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COJOCARI DANIELA

THE MICROBIOSTATIC ACTION OF SOME PLANT EXTRACTS OF PHENOLIC COMPOUNDS ON THE MICROORGANISMS RESPONSIBLE FOR FOOD SPOILAGE

253.06 Biological and chemical technologies in the food industry

Abstract of the PhD in engineering sciences dissertation

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This thesis was written within the Faculty of Food Technology, Technical University of Moldova; Preventive Medicine Department, Microbiology and Immunology Discipline of the State University of Medicine and Pharmacy "Nicolae Testemițeanu", Chisinau, Republic Moldova.

Supervisor:
STURZA Rodica, Doctor habilitatus, corresponding member ASM, professor
Consultant:
RUDIC Valeriu, academician, Doctor habilitatus, professor

Supervisory committee:
GHENDOV-MOȘANU Aliona, Doctor habilitatus, associate professor, UTM
MACARI Artur, Doctor, associate professor, UTM
BALAN Greta, Doctor habilitatus, associate professor, USMF

Official reviewers:
GHENDOV-MOȘANU Aliona, Doctor habilitatus, associate professor, UTM
CEPOI LILIANA, Doctor habilitatus, research coordinator, UTM
BURDUNIUC Olga, Doctor habilitatus, associate professor, ANSP

Members of the Scientific Committee for public defense of the doctoral thesis:
1. BALAN Valerian, president, Doctor habilitatus associate professor, UTM
2. DRAGANCEA Veronica, science secretary, Doctor, associate professor, UTM
3. PINTEA Adela, corresponding member ASM, Doctor habilitatus, associate professor, USAMV Cluj Napoca
4. STURZA Rodica, corresponding member ASM, Doctor habilitatus, associate professor, UTM
5. GHENDOV-MOȘANU Aliona, reviewer, Doctor habilitatus, associate professor, UTM
6. CEPOI LILIANA, reviewer, Doctor habilitatus, research coordinator, UTM
7. BURDUNIUC Olga, reviewer, Doctor habilitatus, associate researcher, NPHA

The defense will take place on 11.07.2024, at 10:00 at the meeting of the Committee for public defense of the doctoral thesis within the Technical University of Moldova, FTA, Studentilor 9/9 str., building 5, office 5-1, MD-2045, Chisinau, Republic Moldova.

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Summary was submitted on 10.06.2024.

Science Secretary of the Doctoral Committee,
DRAGANCEA Veronica, Doctor, associate professor ____________

Supervisor,
STURZA Rodica, Doctor habilitatus, corresponding member ASM, professor ____________

Author
COJOCARI Daniela ____________

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CONCEPTUAL POINTS OF THE RESEARCH

The actuality and importance of the topic. Foodborne illness is a common, costly, sometimes life-threatening - but largely preventable - public health concern (FDA). Researchers have identified more than 250 foodborne diseases. Most of them are infections, caused by a variety of bacteria, viruses and parasites. The Center for Disease Prevention and Control (CDC) estimates in 2021 that each year 48 million people get sick from a foodborne illness, 128,000 are hospitalized and 3,000 die (CDC, 2021; WHO, 2021).

Antimicrobial resistance (AMR) has grown to be one of the main urgent threats to public health, causing serious problems in the prevention and successful treatment of persistent diseases. Agricultural use of antibiotics is another significant contributor to antimicrobial resistance in humans (Mohr, 2016). Experts estimate that in the absence of a treatment solution for antimicrobial-resistant infections, approximately 300 million people will die prematurely in the next 35 years, and global economic losses will increase to 100 trillion dollars (Porooshat, 2019).

As we increasingly face the problem of resistance of microorganisms to antibiotics, it becomes obvious that only a natural approach, which would result in a reduced consumption of synthetic additives, could improve the situation. The use of plant extracts to reduce microbiological risks associated with food would be a promising solution for the health of the population. Pathogenic toxin-producing microorganisms that cause spoilage occur naturally in the environment and can be transferred to food products. Chemical food preservatives are widely used by the food industry to effectively prevent or delay food spoilage (Attarianshandiz, 2022). Several studies indicate that plant polyphenols can be used as chemotherapeutic agents, food preservatives, coloring agents and disinfectants (Cetin-Karaca, 201; Jouda, 2013; Attarianshandiz, 2022).

The reasoning for choosing this subject. Today, food products are obtained through the newest processes. Overproduction and the wide variety of supply forces producers to do everything possible to make their food successful. Thus, the products are preserved for long periods, more attractive dyes and artificially produced vitamins are introduced, etc. The availability of antibiotics to treat infectious diseases has significantly improved the health and life expectancy of humans, as well as the health and welfare of animals. The use of antibiotics has generated antimicrobial resistance in microorganisms. Food can act as a vector for the transfer of antimicrobial-resistant bacteria and antimicrobial resistance genes to humans. Alternatives to replace synthetic preservatives are increasingly sought. Thus, research aiming to develop food products with bioactive components and natural functional ingredients is currently more and more common. Antimicrobial resistance is a worldwide problem for both human and animal health.

The scientific hypothesis: Plant powders and extracts exhibit antimicrobial and antioxidant effects, which could allow for their use as food preservatives. To confirm this hypothesis, it was necessary to test the antibacterial effect of plant fractions as a result of direct contact with pathogenic microorganisms, as well as to evaluate the effect of plant fractions incorporated into food products, through their intentional contamination with pathogens capable of accidentally colonizing these categories of products, and to elucidate the microbiostatic effect of these plant fractions.

The research goal was to evaluate the microbiostatic action of powders and plant extracts from berries and spices on microorganisms responsible for food spoilage *in vitro* and *in situ*, on different categories of processed foods.

Research objectives:

1. Determining the microbiostatic and microbicide effect upon direct contact of plant fractions rich in phenolic compounds on the microorganisms that cause food spoilage i*n vitro*.
2. Determining the microbiostatic effect of certain plant fractions rich in phenolic compounds on meat product matrices *in situ*.
3. Establishing the microbiostatic effect of certain plant fractions rich in phenolic compounds on different matrices of dairy products *in situ*.
4. Describing the composition of bioactive compounds and their antioxidant properties.
5. Analysing the physical and chemical, sensory and morphological properties of cream cheese with encapsulated basil extract.

Summary of the scientific research methodology and justification of applied research methods. The study was carried out within the Preventive Medicine Department, Microbiology and Immunology Discipline of The "Nicolae Testemițanu" State University of Medicine and Pharmacy; and the Departments of Food Technology and Oenology, and Chemistry, The Food Technology Faculty of The Technical University of Moldova. Part of the research was also carried out in international institutions: The Lower Danube University, Galați, Romania; The Plants and Environmental Sciences Institute of Nitra, The Horticulture Institute and The AgoBioTech Research Center, Nitra, Slovakia. The research conducted within the scientific projects served as methodological support for the thesis: 16.80013.5107.22/ Ro - *Substituting synthetic food additives with bioactive components extracted from natural renewable resources,* a project within the Moldova–Romania Scientific and Technological Cooperation Program, 2016-2018; 18.51.07.01A/PS - the *Reducing the contamination of raw materials and food products with pathogenic microorganisms* state project, 2018-2019; 20.80009.5107.09 - the *Improving the quality and safety of food products through biotechnology and food engineering* state project, 2020-2023; the "*Valorisation de composes bioactifs issus de déchets agro-industriels par encapsulation lyposomale" SER-ECO-2023* project, Research Intern, 2023-2024.

In order to confirm the research hypothesis, specific methods approved at national and international level were used for each objective. The research was conducted in stages and consisted of: determining the qualitative and quantitative antimicrobial effect of the biological fractions on the reference strains, determining the microbiocidal and microbiostatic effect of plant extracts and powders incorporated in various food matrices (*in situ*). In order to assess the antioxidant activity, the DPPH free radical test was applied. In accordance with the established purpose and objectives, classic and modern research methods were used, namely: microbiological, physical-chemical methods (UV/vis spectroscopy, FTIR, liquid chromatography – HPLC), mathematical modeling, statistical methods for processing the results.

Theoretical importance and scientific innovation of the paper. The study focused on assessing the antibacterial activity of local plant powders and extracts. The spectrum of action of these preparations on the reference strains that are mainly responsible for food spoilage was identified. For the first time, the microbiostatic and microbiocidal effect of plant fractions incorporated in food matrices was evaluated. Based on the research results, 1 invention patent was obtained - "The ice cream manufacturing process".

Approval of scientific results. The results of the research accumulated in the thesis were presented and discussed at national and international scientific forums such as: of Student, Master and Doctoral Student Conferences, TUM, Chisinau (2019 - 2021); The "Modern Technologies in the Food Industry” International Conference, Chisinau (2018); The "Achievements and perspectives of modern chemistry dedicated to the 60^{th} anniversary of the foundation of the Chemistry Institute” International Conference, Chisinau (2019); "The days of the Academy of Technical Sciences from Romania - XIV edition” International Conference, Chisinau (2019); The "Prospects and Problems of Integration in the European Research and Education Area” International Scientific Conference, 8^{th} Edition, Cahul (2021); The "International Conference Intelligent valorisation of agro -industrial wastes", Chisinau (2021); "The 7^{th} International Conference Ecological & Environmental Chemistry", Chisinau (2022); The "One health approach - achievements and challenges "II Edition” National Conference with international participation, Chisinau, Republic of Moldova (2023); The "Euro-Food International Symposium, Galati (2019); The "Euro-Food" International Symposium, Galati, Romania (2021); The "Works of the International Conference on Carotenoids Research and Applications in Agro-Food and Health”, Cyprus (2019); "The 16^{th} International Conference of Constructive Design and Technologically Optimization in Machine Building Field", OPROTEH 2021; The "Modern Aspects of Human Health Preservation, Proceedings of the 15th International Interdisciplinary Scientific and Practical Conference, 30th Anniversary of the Phytotherapy Research Institute of the State Medical University” International Conference, Ujhorod, Ukraina (2022). National and international exhibitions of inventions: European exhibition of creativity and innovation "Euroinvent-2021", Iași (2021); The 10^{th} International Symposium "Food connects people and shares science in a resilient world", Galați, Romania (2021).

Publications on the thesis subject. The research results and issues addressed were published in the following scientific papers: chapter I of the collective monograph titled "Analysis of risks associated with food in the Republic of Moldova"; 7 articles in journals indexed in Web of Science and SCOPUS databases; 2 articles in recognized foreign scientific journals, BDI indexed; 6 articles in scientific journals from the National Register (B+), 20 summaries of papers published in collections of papers of international (16) and national (4) conferences; 1 patent, and a methodical guide.

Summary of thesis chapters. The doctoral thesis includes an annotation (in Romanian, Russian and English), introduction, 4 chapters, general conclusions, practical recommendations, 236 source references and 3 annexes. The main content has 130 pages, including 34 tables and 29 figures.

Key words: antibacterial effect, extracts, plant powders, polyphenols, successive dilutions, diffusimetric, antioxidants, preservatives.

THESIS CONTENT

1 Biological compounds with antimicrobial action – a promising resource towards reducing food contamination and resistance of microorganisms to antibiotics

Chapter one talks about the problem of foodborne diseases, the epidemiology of foodborne diseases and lists the etiological agents involved. Food is the ideal vehicle for the spread of pathogenic microorganisms. Antibiotics are some of the most used preparations. The antibiotic resistance crisis and the overuse of antibiotics in agriculture and the contribution of food to the spread of antimicrobial-resistant bacteria are discussed. The sources and path of spreading AMR, as well as the phenomenon of antimicrobial resistance worldwide and in the Republic of Moldova were studied.

