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OBTAINING AND STABILIZING DYES, ANTIOXIDANTS AND PRESERVATIVES OF PLANT ORIGIN FOR FUNCTIONAL FOODS

253.01 - Technology of food products of vegetable origin

Summary of the doctoral habilitatus thesis in engineering sciences

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LIST OF ABBREVIATIONS

a*	red/green component.	MBC	minimum bactericidal concentration.
AAE	ascorbic acid equivalents.	ME	malvidol glycoside equivalents.
AAWS	antioxidant activity of water-soluble substances.	MIC	minimum inhibitory concentration.
ABTS	2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic).	n	number of pulses.
AI	acidity index, mg KOH g^{-1}.	PCL	photochemiluminescence test.
AMA	antimicrobial activity.	PEA	sample with chokeberry extract.
b*	yellow/blue component	PEC	sample with sea buckthorn extract.
BAC	biologically active compounds.	PEF	pulsed electric field.
C	concentration, %	PEPA	sample with chokeberry extract and powder.
C*	chromaticity.	PEPC	sample with sea buckthorn extract and powder.
CIELab	three-dimensional color space.	PI	peroxide index.
CrH	cheese cream with hawthorn extract.	R^2	coefficient of determination.
CrR	cheese cream with rosehip extract.	RMSE	root mean square error.
CrS	cheese cream with scorch extract.	RP-HPLC	reversed-phase high performance liquid chromatography.
CrSb	cheese cream with sea buckthorn extract.	t	temperature.
CS	control sample.	TAC	total anthocyanin content.
CSt	control sample with tartrazine.	TAE	tannic acid equivalents.
CSUH	water-soluble dry matter content.	TC	tannin content
d. w.	dry weight.	TCC	total carotenoid content.
DPPH	2,2-diphenyl-1-picrilhydrazyl.	TE	trolox equivalents.
EtOH	ethyl alcohol.	TFC	total flavonoids content.
FAO	Food and Agriculture Organization.	TPC	total polyphenol content
GAE	gallic acids equivalents.	U	electrical voltage.
H_{optim}	optimal hydromodule.	UAE	ultrasonication assisted extraction.
HPLC	high performance liquid chromatography.	UNO	United Nations Organization.
HPSA	hydrogen peroxide scavenging activity.	UV/Vis	ultraviolet-visible.
IR	infrared.	WHO	World Health Organization.
L*	luminosity.	ΔE*	overall color difference.
MAE	microwave assisted extraction.	ε′	dielectric constant.

CONCEPTUAL ELEMENTS OF THE RESEARCH

Insurance with foods is one of the global problems at all stages of the development of human society. According to WHO experts, the state of human health is determined in proportion of 50% by the individual lifestyle; 20% - environmental conditions; another 20% - heredity and only 10% of medical services, the main role belongs to the individual way of life and especially to food [1]. Today, food is manufactured using advanced technologies and processes that ensure long-term shelf life with attractive sensory properties. Changing the composition of food by introducing many food additives can have a profound impact on the unique biochemical balance of the consumer's body [2]. In this context, there is a great need to develop functional foods, which depend on the food group they belong to (cereals, confectionery, dairy, etc.); on the diseases they can prevent or alleviate (cancer, diabetes, etc.); on the physiological effects (antitumor activity, digestion, immunology, etc.); on the category of biologically active compounds (BAC) (antioxidants, vitamins, probiotics, lipids, etc.); on the physico-chemical, sensory properties and the processes used in their production (drying, freezing, etc.) [3].

The concept of functional foods was originally promoted in 1984 by scientists in Japan, which owns over 39.0% of the global functional food manufacturing market [4]. In some countries, national programs are being developed to improve the health of the population through the development and manufacture of food ingredients, which correct the biochemical composition of food for current consumption [5]. Currently, the global market for the consumption of functional foods consists of dairy products 50 - 65%, flour 9 - 10%, functional drinks 3 - 5% and other foods 20 - 25%. There is an annual increase of 5-40% for the consumption of certain types of functional foods [6]. Thus, there is a steady increase of interest for use of plant ingredients in making functional foods.

