na_2CO_3$) | Was not carried out |
| Separation of guluronic acids precipitate | Was not carried out |
| Washing the received precipitate twice and centrifugation | Washing the received precipitate of mannuronic and guluronic mixture and filtration |
| Drying of guluronic acid precipitate | Was not carried out |
| Precipitation of mannuronic acid | Was not carried out |
| Separation of mannuronic acid, twice washing and centrifugation | Was not carried out |
| Drying of mannuronic acid precipitate, mixing of both uronic acids (final product) | Gel composition of mannuronic and guluronic does not require drying (final product) |
| Final product yield in g per 100 g of initial sodium alginate **53.8 %** | Final product yield in g per 100 g of initial sodium alginate **65.6 %** |

The Table 1 demonstrates the technological advantages of the optimized method. The final product (MA + GA composition) obtained by the improved procedure is a result of technological process parameters correction and does not require pre-drying before subsequent use.

The comparison of two final products – separated, dried and mixed MA + GA and optimized gel composition of MA + GA revealed the advantage of the last one. It has convenient consistency and improved consumer properties for further using.

The comparative cost and economic efficiency of the studied methods for obtaining sodium alginate derivatives are presented in Table 2.

*Table 2.* Consumption and cost of source materials expended per 100 g of dry matter of uronic acids

| Materials | Previously created method | | Optimized method | |
|---|---|---|---|---|
| | per 100 g | Eur per 100 g | per 100 g | Eur per 100 g |
| Sulfuric acid, conc., g | 349.3 | 2.20 | 142.5 | 0.90 |
| Sodium carbonate, g | 157.6 | 0.68 | 129.3 | 0.55 |
| Hydrochloric acid, conc., ml | 25 | 0.15 | Was not carried out | Was not carried out |
| pH calibration standard solution, ml | 80 | 1.50 | Was not carried out | Was not carried out |
| Sodium alginate, g | 185.8 | 4.19 | 158.7 | 3.57 |
| | | Total: 8.72 | | Total: 5.02 |

The ratio of the cost of raw materials to the cost of 100 g of the final product is 2.08 and 1.41 for the old and new methods, respectively. This allows us to conclude that the cost effectiveness of the modified technology is 32.3 % higher than for the previously developed version of production. In other words, the cost of producing the same amount of final product using the modified method will be one-third lower.

## Conclusion

The optimization of the technology for the synthesis of sodium alginate derivatives makes it possible to increase the economic efficiency of the process and significantly reduce the cost of final product. The gel composition of manuronic and guluronic acids obtained in this way is safe and may be used in the creation of new innovative products for human health.

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