The literature review describes natural antimicrobial compounds, berries and plants as natural sources against agents responsible for food spoilage. It describes components of plant extracts, their antioxidant capacity, as well as their mechanism of action. The chapter talks about the mechanisms of action involved in the inhibition of bacterial growth, such as destabilization of the cytoplasmic membrane, permeabilization of the plasma membrane, direct actions on microbial metabolism, and deprivation of substrates necessary for microbial growth, the use of these compounds as food preservatives.

2. Research materials and methods

As preparations with antimicrobial effects, powder and plant extracts of sea buckthorn, dogwood, hawthorn, aronia, grape pomace from red varieties and aromatic herbs (basil, rosemary and thyme) were used. The research used local plant material from the Republic of Moldova (RM) offered by the Technical University of Moldova, Faculty of Food Technology. For the *in vitro study* in chapter 2 reference strains were included (*Staphylococcus aureus* ATCC 25923, *Escherichia. coli* ATCC 25922, *Salmonella enterica* serovar Aboni NCTC 6017, *Salmonella enterica* serovar Typhimurium ATCC 14028, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Geobacillus stearothermophilus* ATCC7953 *Klebsiella pneumoniae* ATCC 13883, *Listeria monocytogenes* ATCC 19118, *Candida albicans* ATCC 10231), *Staphylococcus aureus* CCM 2461, *Listeria monocytogenes* CCM 4699, *Bacillus cereus* CCM 2010, *Bacillus subtilis* CCM 1991, *Pseudomonas aerugenosa* CCM 3955, *Shigella sonnei* CCM 4421, *Salmonella* Enteritidis, CCM 4420, *E. coli* CCM 3954) , that come from two collections of American Type Culture Crops Collection (ATCC) and the Czech Collection of Microorganisms, (AgoBioTech Research Center), Nitra, Slovakia. The food products used (with different concentrations of extracts) were sausage, cream cheese and ice cream.

The study was conducted in stages, according to the established schedule. The research was structured and carried out according to the following stages: the study of specialized bibliographic sources > characteristic and definition of the problem, establishing objectives > organizing and conducting the research (examining the antimicrobial effect of plant preparations from berries *in vitro,* establishing the microbiostatic effect of some plant fractions *in situ*, determining the antioxidant action, microencapsulation, processing, analysis and description of statistical data) > implementation of certain results in practice.

The chapter includes a description of two categories of research methods: classical microbiological methods, and physical and chemical analyses. The first category refers to the screening of natural preparations and the determination of MIC and MBC of new chemical compounds. The second category refers to the methods used to determine the antioxidant capacity, the total content of polyphenols, and the microencapsulation.

3 Antimicrobial effect of preparations of natural origin

The screening was performed and the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of the natural preparations were determined. The antibacterial effect of natural preparations was tested on Gram-positive and Gram-negative strains and yeast Powders and extracts from the following berries and herbs were used as natural preparations with an antibacterial effect: sea buckthorn, rosehip, gooseberry, grape pomace, chokeberry, hawthorn, basil, thyme, rosemary.

3.1 Determination of the antimicrobial activity of plant powders by the well method

The aim of the study was to determine whether the etiological agents are resistant or sensitive to the tested natural antimicrobial agents. The potential antibacterial activity of berry powders and extracts was initially determined by the agar well diffusion method. In accordance with the expected objectives, during the research the microbiostatic and microbicide effect was determined *in vitro* in direct contact with different types of plant powders obtained from berries on bacteria that alter food products. The data in Table 3.1 demonstrate the antimicrobial activity of (buckthorn, rosehip, aronia, hawthorn) and groats from the fruits of white seabuckthorn, rosehip and grape pomace against Gram-negative and Gram-positive bacteria.

Table 3.1. Inhibition zones of plant powders on pathogenic microorganisms

Powder	**Diameter of the zone of complete growth inhibition (mm)**			
	S. aureus	***B. subtilis***	***E. coli***	***K. pneumoniae***
Sea buckthorn	22.0±0.2	20.0±0.2	18.2±0.3	17.4±0.4
Sea buckthorn groats	18.0±0.1	17.1±0.3	15.2±0.3	13.2±0.3
Rosehip	16.0±0.3	15.1±0.2	10.3±0.4	9.3±0.3
Rosehip groats	12.0±0.2	12.2±0.2	9.3±0.3	8.1±0.1
Grape pomace	11.0±0.1	11.0±0.3	9.2±0.2	7.3±0.2
Aronia	10.0±0.2	9.4±0.4	7.1±0.1	7.2±0.2
Hawthorn	10.0±0.1	11.2±0.2	8.2±0.2	7.2±0.2

*Values of each test performed in triplicate and calculated as mean ±standard error (SE), statistical analysis - ANOVA, ($\alpha \leq 0.05$) with GraphPad 5. *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *K pneumoniae* ATCC 13883

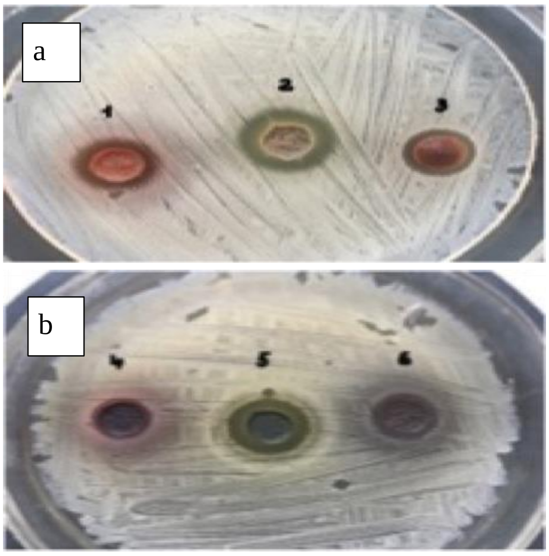


Figura 3.1. Activity of different types of plant material on the *S. aureus* strain
a) 1 - hawthorn; 2 - buckthorn groats; 3 - rosehip; b) 4 - aronia; 5 - sea buckthorn; 6 - grape pomace.

All preparations showed antimicrobial activity. Plant powders from sea buckthorn showed inhibitory effect against all tested strains. The white sea buckthorn powders proved an accentuated effect. The sea buckthorn extract also has a clear effect on all the strains studied. Sea buckthorn and sea buckthorn groats were very active against *S. aureus*, the inhibition zone diameter is 22 ±0.2 mm and for sea buckthorn groats of 18 ±0.1 mm in diameter (fig. 3.1). According to figure 3.1, rosehip powder showed a more moderate effect, the diameter of the inhibition zone 16±0.3 mm for *S. aureus* and 15±0.2 mm for *B. subtilis*. Aronia and hawthorn have a lower antibacterial activity value, the diameter of the inhibition zone is 10 ±0.1 mm for *S. aureus* and 9.3 ±0.3 and 11 ±0.1 mm for *B. subtilis,* respectively. The same plant powder was used for Gram negative bacteria. *E. coli* and *K. pneumonia* were more sensitive to sea buckthorn preparations, the inhibition diameter being 18±0.1 mm for *E. coli* and 17±0.3 mm for *K. pneumonia.*

Rosehip and gooseberry showed moderate effect against these bacteria. Chokeberry and hawthorn presented lower antibacterial activity with a fair inhibitory diameter for both strains (tab. 3.1.). From this data, we conclude that sea buckthorn preparations are active on these strains. Gram-positive bacteria have been proven more sensitive, especially *S. aureus.*

3.1.1 The antimicrobial effect of different varieties of sea buckthorn, determined by the well method

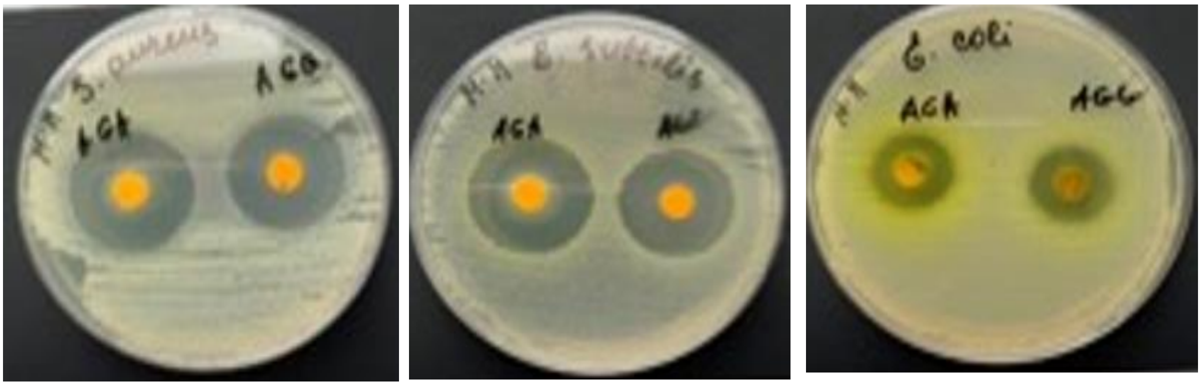
Going further, different varieties of sea buckthorn were used in powder form (R1, 2, 4, 5; C6, AGG, AGA, Pomona, Mr.Sandu, Seirola). The strains in table 3.2 were selected for testing.

Table 3.2. The inhibition zones of different sea buckthorn varieties on pathogenic microorganisms

Sea buckthorn varieties	Diameter of the complete growth inhibition zone (mm)				
	***S. aureus* ATCC 25923**	***B. subtilis* ATCC 6633**	***S.* Typhimurium ATCC 14028**	***E. coli* ATCC 25922**	***C. albicans* ATCC 10231**
R1	22.0±0.2	19.3 ±0.4	Lack	12.4 ±0.5	Lack
R2	24.2 ±0.3	22.2±0.2	13.1±0.2	13.5±0.6	Lack
4	26.1 ±0.3	24.3±0.2	15.2±0.1	15.2±0.3	Lack
5	26.2 ±0.4	25.2±0.3	14.3±0.3	15.4±0.4	Lack
6	24.2 ±0.3	26.4±0.5	14.3±0.3	17.4±0.2	Lack
AGG	29.2 ±0.5	28.2±0.3	18.2±0.3	18.2±0.4	Lack
AGM	30.3 ±0.4	29.4±0.4	18.2±0.5	18.4±0.2	Lack
Pomona	21.3±0.2	22.2±0.1	Lack	12.3 ±0.2	Lack
Mr. Sandu	28.2±0.3	27.4±0.3	11.3±0.2	18.3±0.4	Lack
Seirola	25.4±0.3	26.4±0.2	13.2±0.4	15.2±0.3	Lack

Note: Values of each test performed in triplicate and calculated as mean ±standard error (SE), statistical analysis - ANOVA, ($\alpha \leq 0.05$) with GraphPad 5, Absence – lack of antimicrobial effect

According to the results, all types of sea buckthorn have pronounced activity on the tested microorganisms, with the exception of *C. albicans* ATCC 10231. The highest bacteriostatic activity on the bacteria *S. aureus* ATCC 25923 was shown by preparations from sea buckthorn varieties AGA and AGG with a diameter of 30 mm and 29 mm. The antibacterial effect against *B. subtilis* was significantly higher in AGA and AGG, with an inhibition zone of 29 and 28 mm. Other types of sea buckthorn are also active on Gram-positive strains with a difference of 2-3 mm. It was noted that the varieties of sea buckthorn AGA and AGG are very active, including on Gram negative strains, with the exception *of S.* Typhimurium (fig. 3.2).