The substitution of synthetic additives with compounds of natural origin obtained from sea buckthorn, rosehip, sorbus, hawthorn, chokeberry and grape marc in food is current. Research conducted in vivo and in vitro has shown that BAC in this plant matter have various positive effects on consumer health and are characterized by a wide range of pharmacological effects: immunomodulatory, anti-inflammatory, antidiabetic, antiatherogenic, etc. [7]. Unlike synthetic dyes, natural pigments are sensitive to chemical and physical factors, they need to be stabilized, thus presenting a strategic problem. Unconventional processes for extracting compounds of plant origin, while preserving functionality and improving bioavailability, will allow a wide range of natural dyes to be obtained for the food industry and provide a basis for sustainable economic growth. The antioxidant and microbiostatic activity of plant extracts, rich in polyphenols and carotenoids, is a promising source of alternative solutions for their use in order to replace certain antioxidants and food preservatives of synthetic origin.

Development and implementation of manufacturing technologies of functional food with compounds from natural resources spread in the Republic of Moldova, but also of some by-products of the food industry as dyes, antioxidants and preservatives is very current, because it contributes to reducing oxidative stress and nutritional allergies by integrating the concept of optimized nutrition and increasing the competitiveness of local businesses.

The purpose and objectives of the research. The work is part of the basic concept of the circular bioeconomy, in the FAO/WHO strategy for reducing raw material losses and in the UNO Program on the Development and Implementation of Functional Foods with Natural Bioactive Components, which is a way to increase safety and quality of food in optimized nutrition.

The general objective of this work is to establish the theoretical and practical principles for obtaining and stabilizing dyes, antioxidants, and preservatives of natural origin by elucidating chemical, physico-chemical and biochemical transformations that take place under conditions of extraction, storage, and addition of plant matter in food, with the formulation of technology of some functional foods. The following operational objectives were envisaged to achieve the goal:

1. Theoretical and experimental research on determining the optimal hydromodule for the extraction of water-soluble complex, which allows obtaining plant extracts with an increased content of BAC and optimal solvent consumption. Application of empirical mathematical models to describe the kinetics of the extraction process of the water-soluble complex from vegetable powders.

2. Elucidation of the influence of extraction conditions (solvent composition and temperature) on the BAC yield from berries and grape marc, the antioxidant activity determined by PCL, DPPH and HPSA tests and CIELab chromatic parameters in order to elaboration of technologies for manufacturing of local functional foods. Identification and quantification of polyphenols, anthocyanins and organic acids in hydroalcoholic extracts. Application of the mutual information analysis, canonical correlation and spline functions to determine the influence of extraction conditions on the analyzed parameters and the dependence between BAC and antioxidant activity.

3. Theoretical and experimental research on the influence of the extraction conditions of the fat-soluble complex from sea buckthorn and rosehip fruits and of the shelf life on the dynamics of accumulation of primary and secondary products of lipid oxidation, on carotenoid pigment content, physico-chemical indices of quality and antioxidant activity, CIELab chromatic

parameters of fat-soluble extracts. Application of the mutual information analysis and fuzzy sets to establish the influence of the shelf life on physico-chemical indices of quality and antioxidant activity of fat-soluble extracts.

4. Assessment of the influence of different ”*green extractions*” techniques, in particular pulsating electric field (PEF), microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE) on the recovery efficiency of BAC, antioxidant activity and CIELab chromatic parameters in extracts vegetable. Use of sensitivity analysis (first order Sobol index) to determine the level of influence of different extraction conditions (PEF, MAE, UAE, EtOH concentration and temperature) on the yield of BAC and antioxidant activity in plant extracts.

5. Evaluation of the influence of heat treatments (freezing and different drying conditions) of sea buckthorn, rosehip and sorbus fruits on the yield of BAC and antioxidant activity in extracts obtained by UAE. Identification and quantification of carotenoids in unsaponified and saponified extracts from frozen pulps of sea buckthorn, rosehip and sorbus fruits by RP-HPLC.

6. Theoretical and experimental research on the influence of pH on the color stability of pigments, evaluation of CIELab chromatic parameters and antioxidant activity in hydroalcoholic extracts of sea buckthorn, rosehip, chokeberry and grape marc in order to determine the interactions of BAC - matrix during food processing.

7. Assessment of the effect of metal ions present in food on the color stability and antioxidant activity of anthocyanin extracts from chokeberry and grape marc in order to apply them in the formulation of functional foods for the substitution of synthetic dyes.