AGA and AGG activity on *S. aureus* ATCC 25923 | **AGA and AGG activity on *B. subtilis* ATCC 6633** | **AGA and AGG activity on *E. coli* ATCC 25922**

Figure. 3.2. The action of different varieties of sea buckthorn on Gram-positive and Gram-negative bacteria

The most sensitive strain to these preparations is *S. aureus* (fig. 3.2). In conclusion, we can note that these preparations have different antibacterial action and it depends on the type of microorganism. Gram-positive bacteria are more sensitive to all types of sea buckthorn. Antifungal effect of new varieties of sea buckthorn on *C. albicans* ATCC 10231 it was not highlighted by the gel diffusion method (fig. 3.2).

3.1.2 Resistance of plant powder antimicrobial properties over time

antimicrobial effect of plant powders after two years of storage (2020-2022) was evaluated. The present study was carried out to determine the antimicrobial efficacy with the possibility of evaluating their efficacy in storage. For this purpose, the reference strains presented in table 3.3 were used. The powders of rosehip, gooseberries and new varieties of sea buckthorn grown in the Republic of Moldova were used for testing. The plant powders were tested over a period of 2 years (2020-2022). To assess the effectiveness of the preparations over time, after two years, quantitative and qualitative methods were used to determine the antibacterial activity (tab. 3.3).

The results obtained (tab. 3.3) show that different types of pomaces had no activity on the tested reference strains. Rosehip did not show antibacterial effect against the tested microorganisms. *S. aureus* showed the highest sensitivity to sea buckthorn preparations and the recorded inhibition zones were 15, 12, 18, 19 and 17 mm for C6, R1, AGA, and R4, Mr. Sandu.

Table 3.3. Antimicrobial activity of plant powders over time* on pathogenic microorganisms

Plant powders	**Diameter of the complete growth inhibition zone (mm)**								
	***S. aureus* CCM 2461**	***L. monocytogenes* CCM4699**	***B. cereus* CCM2010**	***B. subtilis* CCM1991**	***C. perfringens* CCM4991**	***E. coli* CCM3954**	***S. sonnei* CCM 4421**	***P. aeruginosa* CCM 3955**	***S.* Enteritidis CCM 4420**
FN Bugac pomace	absent	absent	absent	absent	absent	absent	absent	absent	6.8
FNmesca pomace	absent	absent	absent	absent	absent	absent	absent	absent	absent
FN sem pomace	absent	absent	absent	absent	absent	absent	absent	absent	absent

continuation of table 3.3

Rosehip	absent	absent	absent	absent	absent	absent	absent	absent	absent
C6-buckthorn	15.2 ±0.1	6.8 ±0.4	14.2 ±0.1	11.4 ±0.3	Absent	14.4 ±0.3	15.4 ±0.3	16.2 ±0.3	14.2 ±0.1
R1buckthorn	12.3 ±0.2	absent	11.3 ±0.1	6.8 ±0.4	absent	absent	12.2 ±0.2	12.5 ±0.3	16.4 ±0.3
AGA - sea buckthorn	18.3 ±0.1	13.2 ±0.3	15.3 ±0.2	14.2 ±0.3	absent	12.5 ±0.2	19.3 ±0.3	15.2 ±0.4	18.6 ±0.5
R4 sea buckthorn	19.3 ±0.2	14.5 ±0.4	16.4 ±0.6	14.2 ±0.3	absent	12.4 ±0.1	17.0 ±0.3	16.5 ±0.5	18.2 ±0.3
Mr.Sandu-sea buckthorn	17.5 ±0.4	13.5 ±0.3	14.3 ±0.2	16.0 ±0.3	absent	11.4 ±0.5	14.4 ±0.3	19.2 ±0.3	17.0 ±0.2

Note: * - after a storage period of two years (2020 -2022), ambient conditions. Lack of antibacterial effect. Values of each test performed in triplicate and calculated as mean ±standard error (SE), statistical analysis - ANOVA, (α ≤ 0.05) with GraphPad

If a comparative study of plant powders is carried out early, after two years some of the plant powders (buckthorn varieties) have retained their antibacterial activity. Rosehip and grape pomace powders were also used in the study, also after two years. However, pomace and rose hip powders did not show antibacterial activity. A more diminished antibacterial activity after a period of time on Gram positive bacteria (*S. aureus, E.coli)* is attested with a difference in the diameter of the inhibition zone of approximately 10 mm (fig.3.3).

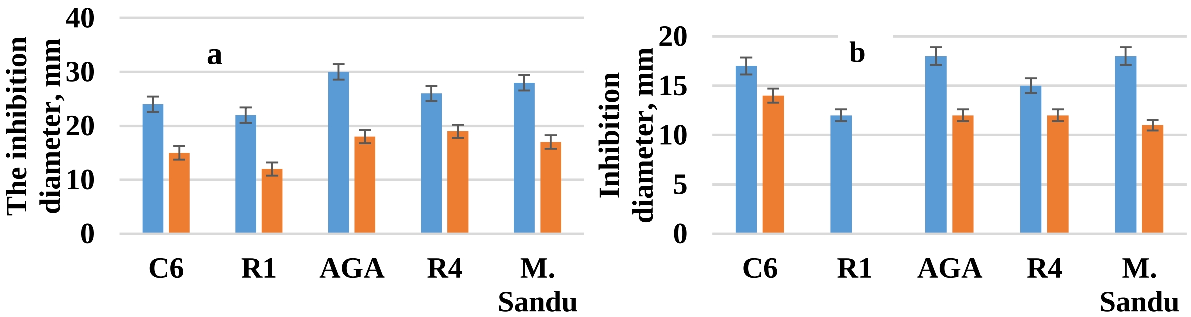


Fig. 3.3. Comparative study of the evolution of antibacterial activity during storage (2020 - 2022) of sea buckthorn powders on pathogenic microorganisms a) *S. aureus, b) E. coli*

We mention that sea buckthorn varieties retained their antimicrobial activity after a period of two years with a more pronounced decrease of the antibacterial effect on Gram positive bacteria. The antibacterial activity of the sea buckthorn varieties decreased over time, and the sea buckthorn variety R1 did not show antibacterial activity against *the* E.coli strain (fig. 3.3).

3.1.3 Antimicrobial activity of plant powders and extracts of basil, thyme and rosemary on pathogenic microorganisms

By the well diffusion method, the efficacy of a range of powders and extracts was determined against *B. cereus*, *S. aureus*, *E. faecalis*, *G. stearothermophilus*, *E. coli*, *S.* Subscribers, *C. albicans* (tab. 3.4). Thyme extract showed the highest zone of inhibition against *C. albicans* (29.3 mm) and for *S. aureus* with a zone of inhibition of 26.3 mm. Thyme showed activity against all microorganisms studied. A significant activity was also shown by the rosemary extract with an inhibition zone of 27 mm for *G. stearothermophilus* followed by basil extract with 26.3 mm growth inhibition zone for *S. aureus.*

The results of antibacterial activity showed that all plant extracts have antibacterial activity against the tested microorganisms, the most active extract being the thyme extract (tab. 3.4).

Table 3.4. Antimicrobial activity of basil, rosemary and thyme extracts on pathogenic microorganisms

Reference strains	Inhibition zone, mm (extracts)		
	Busuioc	Cimbru	Rozmarin
***S. aureus* ATCC 25923**	**Basil**	**Thyme**	**Rosemary**
***B. cereus* ATCC 11778**	26.3±0.6	26.3±0.6	21.3±0.2
***E. faecalis* ATCC 29212**	11.0±0.4	25.3±0.6	18.3±0.5
***G. stearothermophilus* ATCC 7953**	8.3±0.6	13.7±0.7	18.7±0.3
***C. albicans* ATCC 10231**	15.3±0.5	20.0±0.5	27.0±0.6
***E. coli* ATCC 25922**	11.0±0.3	29.3±0.6	19.0±0.4
***S.* Abony NCTC 6017**	8.6±0.7	10.0±0.7	15.0±0.6

Note: N – not tested (powders)

3.1.4 Antimicrobial activity of plant powders on L. monocytogenes ATCC 19118, L. monocytogenes EGDe (wells)

Lister monocytogenes is a Gram positive facultative anaerobic microorganism, optimal growth temperature varies between of - 0.4 ° C to 45 ° C (Osek et al., 2022). *Lister monocytogenes* it is a psychrotrophic microorganism (able to grow and multiply during cold storage) and even a few cells present in the final product can multiply to a level that is dangerous for consumers (Sandulachi et al., 2020).The aim of the study was to determine the antibacterial activity of berry powders and extracts on *L. monocytogenes.* The reference strain was used *Lister monocytogenes* ATCC 19118. The antibacterial activity of the following berry powders was tested: sea buckthorn, aronia, rosehip, hawthorn and gooseberry (tab. 3.5). The arguments for determining the sensitivity of listeria to these preparations was that these bacteria have the ability to multiply and survive in extreme environmental conditions.

Table 3.5. Antimicrobial activity of plant powders on *Listeria monocytogenes* ATCC 19118

Powder	***Lister monocytogenes* ATCC 19118**
	Diameter of the zone of complete growth inhibition (mm)
Sea buckthorn	22,5±0,3
Sea buckthorn groats	16,3±0,3
Aronia	-
Grape pomace	12,5±0,3
Rosehip	16,3±0,3
Rosehip groats	17,8±0,4
Hawthorn	-

Note: - the preparations were not active against *Listeria monocytogenes*

According to the results, the highest activity recorded against *L. monocytogenes* ATCC 19118 was demonstrated by sea buckthorn powders with an inhibition zone of 22.5 mm, followed by rosehip with an inhibition zone of 17.8 mm (fig. 3.4).

Figure 3.4. The activity of plant powders on *L. monocytogenes* ATCC 19118

Hawthorn and chokeberry have no activity against this strain. In conclusion, we can state that on *L. monocytogenes* a significant effect was demonstrated by sea buckthorn, but against the action of hawthorn and aronia it is resistant. Also, the antimicrobial activity of plant extracts was also determined on strains from the EGDe collection (tab. 3.6).

Similar tests were performed on *L. monocytogenes* EGDe, offered by Galati University. To determine the antibacterial effect, water-soluble and fat-soluble extracts obtained from sea buckthorn, hawthorn, chokeberry, dogwood, rosehip were used.

Table 3.6. Antimicrobial activity of plant powders on *L. monocytogenes* EDGE

Extract	***Listeria monocytogenes* EGDe**				
	diameter of growth inhibition zone (mm)				
	Sea buckthorn	**Rosehip**	**Grape pomace**	**Aronia**	**Hawthorn**
C1	32,4±0,3	20,5±0,5	20,5±0,5	12,4±0,5	0
C2	30,2±0,4	21,5±0,4	20,5±0,5	9,5±0,4	0
H1	29,4±0,5	21,3±0,4	n/t	15,5±0,1	0
H2	30,2±0,3	22,2±0,3	n/t	16±0,2	0
P1	27,5±0,3	26,4±0,3	n/t	n/t	0
P2	27,4±0,3	25,5±0,5	n/t	n/t	0

Note: C - concentrated 1.2; L – fat-soluble extract 1,2; P. - test 2016 1.2; n/t - not tested due to unavailability of extracts.

C1 extract from sea buckthorn showed the most pronounced effect against *L. monocytogenes* EGDe (with an inhibition zone of 32 mm), followed by C2 and H2, H1 (with an equivalent inhibition zone of 30.29. In the case of *L. monocytogenes* ATCC 19118, preparations from hawthorn were not active and likewise against *L. monocytogenes* EGDe (reference strain provided by the Lower Danube University) (tab. 3.6).

3.2 Determination of the minimum inhibitory concentrations of plant powders

There are different *in vitro techniques* to test minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) to determine antimicrobial susceptibility or resistance of microorganisms. The aim of the study was to determine whether the etiological agent is resistant or sensitive to the tested natural antimicrobial agents.

3.2.1 *Determination of MIC and MBC of plant powders by the dilutions method*

The tests were carried out by the method of successive dilutions in liquid media, with subsequent reinoculation on optimum agar plate (tab. 3.7).

Table 3.7. Minimum inhibitory concentrations (MIC*) and minimum bactericidal concentrations (MBC*) of plant powders on pathogenic microorganisms.

Pulbere	***S. aureus* ATCC 25923**		***E. coli* ATCC 25922**		***K. pneumoniae* ATCC 13883**		***B. subtilis* ATCC 6633**	
	MIC* mg/mL	**MBC* mg/mL**	**MIC* mg/mL**	**MBC* mg/mL**	**MIC* mg/mL**	**MBC* mg/mL**	**MIC* mg/mL**	**MBC* mg/mL**
Sea buckthorn	1,95±0,12	3,90±0,23	7,81±0,37	15,6±0,7	15,6±0,5	31,25±1,25	3,90±0,15	7,81±0,21
Buckthorn groats	15,63±0,33	31,25±1,03	62,50±2,37	125±5,0	62,5±2,1	125±5,0	7,81±0,19	15,62±0,41
Aronia	15,63±0,37	31,25±0,62	-	-	-	-	31,25±0,98	62,5± 1,8
Pomace	7,81±0,19	15,62±0,41	62,50±1,57	125±5,0	-	-	7,81±0,37	15,6±0,7
Rosehip	3,91±0,15	7,81±0,21	31,25±0,98	62,5±1,8	62,5±2,1	125±5,0	7,81±0,37	15,6± 0,7
Hawthorn	41,67±0,56	83,33±1,23	62,50±1,87	125±5,0	-	-	41,67±0,56	83,33±1,23
Rosehip groats	3,91±0,23	7,81±0,29	31,25±0,71	62,5±2,5	62,5±2,1	125±5,0	7,81±0,19	15,62±0,41

Notă: *Valorile testelor realizate în triplicate, medie ±eroare standard (SE), analiza statistică – ANOVA, ($\alpha \leq 0,05$), GraphPad 5

3.2.2 *Determination of the minimum inhibitory concentrations of plant powders (over time) by the microdilution method*

Data obtained by well diffusion of the raw powders and extracts suggested that they had potential antibacterial activity against Gram-positive and Gram-negative bacteria. The plant preparations demonstrated diverse antibacterial activity, further these extracts were tested on Gram positive and Gram negative strains to determine the minimum inhibitory concentrations and the minimum bactericidal concentrations (tab. 3.8).

Table 3.8. Determination of MIC* and MBC* of plant extracts (over time) by the microdilution method on pathogenic microorganisms

Reference strains	MIC/MBC	Extr C6	Extr R1	Extr AGA	Extr R4	Ms.S
E. coli **CCM 3954**	MIC50	65,21	15,46	15,46	15,46	15,46
	MBC90	67,18	18,12	18,12	18,12	18,12
B. cereus **CCM2010**	MIC50	5,82	15,46	8,26	8,26	8,26
	MBC90	7,23	18,12	10,42	10,42	10,42
L. monocytogenes **CCM 4699**	MIC50	34,83	15,46	8,26	15,46	5,82
	MBC90	37,23	18,12	10,42	18,12	7,23
B. subtilis **CCM 1991**	MIC50	34,83	34,83	15,46	8,26	8,26
	MBC90	37,23	37,23	18,12	10,42	10,42
P. aeruginosa **CCM3955**	MIC50	34,83	65,21	15,46	15,46	8,26
	MBC90	37,23	67,18	18,12	18,12	10,42
S. **Enteritidis CCM4420**	MIC50	15,46	8,26	8,26	8,26	8,26
	MBC90	18,12	10,42	10,42	10,42	10,42
S. sonnei **CCM *4421***	MIC50	34,83	15,46	129,26	15,46	8,26
	MBC90	37,23	18,12	131,46	18,12	10,42
C. perfringens **CCM *4991***	MIC50	15,46	15,46	34,83	15,46	15,46
	MBC90	18,12	18,12	37,23	18,12	18,12
S. aureus **CCM2461**	MIC50	34,83	34,83	15,46	5,82	8,26
	MBC90	37,23	37,23	18,12	7,23	10,42

Note: MIC - minimum inhibition concentration, MBC - minimum bactericidal concentration; $p \leq 0.05$.

The results obtained (tab. 3.8) indicated different levels of MIC depending on the bacterial strain tested. Thus the activity of the extracts of the five types of natural preparations (obtained from sea buckthorn), MIC varied from 67.18 µg / mL to 5.82 µg / mL. Extract C6 presented the lowest MICs of all tested extracts. All tested bacteria showed different MIC levels at R1 and C6. The lowest MIC observed in extract R1 was 8.26 µg / mL for *S*. Enteritidis, while the AGA extract from the same plant recorded the lowest MIC for *B. cereus*, *L. monocytogenes* and *S.* Enteritidis with a value of 8.26 µg / mL. The lowest MIC value for R4 was 5.82 µg / mL in *S. aureus*, and Ms. Sandu recorded MIC of 5.82 µg / mL against *L. monocytogene*s. It is mentioned that these preparations over a period of time demonstrated antibacterial activity, which was demonstrated by qualitative and quantitative methods (tab. 3.8).

3.2.3 *Determination of MIC and MBC of basil, thyme, rosemary extracts*

Minimum inhibitory concentration testing demonstrated that all tested plant extracts exhibited antibacterial activity against *B. cereus* test strains, *E. faecalis, C. albicans, G. stearothermophilus* with MIC values ranging from 0.7 mg/ mL to 45 mg/ mL.

The extracts tested showed different levels of antimicrobial activity depending on the variety tested as indicated in Table 3.9.

Table 3.9. MIC and MBC of basil, thyme, rosemary extracts

Reference strains	Plant concentration in the extract, mg/ mL					
	Basil		Thyme		Rosemary	
	MIC	MBC	MIC	MBC	MIC	MBC
B. cereus ATCC 11778	22,5	22,5	2,8	5,6	0,7	0,7
E. faecalis ATCC 29212	45,0	90,0	5,6	5,6	2,8	11,2
C. albicans ATCC 10231	22,5	22,5	5,6	11,2	2,8	5,6
G. stearothermophilus ATCC 7953	11,2	22,5	1,4	2,8	0,7	1,4

Note: MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; $p \leq 0.05$.

The most active extract listed in Table 3.9 was rosemary extract. At a concentration of 0.7 mg/ml it has bacteriostatic and bactericidal action on *B. cereus* and *G. stearothermophilus* (MBC 1.4 mg/ mL) and thyme extract demonstrated MIC values between 1.4 and 5.6 mg/ mL (tab. 3.9). This extract has a clear antibacterial effect on *G. stearothermophilus.* The lowest MIC values were shown by the basil extract, between 11.2 and 45 mg/ mL. Thyme, basil and rosemary extracts have a pronounced effect on *G. stearothermophilus.* Among all natural preparations tested in this study, thyme, rosemary and basil extracts had activity against *C. abicans* (tab. 3.9).

3.2.4 *Determination of minimum inhibitory concentrations and minimum bactericidal concentrations of plant powders on L. monocytogenes ATCC 19118*

The MIC and MBC values were also determined by the broth dilutions method on *L. monocytogenes* (tab. 3.10). This microorganism is considered one of the most important pathogens responsible for foodborne infections. *L. monocytogenes* is an opportunistic bacterial pathogen that has the ability to survive extreme environmental conditions found in nature and in the food chain, such as high salt concentrations, wide pH range, desiccation and low temperatures (Sandulache et al., 2020).

Table 3.10. Determination of MIC and MBC of plant powders on *Lister monocytogenes* ATCC 19118

Powder	*L. monocytogenes* ATCC 19118	
	MIC*, mg/mL	MBC*, mg/mL
Sea buckthorn	31,25	62,5
Sea buckthorn groats	62,5	125
Grape pomace	125	250
Rosehip	62,5	125
Rosehip groats	62,5	125

**Note MIC - minimum amount of inhibitors, MBC - minimum bactericidal amount;* $p \leq 0.05$.

The result of the minimum inhibitory concentration test indicated different MIC values depending on the tested preparation. Sea buckthorn has the strongest effect among preparations. This preparation at the concentration of 62.5 mg/ mL exhibits bactericidal action on *L. monocytogenes* ATCC19118. The results obtained correlate positively with the bibliographic data. Thus, sea buckthorn (*Hippophae rhamnoides L.*) ensures destabilization and permeabilization of the cytoplasmic membrane, enzyme inhibition by oxidized products (Negi et al., 2005). Nucleic acid synthesis of Gram-negative and Gram-positive bacteria is inhibited. Bacteriostasis occurs by damaging cell membranes (Cristi et al., 2020). Grape pomace shows a moderate effect on *L. monocytogenes* ATCC 19118, with respective MIC and MBC values of 125 mg/ mL and 250 mg/ mL.

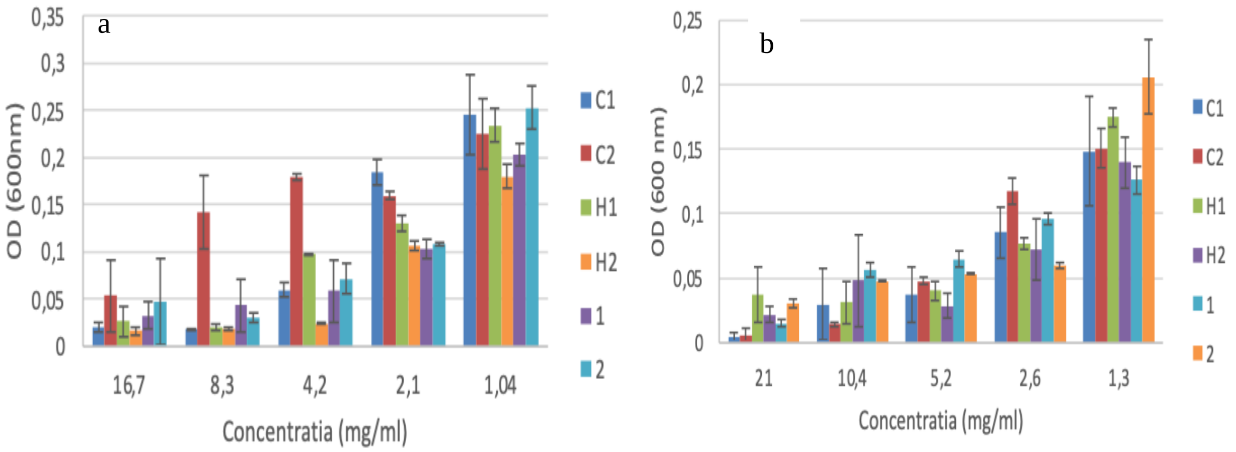
3.2.5 *Determination of the minimum inhibition concentrations of plant powders on L. monocytogenes EDGE*

Preparations used to determine the antibacterial effect on *L. monocytogenes* ATCC19118 were also tested on *the* L. *monocytogenes* strain EDGE (University of Lower Danube, Galați, Romania). Concentrated extracts and powders of sea buckthorn, rosehip, aronia, hawthorn were used. MIC was determined using the spectrophotometer, OD is measured at o λ= 600 nm. Anything above 0.1 OD is considered microbial growth. Sea buckthorn demonstrated more pronounced antimicrobial properties for *L. monocytogenes* than rosehip. MIC of hydroalcoholic extracts for each of C1, H1, H2, sample 1, 2, against *L. monocytogenes* were 2.6 mg/ mL and respectively for C2 – 5.2 mg/ mL (fig. 3.5a).

Figure 3.5. Minimum inhibitory concentration of plant extracts for

b **_L. monocytogenes_ EGOf a) sea buckthorn, b) rosehip**

The hydroalcoholic extracts of rosehip showed a more diminished effect on *L.*



monocytogenes with fair MIC values of 4.2 mg/ mL for concentrated, hydroalcoholic and fat-soluble extracts (C1, H1, H2, L1 and L2) (fig. 3.5b).

Compared to pomace, aronia was more active, the MIC is 1.9; 3.9 and 7.8 mg/ mL for H1, H2, C1,2. These properties are probably due to the chemical composition of sea buckthorn and rosehip, which confirms the results of previous studies (Efenberger et al., 2021; Sandulachi et al., 2020), Sea buckthorn shows the most pronounced effect on *L. monocytogenes*, especially concentrated extract 1 and 2, followed by rosehip and grape pomace. It was found that these preparations have different antibacterial action, which depends on the species of microorganisms. Gram-positive bacteria are more susceptible to all types of sea buckthorn.

3.3 Antimicrobial and antioxidant activity of extracts – mechanisms of interaction

Polyphenols and carotenoids serve as preservatives in food processing. Food spoilage and food poisoning caused by the growth of pathogenic bacteria are major problems in the food industry. There is an interest in the use of active natural preservatives. Thus, research into the mechanisms of antimicrobial and antioxidant action of bioactive plant compounds is of particular importance (Finley et al., 2011; Poljsak et al., 2021).

3.3.1 *Composition of grape pomace and berry extracts*

Antioxidants are able to stop these chain reactions by oxidizing free radicals and thus blocking their action. These properties are characteristic to several families of chemical compounds: thiols, phenols, carotenoids, etc. Table 3.11 shows the composition of individual polyphenols, identified in hydroethanolic extracts from berries and grape pomace (HPLC method) **(**Sandulachi et al., 2020**;** Ghendov-Mosanu et al., 2022).

Table 3.11. Individual polyphenols identified in hydroethanolic extracts of berries and grape pomace

Polifenols	Sea buckthorn, mg/100mL	Rosehip, mg/100mL	Aronia, mg/100mL	Grape pomace, mg/100mL
Gallic acid	0,16±0,01	0,85±0,01	0,39±0,01	1,95±0,01
m-Hydroxybenzoic acid	0,020±0,002	0,020±0,00u1	0,13±0,01	0,010±0,002
Protocatechuic acid	0,98±0,01	0,43±0,01	1,88±0,01	0,32±0,01
p-Hydroxybenzoic acid	0,21±0,01	0,19±0,01	0,21±0,01	0,34±0,01
Gentisic acid	0,15±0,01	0,27±0,01	-	-
Vanillic acid	0,17±0,01	0,13±0,01	0,09±0,01	-
Salicylic acid	24,48±0,05	1,07±0,01	2,65±0,02	-
Syringic acid	-	-	0,05±0,01	0,19±0,01
p-Coumaric acid	0,010±0,002	0,010±0,001	0,06±0,01	-
Ferulic acid	2,19±0,01	0,32±0,01	5,51±0,03	0,82±0,01
Caffeic acid	0,006±0,001	-	0,09±0,01	-
Sinapic acid	0,13±0,01	-	0,08±0,01	0,008±0,001
Catechin	-	2,05±0,01	15,41±0,15	1,34±0,01
Epicatechin	0,37±0,01	0,49±0,01	4,7±0,02	-
Quercetin	0,030±0,005	0,020±0,001	-	0,19±0,01
Hyperoside	38,53±0,02	0,41±0,01	0,97±0,01	0,37±0,01
Procyanidin B1	0,19±0,01	0,70±0,01	0,27±0,01	1,33±0,01
Procyanidin B2	0,10±0,01	1,75±0,01	0,12±0,01	15,34±0,15
Chlorogenic acid	1,43±0,02	-	-	-
Polydatine	5,40±0,01	0,06±0,01	1,27±0,01	-
Trans-resveratrol	1,20±0,01	-	0,005±0,001	-
Cis-Resveratrol	4,17±0,01	0,010±0,001	0,011±0,001	-
Methyl Ferulate	25,43±0,02	1,44±0,01	1,48±0,02	0,74±0,01

Note:* results are presented as mean ±standard deviation

Sea buckthorn fruit extracts contain significant amounts of salicylic acid (24.48 mg/100 mL), hyperoside (38.53 mg/100 mL), ferulic acid methyl ester (25.43 mg/100 mL), and other substances in smaller quantities. Rosehip extracts have important amounts of substances, such as derivatives of hydroxybenzoic acid, hydroxycinnamic acid, flavones, flavonoids and the methyl ester of ferulic acid. The main phenolic compounds detected in chokeberry extract were catechin (15.41 mg/100 mL), epicatechin (4.7 mg/100 mL), etc. It was found that the extracts used are rich in bioactive substances. Grape pomace extracts contain significant amounts of procyanidin B2, gallic acid, catechin, procyanidin B1, ferulic acid.

3.3.2 Composition and antioxidant activity of basil extracts

The total polyphenol content determined from the basil extract was 26.18 mg GAE/g su. Individual polyphenols were identified by high performance liquid chromatography coupled with ESI mass spectrometer equipped and photodiode array (HPLC-DAD-ESI-MS) (tab. 3.12).

A total of nine phenolic compounds were detected in the basil extract (tab. 3.12). The compounds were assigned to the phenolic acids and flavonoid class. Methyl-rosmarinate and rosmarinic acid proved to be the most abundant (17.08 and 13.81 mg/g su., respectively).

Table 3.12. Total and individual polyphenol content and antioxidant activity of the basil extract used for the experiments

Indicators	Content
The total content of polyphenols (Folin -Ciocâlteu), mg GAE/g d.s.	26,18 ± 0,21
Epigallocatechin, mg/g d.s.	0,72 ± 0,09
Chicory acid, mg/g d.s.	1,00 ± 0,13
Querectin-rutinoside, mg/g d.s.	0,75 ± 0,02
Luteolin -glucoside, mg/g d.s.	0,85 ± 0,05
Dehydrodiferulic acid, mg/g d.s.	3,10 ± 0,26
Rosmarinic acid, mg/g d.s.	13,81 ± 0,57
Methyl Rosmarinate, mg/g d.s.	17,08 ± 0,39
Carnosol, mg/g d.s.	4,78 ± 0,06
Rosmadial, mg/g d.s.	6,45 ± 0,01
Unidentified	8,46 ± 0,17
Antioxidant activity (DPPH), MM TE /g d.s.	644 ± 21
Antioxidant activity (ABTS), MM TE / g d.s.	8,95 ± 0,03

Note: DPPH—2,2-diphenyl-1-picrylhydrazyl; ABTS—2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). Values in the table represent means of three replicate experiments ± standard deviation.

The results obtained in this study are consistent with other studies (Khatib, S. et al, 2021; Romano et al., 2022) where rosmarinic acid is reported to be the most represented phenolic acid in basil. The content of chicoric acid and dehydro-diferulic acid is 1.3 mg/g su. and 3.1 mg/g su, respectively.

3.3.3 *Antioxidant activity of berry and grape pomace extracts*

The antioxidant activity of the berry and gooseberry extracts was determined after a 2-year storage period. The free radical scavenging capacity of the extracts was evaluated using the widely used 2,2-diphenyl-1-picrylhydrazyl test (DPPH). The DPPH test provides an easy and rapid way to estimate antioxidant activity. This test is based on electron transfer. DPPH produces a purple solution in ethanol or methanol. Tests aimed at the comparative analysis of the antioxidant activity of the extracts of sea buckthorn and gooseberry were carried out depending on their concentration. The results of the tests carried out for the extract from the variety B1 (Bulgac) are presented in figure 3.6.

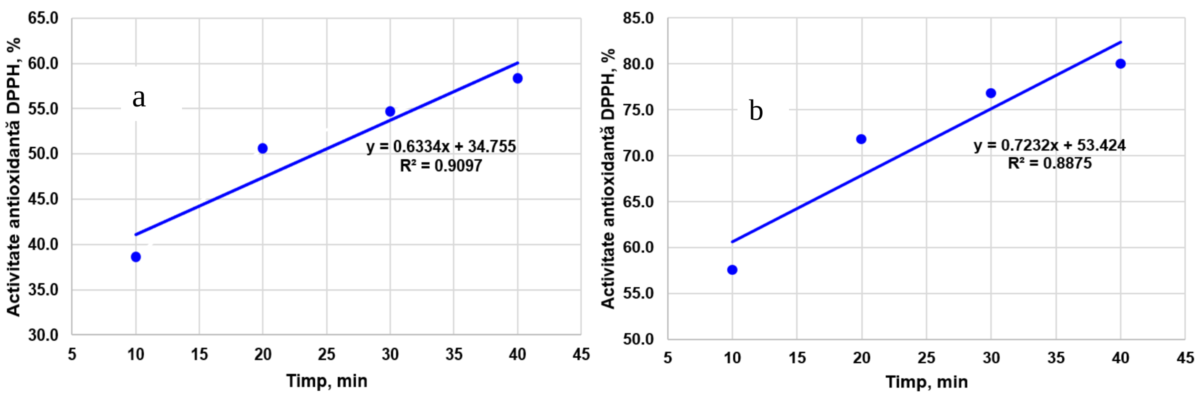


Figure 3.6. Antioxidant activity of grape pomace extract (variety B - 1): a – pure extract (1 g dry extract + 25 mL of 96% ethanol); b – ratio 1:2 (1 part extract + 1 part ethanol).

The findings show that the pure extract (fig. 3.6 a) provides an AA of 80% inhibited DPPH. In the case of the extract diluted with ethanol in a ratio of 1:2, AA constitutes 60% (fig. 3.6 b). Thus, reducing the extract concentration to 50% can be associated with a 25% decrease in antioxidant potential. Thus, the final dilution selected was 1 to 2 (1:2) ($y = 0.6334x + 34.755$, $R^2 = 0.9097$). The research conducted showed that at high concentrations of extract, the kinetic curves of changes in antioxidant activity over time are more parallel to the X axis (time). The total antioxidant activity is 80 – 85 % and does not depend to a considerable extent on the volume of the extract.

4. In situ analysis of the microbiostatic and microbicidal action of some plant extracts and powders

Although the microbiostatic activity of plant plants represents a promising source of alternative solutions for their use in order to reduce the microbial contamination of raw materials and food products, the effectiveness of these compounds is shown to be significantly lower *in situ*, after their inclusion in the matrix of real foods, than *in vitro.* In the framework of the thesis, the microbiostatic and microbicidal effects of some plant extracts and powders were examined in different categories of products contaminated with microorganisms, capable of accidentally colonizing them.

4.1 *In situ* evaluation of the antimicrobial action of berry extracts and powders in meat products

The growth of microbial strains *in situ* was determined (sausage), in the control sample and in those with added rosehip and hawthorn in a concentration of 0.5%. Contaminated samples were incubated at 37 °C for 24, 48, 72 and 96 h. Table 4.1 shows the results of monitoring the growth of pathogenic strains *in situ.*

Table 4.1. Number of microbial colonies developed in samples of contaminated sausages

Day/Sample (Strain)		***S. aureus*** **ATCC 25923**		***S.*** **Abony ATCC 6017**		***K .pneumoniae*** **ATCC 13883**		***E. coli*** **ATCC 25922**	
		10^{-3}	**10^{-6}**	**10^{-3}**	**10^{-6}**	**10^{-3}**	**10^{-6}**	**10^{-3}**	**10^{-6}**
Day 1	CS	552	78	diffuse	>700	diffuse	168	diffuse	488
	Rosehip	228	16	diffuse	120	diffuse	88	diffuse	64
	Hawthorn	96	1	diffuse	248	diffuse	103	diffuse	88
Day 2	CS	>1000	268	diffuse	>800	diffuse	346	diffuse	596
	Rosehip	440	23	diffuse	228	diffuse	114	diffuse	264
	Hawthorn	176	3	diffuse	480	diffuse	144	diffuse	152
Day 3	CS	>1000	396	diffuse	diffuse	diffuse	412	diffuse	>700
	Rosehip	560	49	diffuse	392	diffuse	300	diffuse	960
	Hawthorn	222	4	diffuse	>1000	diffuse	760	diffuse	344
Day 4	CS	diffuse	416	diffuse	diffues	diffuse	560	diffuse	difuz
	Rosehip	diffuse	280	diffuse	>1000	diffuse	372	diffuse	difuz
	Hawthorn	144	3	diffuse	>1000	diffuse	896	diffuse	364

Notă: CS – Control sample, diffuse – confluent growth

Comparing the results, we found that the samples containing the added rosehip and hawthorn have a greater ability to inhibit the development of tested strains compared to the control sample. Hawthorn-containing samples demonstrated a more pronounced inhibitory effect on *S. aureus* ATCC 25923 strains.

The samples with the added berries have a reduced effect on the strains of Gram negative bacteria that were studied (fig. 4.1), mainly on the strains of *S.* Abony ATCC 6017.

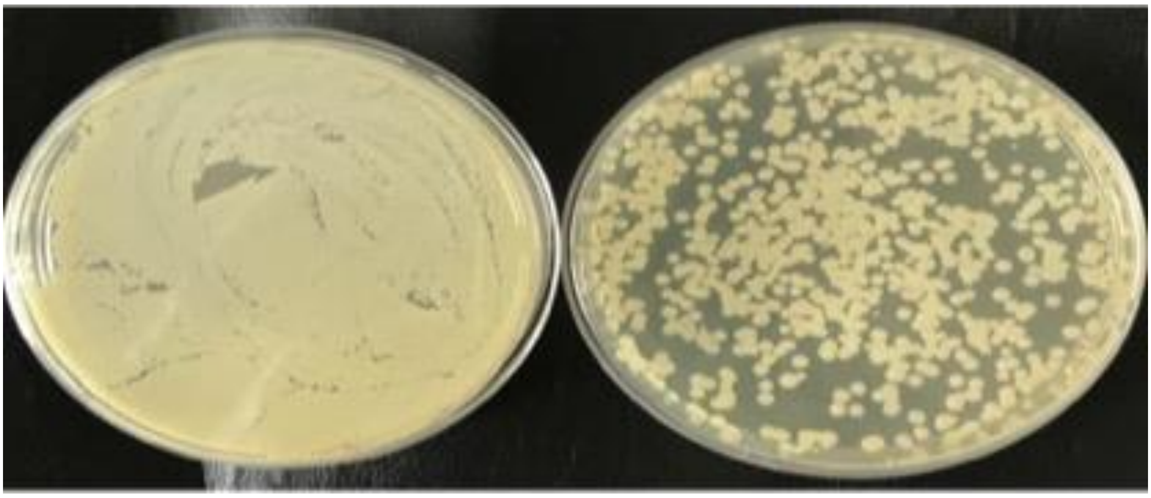


Figure 4.1. Colonies of *Klebsiella pneumoniae* developed from sausage samples tested after 96 hours (a – control sample; b – hawthorn sample).

The *Lag* phase (fig.4.2) is defined as the initial period in the life cycle of a bacterial population when cells adapt to a new environment before starting exponential growth. A small group of cells are placed in a nutrient-rich environment that allows them to synthesize proteins and other molecules needed for replication. These cells increase in size, but no cell division occurs during this phase (Cojocari et al., 2019). After the lag phase, bacterial cells enter the exponential phase, where cell division takes place. During this growth phase, antibiotics and disinfectants are most effective.

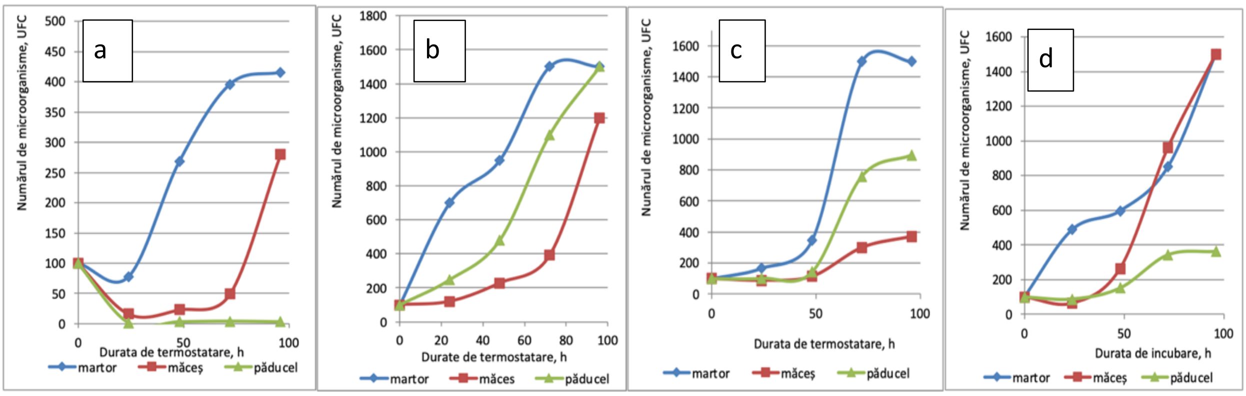


Figure 4.2. Phase *Lag* and the *Exponential* Phase of pathogenic strains in sausage samples; (test period 96 h) *a) S. aureus* ATCC 25923; *b) S.* Abony ATCC 6017; c) *K. pneumoniae* ATCC 13883; d) *E. coli* ATCC 25922

Figure 4.2. shows that the added hawthorn and rose hip in sausage samples increased the *lag phase* for the strains of inoculated microorganisms and decreased the growth rate of pathogenic microorganisms. Fore *S. aureus* (fig. 4.2a), the added hawthorn powder clearly inhibits the growth of microorganisms during the evaluated period. For *E. coli* (fig. 4.2d) hawthorn is also the most effective in stagnating the development of pathogenic microorganisms in meat products. In the case of *K. pneumoniae* (fig. 4.2c) the effect of rosehip powder is the most striking. For *S.* Abony,

the *lag phase* is observed during 80 hours (sausages with rosehip powder) and about 40 hours for the products with hawthorn addition (fig. 4.2b).

As a result of the tests carried out, it was found that the addition of rosehip and hawthorn in the sausage recipe can keep under control the growth rate of microorganisms, including pathogenic ones.

4.2 *In situ* evaluation of the antimicrobial action of some plant extracts and powders in cream cheese

Cream cheese is a favorable environment for the growth of pathogenic microorganisms and provides conditions for spoilage of the product (Popescu et al., 2023). The aim of this study was to evaluate the effect of adding berry powders to the cream cheese recipe on pathogenic microorganisms that may accidentally infect the finished product. After evaluating the direct contact antibacterial activity of extracts and powders from various berries and plants and their bacteriostatic effect was determined *in situ* for cream cheese (tab. 4.2).

Table 4.2. Reduction of microbial growth in cream cheese samples with added berry powders

Sample / Dilution		***Staphylococcus aureus* ATCC 25923**		***Salmonella* Abony ATCC 6017**		***Escherichia coli* ATCC 25922**	
		day I, CFU	**day II, CFU**	**I day, CFU**	**II day, CFU**	**I day, CFU**	**II day, CFU**
S1	**10^{-6}**	>1000	171	>1000	432	242	125
S3	**10^{-6}**	38	1	11	a/c	12	a/c
S6	**10^{-6}**	40	28	42	5	a/c	a/c
S9	**10^{-6}**	71	51	a/c	a/c	2	a/c
S12	**10^{-6}**	28	a/c	66	10	6	a/c

Note: UFC - colony forming units, a/c - lack of growth,

In this study, the following samples with added berry powder were microbiologically investigated (tab. 4.2): cream cheese – control sample – (S1); cream cheese with 2% rosehip powder – (S3); cream cheese with 2% sea buckthorn powder – (S6); cream cheese with chokeberry powder 2% – (S9); cream cheese with 2% hawthorn powder – (S12). The test results evaluated the antimicrobial effect of berry powders on pathogenic microorganisms (*S.* Abony, *S. aureus, E. coli).*

It has been determined that the addition of berry powders (rose hip, chokeberry, sea buckthorn and hawthorn) can keep the growth rate of microorganisms, including pathogens, under control. The most relevant antimicrobial effect was observed in the case of berry powders added to cream cheese on the tested strains of *E. coli.* Rosehip and chokeberry powders added to the cream showed a major antimicrobial effect on Salmonella strains. The addition of hawthorn powders showed an antimicrobial effect on *S. aureus.*

4.3 The effect of microencapsulated basil extract on cream cheese quality and stability

Basil extract is characterized by a high antioxidant and antimicrobial activity contributing to the reduction of the population of pathogenic microorganisms and to the extension of the shelf life of perishable food products. The direct addition of herbs to food products is the most common method in the industry (Leri et al., 2020; Eghbal et al., 2022; Romano et al., 2022).

The objective of the study was to evaluate the antioxidant and antimicrobial activity of basil extract (Ocimum basilicum L.), the efficiency of its microencapsulation and the effect of added microencapsulated basil extract on the sensory, physical, chemical and textural properties of cream cheese during shelf life (tab. 4.3).

The physical and chemical parameters of cream cheese were not significantly influenced by the addition of MBE. Increasing MBE concentration in cream cheese led to a slight decrease in protein and fat content in the ranges of 5.82–5.73 % and 23.04–22.72 %, respectively. The sodium alginate in the EBM formulation led to free water retention and curdling (puckering) of the dry matter content in the cream cheese samples.

Table 4. 3. Physical and chemical parameters of cream cheese fortified with microencapsulated basil extract *

Parameters (%)	Samples				
	CC	0.3% CCMBE	0.6% CCMBE	0.9% CCMBE	1.2% CCMBE
Dry substance	34,32 ±0,02	34,50 ± 0,01	34,69 ± 0,02	34,89 ± 0,03	35,09 ± 0,03
Protein content	5,82 ± 0,00	5,78 ± 0,01	5,77 ± 0,01	5,75 ± 0.01	5,73 ± 0,01
Fat content	23,04 ±0,00	22,93 ± 0,01	22,86 ± 0,01	22,79 ± 0.01	22,72 ± 0,02

Note: CC – Cream cheese without microencapsulated basil extract; CCMBE - cream cheese with microencapsulated basil extract; means of three trials ±standard deviation ($p \leq 0.05$).

Basil has a specific flavor that is not tolerated by consumers and does not associate with different categories of products. In this context, in the research the basil extract was encapsulated in sodium alginate. The analysis of scanning electron microscopy (SEM) images showed that MBE looks like microspheres with a rough surface, with sizes ranging from 0.8 to 1.1 mm (fig. 4.3).

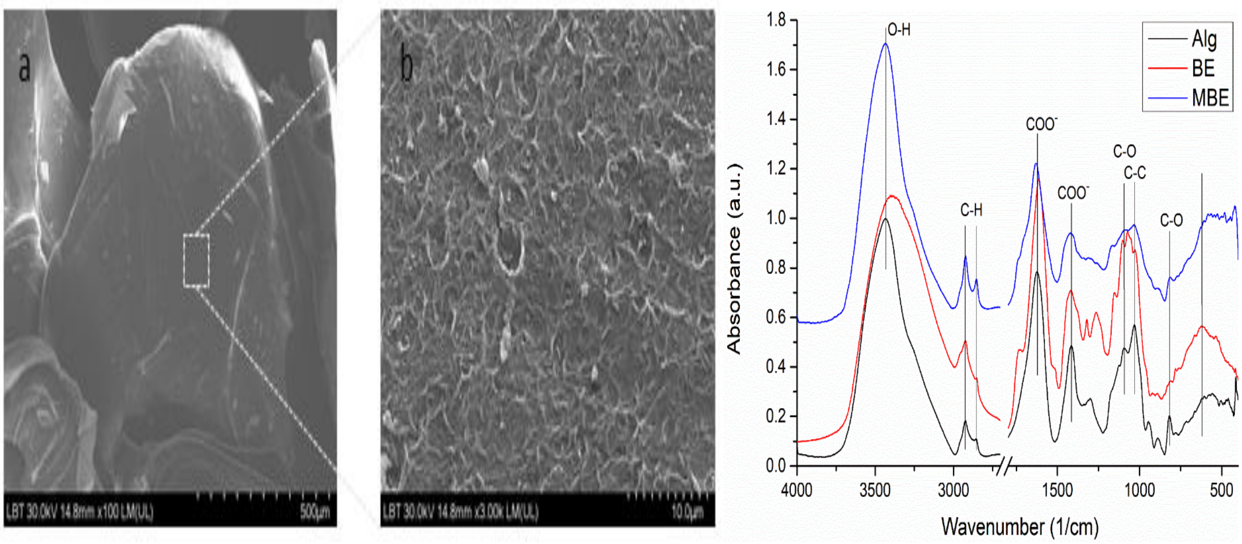


Figure 4.3. SEM micrographs of microencapsulated basil extract sample (a, b) and FT-IR spectra of sodium alginate (Alg), basil extract (BE) and microencapsulated basil extract (MBE), spectral range 4000–400 cm $^{-1}$ and 2700–1800 cm $^{-1}$.

The infrared spectrum with Fourier transform (FT-IR) of MBE revealed vibrational bands particular to functional groups. The changes identified in the MBE spectrum compared to the spectra of its components, Alg and BE, can be attributed to the existence of weak physical interactions between the components. Therefore, by encapsulating basil extract in sodium alginate as coating material, the formed microcapsules can serve as polyphenol carriers in food (fig. 4.3).

Table 4.4. pH evolution of cream cheese fortified with microencapsulated basil extract during storage

Shelf life, days	Samples				
	CC	0,3% CCMBE	0,6% CCMBE	0,9% CCMBE	1,2% CCMBE
1	5,41 ± 0,0 ^{h}	5,35 ± 0,01 ^{g}	5,31 ± 0,01 ^{f}	5,30 ± 0,02 ^{e,f}	5,26 ± 0,01 ^{d}

7	5,41 ± 0,0 [h]	5,34 ± 0,01 [g]	5,30 ± 0,01 [e,f]	5,28 ± 0,01 [d,e]	5,24 ± 0,02 [c,d]
14	5,35 ± 0,01 [g]	5,31 ± 0,01 [f]	5,30 ± 0,01 [e,f]	5,27 ± 0,02 [d,e]	5,21 ± 0,01 [b,c]
21	5.24 ± 0,01 [c,d]	5,27 ± 0,01 [d,e]	5,28 ± 0,01 [d,e]	5,26 ± 0,01 [d]	5,21 ± 0,01 [b,c]
28	5,12 ± 0,01 [a]	5,19 ± 0,01 [b]	5,27 ± 0,01 [d,e]	5,25 ± 0,01 [c,d]	5,20 ± 0,01 [b]

Note: CC – cream cheese without microencapsulated basil extract; CBMBE—cream cheese with microencapsulated basil extract. Table values represent means of three replicated ±standard deviation. Letters (a–h) denote statistically different results ($p \leq 0.05$).

The pH values of cream cheese enriched with encapsulated basil extract during the storage time of 28 days at 4 °C are shown in table 4.4. Later, during the storage time, the pH of the control CB sample as well as the cream cheese with MBE decreased gradually.

Addition of MBE in cream cheese samples from 0.6 to 1.2% inhibited the post-fermentation process during storage. Therefore, MBE prevents the growth of microorganisms during storage, which demonstrates their preservation potential.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. The extracts and powders of sea buckthorn, rosehip and red grape pomace showed high inhibitory activity against the bacteria *L. monocytogenes* ATCC 19118. Rosehip powders possess very high antimicrobial activity against *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633. The strains of Gram negative bacteria are less sensitive to the effect of plant powders compared to Gram positive bacteria (Ghendov-Moșanu et al., 2018; Cojocari et al., 2019; Cojocari et al., 2021; S turza et al., 2021).
2. The antimicrobial activity of powders and extracts from new varieties of sea buckthorn from the Republic of Moldova (R1, R2, R4, R5, C6, AGG, AGA, Pomona, Mr. Sandu, Seirola) on Gram-negative, Gram-positive bacteria and yeasts was tested. All varieties of sea buckthorn have demonstrated an antibacterial effect. The antibacterial effect was also analyzed after a storage period (by freezing) for two years. The sea buckthorn varieties kept their activity, with a slight decrease in microbiological activity on the tested strains. (Sandulachi et al., 2021; Sandulachi et al., 2022).
3. Extracts of rosemary, basil, and thyme exhibited broad antimicrobial activity, inhibiting the proliferation of both Gram-positive and Gram-negative bacterial species, as well as yeasts. Rosemary and basil extracts demonstrated the highest activity on all strains studied, which highlights the potential of these extracts as natural antimicrobial agents with diverse applications in food preservation (C ojocari et al., 2021; Popescu et al., 2021; MACARI et al., 2021).
4. Individual polyphenols from berries, grape pomace and herbs (basil) were analyzed. Grape pomace extracts contain significant amounts of procyanidin B2, gallic acid, catechin, procyanidin B1, ferulic acid and its methyl ester. Sea buckthorn berries contain significant amounts of salicylic acid, hyperoside, ferulic and chlorogenic acids, their esters, polydatin, *cis*- and *trans*-resveratrol. Rosehip extracts have important amounts of derivatives of hydroxybenzoic acids (salicylic, gallic, protocatechuic), hydroxycinnamic (ferulic), flavones (catechin, epicatechin), flavonoids (procyanidin B2 and procyanidin B1). Aronia extracts contain catechin, epicatechin, hydroxybenzoic, hydroxycinnamic acids and their derivatives, especially gallic, para- and metabenzoic acids, procyanidin B1 and B2. Basil extracts contain important amounts of phenolic acids (methyl- rosmarinate, rosmarinic acid, rosmadial, carnosol, dehydrodiferulic acid and chicoric acid) and flavonoids (luteo -lin-glucoside, querectin-rutinoside and epigallocatechin).
5. The antioxidant activity of the extracts of berries (buckthorn, rosehip, aronia) and grape pomace was tested for different degrees of dilution (1:1–1:8). It was found that although the decrease in the concentration of the biologically active compounds shows a reduction in AA (DPPH test), their ability to inhibit free radicals is preserved. Most polyphenols appear to scavenge free radicals through the hydrogen atom transfer mechanism. Flavonols show a stronger antiradical effect than the corresponding flavones due to the presence of the 3-hydroxyl group. Acidic polyphenolic compounds are characterized by a high degree of p-electron delocalization, for which deprotonation yields to anionic species stabilized by resonance phenomena, their stability being increased by the presence of hydrogen bonds (Cojocari et al., 2019; Cojocari, 2023).
6. The microbiostatic effect of some plant fractions rich in phenolic compounds was analyzed *in situ*, on meat products with plant additions. Analysis of products contaminated with the

reference strains revealed a more pronounced antimicrobial activity of rosehip and hawthorn powders on Gram-positive bacteria and a weaker effect on Gram-negative bacteria. A comparison of the Lag and Logarithmic phases of growth of microbial strains demonstrated a more pronounced bacteriostatic effect of hawthorn on strains of *S. aureus* and *E. coli*, and rosehip powder - a more pronounced bacteriostatic effect on strains of *S.* Abony and *K. pneumoniae.* Sausages with added basil, in various concentrations, showed an obvious ability to inhibit *E.coli strains* and a moderate effect on *S.* Abony. A clear activity on all tested bacteria was noted in sausages with the added extracts in concentrations of 0.3% (Sandulachi et al., 2021; M acari et al., 2021).

7. The microbiostatic effect of berry powders was analyzed *in situ*, on dairy products with added plant material. The most relevant antimicrobial effect was observed in cream cheese on strains of *E. sheets.* Rosehip and chokeberry powders showed a major antimicrobial effect on *Salmonella* strains, and hawthorn showed a major antimicrobial effect on *S. aureus.* Most of the inoculated pathogenic microorganisms (*S.* Abony, *S. aureus* and *E. coli)* in the samples of cream cheese with the addition of berry powders were destroyed after 48 hours of incubation at 37 °C, which demonstrated that rosehip, chokeberry, sea buckthorn and hawthorn powders can keep the proliferation of accidental pathogens under control (Sturza et al., 2021; Cojocari et al., 2021).
8. The effect of basil extract encapsulated in sodium alginate on microbiological stability, sensory, physical chemical, and textural properties of cream cheese was tested. The encapsulation efficiency was 78.59 ± 0.01 %, and the FTIR spectra of the encapsulated extracts indicated the presence of weak physical interactions between the components, the formed microcapsules being efficient vehicles of polyphenols for food. The optimal concentration of encapsulated extract (0.6 – 0.9 %) ensures the inhibition of the post-fermentation process, the improvement of the degree of water retention and the textural parameters of the cream cheese, thus contributing to the extension of the shelf life of the product by 7 days compared to of control sample. Mutual information analysis was used to determine the influence of encapsulated basil extract on the texture, pH and overall acceptability of cream cheese (Popescu et al., 2023).
9. The incorporation of extracts and powders from hawthorn, rosehip, chokeberry and sea buckthorn in ice cream ensured a pronounced antimicrobial effect against the strain *Staphylococcus aureus* ATCC 25923 and a moderate effect against *Salmonella* Abony NCTC 6017 and *Escherichia coli* ATCC 25922, which permits their recommendation for use in the food industry to reduce the risk of microbial contamination of raw materials and finished products (Popescu et al., 2020.

RECOMMENDATIONS FOR FUTURE RESEARCH

Based on the results of this study, the following recommendations are suggested:

1. Extracts from these plants should be further analyzed to identify their specific antibacterial principles. Further research on the studied plant powders would be necessary to isolate and identify the active compounds responsible for their low antimicrobial, antioxidant and cytotoxic properties.
2. It is recommended to determine the activity of these plant extracts on other species of pathogenic microorganisms involved in other infectious diseases, in addition to the synergistic activity of these medicinal plants in combination with antibiotics.

3. Toxicity studies of the effective plants should also be conducted to determine the safety indexes for the extracts. Clinical trials should be conducted to explore the potential of these plant extracts in the treatment of these infectious diseases.
4. The use of plant-based antimicrobials can be an alternative to chemicals used in food preservation.
5. Adding natural antimicrobials to food products without adversely affecting the sensory characteristics is still a challenge for researchers because the concentrations that are needed to ensure the safety of food and food products are several times higher than those accepted by consumers from a sensory perspective.
6. New studies combining the use of antimicrobials with other food preservation methodologies are needed to reduce the impact of compounds on sensory properties.
7. Further research is needed to better understand the impact of phenolic compounds on pathogens, organoleptic properties of foods and their relevant use in food applications.

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3.3. national conferences

1. **COJOCARI D.,** BEHTA, E. Pulberi din fructe de pădure ca antimicrobiene împotriva agenților patogeni Gram pozitivi responsabili de toxiinfecții alimentare, în cadrul *Conferinței Tehnico-Ştiințifice a studenților, masteranzilor și doctoranzilor*, 23-25 martie 2021, Universitatea Tehnică a Moldovei. http://repository.utm.md/handle/5014/16237.
2. BEHTA, E., **COJOCARI, D**. The Toxic substances formed in the process of microbial spoilage of the wine and their effect on human body, *Technical Scientific Conference on Ungraduate, Master and PhD Student*, 23-25 March, 2021, TUM. http://repository.utm.md/handle/5014/16235.

3. **COJOCARI, D**. Efectul antimicrobian al extractelor vegetale asupra *L. monocytogenes. Conferința tehnico-științifică a studenților, masteranzilor și doctoranzilor*, 1–3 aprilie 2020, pp. 405 – 406. http://repository.utm.md/handle/5014/8566.
4. **COJOCARI, D.** Acțiunea in vitro a extractelor vegetale bogate în compuși biologic activi asupra microorganismelor responsabile de alterarea alimentelor, *Conferința Studenților, Masteranzilor și Doctoranzilor*, UTM, prezentare în plen, Chișinău, 26-29 martie 2019.

4. INVENTION PATENT

POPESCU, L., GHENDOV-MOŞANU, A., STURZA, R., **COJOCARI, D.** et al Procedeu de fabricare a înghețatei. Brevet de invenție de scurtă durată MD 1451 (13) Y din 2020.02.05. Publicat BOPI nr. 8/2020. http://repository.utm.md/handle/123456789/14635

ANNOTATION

Cojocari Daniela: " The microbiostatic action of some plant extracts of phenolic compounds on the microorganisms responsible for food spoilage", doctoral thesis in engineering sciences, Chisinau, 2024.

Thesis structure: Introduction, 4 chapters, general conclusions and recommendations, bibliography of 236 titles, 3 annexes, 130 pages of main text, including 30 figures and 35 tables. The obtained results are published in 37 scientific papers.

Keywords: antimicrobial activity, pathogenic microorganisms, powders and plant extracts, biologically active compounds, antioxidants, food additives.

The research aims to evaluate the microbiostatic action of powders and plant extracts from berries and spices on microorganisms responsible for food spoilage *in vitro* and *in situ*, on different categories of processed foods.

Research objectives: *In vitro* determination of the microbiostatic and microbicidal effect upon direct contact of plant fractions rich in phenolic compounds on microorganisms causing food product spoilage; *in situ* determination of the microbiostatic effect of plant fractions rich in phenolic compounds on different matrices of meat and meat products; in situ determination of the microbiostatic effect of plant fractions rich in phenolic compounds on different matrices of dairy products; characterization of the composition of bioactive compounds and their antioxidant properties; analysis of the physico-chemical, sensory, and morphological properties of cream cheese with encapsulated basil extract.

Scientific novelty and originality: For the first time, based on an extensive study, the antimicrobial action of plant extracts on reference strains was evaluated. The microbiostatic and microbicidal effect of these extracts on diverse food matrices was determined *in situ*. The composition, antioxidant activity, and possible mechanisms of action of the extracts were estimated.

Solved scientific problem: The antimicrobial activity of plant extracts on microorganisms responsible for food spoilage was tested *in vitro*. The antioxidant action of plant extracts was estimated. This contributes to the development of alternative anti-infective strategies to reduce the use of synthetic additives and the development of antimicrobial resistance.

Theoretical significance: Information on the activity of plant preparations (extracts, powders) on reference strains was accumulated. The microbiostatic effect on diverse food matrices was determined. Data on the influence of these extracts on prolonging the lag phase in bacterial development were collected.

Applicative value: The process of manufacturing ice cream was proposed and implemented, leading to a patent.

Implementation of scientific results: According to the implementation act of September 3, 2021, at the Mellang&Compani LLC enterprise, 4 experimental batches of ice cream were manufactured with powders and extracts of aronia, hawthorn, rosehip, and sea buckthorn, each in a quantity of 100 kg. The results of microbiological tests were used to develop the collective monograph "*Analysis of risks associated with nutrition in the Republic of Moldova*," recommended by the UTM Senate to specialists in the field of food safety and for Master's and Doctoral students.

ADNOTARE

Cojocari Daniela: „Acțiunea microbiostatică a unor extracte vegetale de compuși fenolici asupra microorganismelor responsabile de alterarea alimentelor", teza de doctor în științe inginerești, Chișinău, 2024.

Structura tezei: Introducere, 4 capitole, concluzii generale și recomandări, bibliografia din 236 de titluri, 3 anexe, 130 pagini de text de bază, inclusiv 30 figuri și 35 de tabele. Rezultatele obţinute sunt publicate în 37 lucrări ştiinţifice.

Cuvintele-cheie: activitate antimicrobiană, pudre și extrate, fructe de pădure, plante aromatice, compuși biologic activi, antioxidanți, aditivi alimentari.

Scopul cercetării constă în evaluarea acțiunii microbiostatice a pudrelor și extractelor vegetale din fructe de pădure și condimente asupra microorganismelor responsabile de alterarea alimentelor *in vitro* şi *in vivo,* pe diferite categorii de alimente procesate.

Obiectivele cercetării: Determinarea *in vitro* a efectului microbiostatic şi microbicid la contactul direct a unor fracţii vegetale bogate în compuşi fenolici asupra microorganismelor ce cauzează alterarea produselor alimentare; stabilirea *in situ* a efectului microbiostatic a unor fracţii vegetale bogate în compuşi fenolici pe matrici de produse din carne şi mezeluri; stabilirea *in situ* a efectului microbiostatic a unor fracţii vegetale bogate în compuşi fenolici pe diferite matrici de produse lactate; caracterizarea compoziției compușilor bioactivi și a proprietăților lor antioxidante; analiza proprietăților fizico-chimice, senzoriale și morfologice ale cremei de brânză cu extract încapsulat.

Noutatea şi originalitatea ştiinţifică: Pentru prima dată, pe baza unui studiu amplu, a fost evaluată acţiunea antimicrobiană a extractelor vegetale bogate în polifenoli asupra tulpinelor de referință. S-a determinat efectul microbiostatic și microbiocid al acestor extracte *in situ*, pe diverse matrice alimentare. Au fost estimate compoziția, activitatea antioxidană și mecanismele posibile de acțiune a extractelor.

Problema științifică soluționată: A fost testată activitatea antimicrobiană a unor extracte vegetale asupra microorganismelor responsabile de alterarea alimentelor *in vitro.* Au fost estimată acțiunea antioxidantă a extractelor vegetale. Acest fapt contribuie la elaborarea unor strategii antiinfecţioase alternative, în scop de a diminua utilizarea aditivilor sintetici și dezvoltarea rezistenței la antimicrobiene.

Semnificaţia teoretică: Au fost acumulate informații despre activitatea unor preparate (extracte, pudre) vegetale asupra tulpinilor de referință. A fost determinat efectul microbiostatic pe diverse matrice alimentare. Au fost adunate date despre influența acestor extracte asupra prelungirei fazei lag în dezvoltarea bacteriilor.

Valoarea aplicativă: A fost propus şi realizat procedeul de fabricare a înghețatei în baza căruia s-a obținut un brevet de invenție.

Implementarea rezultatelor ştiinţifice: Conform actului de implementare din 3 septembrie 2021, la întreprinderea SRL Mellang&Compani au fost fabricate 4 loturi experimentale de înghețată cu pudre și cu extracte de aronia, păducel, măceş și cătină în cantitate de 100 kg fiecare. Rezultatele tesărilor microbiologice au servit pentru elaborarea monografiei colective *„Analiza riscurilor asociate alimentației în Republica Moldova",* recomandată de către Senatul UTM specialiștilor din domeniul siguranței alimentelor, pentru studenții ciclului II (Masterat) și III (Doctorat).

АННОТАЦИЯ

Даниела Кожокарь: « Микробостатическое действие некоторых растительных экстрактов фенольных соединений на микроорганизмы, вызывающие порчу пищевых продуктов», кандидатская диссертация по техническим наукам, Кишинев, 2024.

Структура диссертации: введение, 4 главы, общие выводы и рекомендации, библиография из 236 наименований, 3 приложения, 130 страниц основного текста, в том числе 30 рисунков и 35 таблиц. Полученные результаты опубликованы в 37 научных статьях.

Ключевые слова: антимикробная активность, патогенные микроорганизмы, растительные порошки и экстракты, биологически активные соединения, антиоксиданты, пищевые добавки.

Цель исследования - оценить микробиостатическое действие порошков и растительных экстрактов из ягод и специй на микроорганизмы, вызывающие порчу пищевых продуктов *in vitro* и *in situ* на различных категориях переработанных пищевых продуктов.

Задачи исследования: определение *in vitro* микробиостатического и микробицидного действия при прямом контакте некоторых фракций растений, богатых фенольными соединениями, на микроорганизмы, вызывающие порчу пищевых продуктов; установление *in situ* микробиостатического действия некоторых растительных фракций, богатых фенольными соединениями, на различные матрицы мясных и колбасных изделий; установление *in situ* микробиостатического действия некоторых фракций растений, богатых фенольными соединениями, на различные матрицы молочных продуктов; характеристика состава биологически активных соединений и их антиоксидантных свойств; анализ физико-химических, органолептических и морфологических свойств сливочного сыра с капсулированным экстрактом базилика.

Научная новизна и оригинальность: впервые на основе обширного исследования оценено антимикробное действие растительных экстрактов на эталонные штаммы микроорганизмов. Микробиостатическое и микробиоцидное действие этих экстрактов было определено *in situ* на различных пищевых матрицах. Оценены состав, антиоксидантная активность и возможные механизмы действия экстрактов.

Решение научной задачи: Была исследована антимикробная активность некоторых растительных экстрактов в отношении микроорганизмов, ответственных за порчу пищевых продуктов in vitro. Было оценено антиоксидантное действие растительных экстрактов. Этот факт способствует разработке альтернативных противоинфекционных стратегий с целью сокращения использования синтетических добавок и развития устойчивости к противомикробным препаратам.

Теоретическая значимость: собрана информация о действии некоторых растительных препаратов (экстрактов, порошков) на эталонные штаммы микроорганизмов. Определено микробиостатическое действие на различные пищевые матрицы. Были собраны данные о влиянии этих экстрактов на продление лаг-фазы роста бактерий.

Практическая ценность: предложен и реализован процесс производства мороженого, на основании которого получен патент на изобретение.

Внедрение научных результатов: согласно акту внедрения от 3 сентября 2021 года на предприятии SRL Mellang&Compani изготовлено 4 опытные партии мороженого с порошками и экстрактами черноплодной рябины, боярышника, шиповника и облепихи в количестве 100 кг каждая. Результаты микробиологических исследований были использованы при разработке коллективной монографии «*Анализ рисков, связанных с пищевыми продуктами в Республике Молдова*», рекомендованной Сенатом ОТМ специалистам в области безопасности пищевых продуктов и для студентов циклов II (магистратура) и III (докторантура).

COJOCARI DANIELA

THE MICROBIOSTATIC ACTION OF SOME PLANT EXTRACTS OF PHENOLIC COMPOUNDS ON THE MICROORGANISMS RESPONSIBLE FOR FOOD SPOILAGE

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