8. Research on antimicrobial activity (AMA) and determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and bactericidal effect of vegetable powders from sea buckthorn, rosehip, hawthorn, chokeberry and grape marc on Gram-positive pathogenic microorganisms - *Staphylococcus aureus* and Gram-negative: *Escherichia coli* and *Klebsiella pneumoniae* in order to replace synthetic preservatives in the formulation of functional foods.

9. Assessment of carotenoids bioavailability *in vitro* and their individual profile in sea buckthorn, rosehip and sorbus extracts in order to identify promising food matrices for carotenoid release, processing and storage conditions of foods.

10. Determining the technological parameters and optimizing the processes for adding vegetable extracts and powders in the formulation of functional foods.

11. Assessment of the influence of plant extracts and powders on quality indicators, chromatic parameters and antioxidant activity *in vitro* in processed foods and their evolution during storage.

Research hypothesis. *Plants and their derivatives contain a wide variety of secondary metabolites that can be extracted with maintaining their functionality and improving their bioavailability. They are characterized by important coloring capacity, increased antioxidant activity, being able to inhibit free radicals in oxidative processes, but also by antimicrobial activity, manifested by the stagnation of the development of pathogenic microorganisms that cause food spoilage. Respectively, some of these BAC may be recommended in the formulation of functional foods for the substitution of synthetic additives (dyes, antioxidants, and preservatives).*

Synthesis of the research methodology and justification of the chosen research methods. For the realization of the work, traditional and non-trivial physico-chemical methods were applied, such as extraction in PEF, MAE and UAE. To characterize the composition of the extracts were applied: UV/Vis spectroscopy, IR, HPLC (separation of polyphenols, anthocyanins), RP-HPLC (carotenoids identification) and the method of capillary electrophoresis (organic acids quantification). The antioxidant capacity was determined both for the extracts obtained (DPPH, HPSA, PCL, ABTS methods, with the stabilization of Ag nanoparticles), and for the processed foods (*in situ*). Microbiological analysis methods were applied according to experimental protocols appropriate to research involving pathogenic microorganisms (*Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*). For *in situ* research, sensory, physico-chemical, rheological, microbiological methods, UV/Vis and HPLC spectroscopy were used on the food matrix.

Theoretical importance and scientific innovation of the work: for the first time all the stages of obtaining and stabilizing the compounds from native berries and grape marc with the use of secondary metabolites from vegetable matter with coloring, antioxidant and antimicrobial properties were examined, for the substitution of dyes, antioxidants, and preservatives of synthetic origin in the formulation of functional foods (flour, sugar and dairy products). This problem has been solved by conducting the following research:

- for the first time, the optimal hydromodule was determined for the extraction of water-soluble compounds from sea buckthorn, rosehip, hawthorn, sorbus, chokeberry and grape marc of local origin and the empirical models Peleg, Page and power were applied to optimizing the process of extraction in system solid-liquid;
- for the first time the carotenoids bioavailability in vitro was determined and individual carotenoids from native sea buckthorn, rosehip and sorbus fruit powders were identified;

- the originality of the work consists in theoretical and experimental argumentation, in the mutual information analysis and in the elaboration of mathematical models of canonical correlation and spline functions regarding the influence of extraction conditions on the yield of water-soluble and fat-soluble BAC, antioxidant activity and CIELab chromatic parameters in extracts from berries and grape marc;
- the originality of the work consists in the use of different techniques of ”*green extractions*”, PEF, MAE and UAE for the BAC recovery, determination of antioxidant activity and chromatic parameters of extracts; establishing the influence of the BAC extraction conditions by applying the sensitivity analysis;
- new scientific information was obtained on the influence of pH and metal ions in food on the color stabilization of pigments by CIELab analysis and antioxidant activity of plant extracts from berries and grape marc with the application of informational analysis;
- it was argued theoretically and experimentally the antimicrobial activity of vegetable powders against microorganisms that cause food spoilage, Gram-positive and Gram-negative;
- technologies have been developed for the functional foods manufacture with extracts and powders from berries and grape marc to replace dyes, antioxidants and synthetic preservatives;
- the benefits of functional products obtained by point of view of microbiological quality and stability at storage, sensory quality and antioxidant activity determining *in situ* and *in vitro* were scientifically argued.

The work was carried out based on research and experience gained in carrying out the following national and international research projects:

19.00208.1908.05 Obtaining and stabilizing dyes, antioxidants, and plant preservatives for functional foods (2019-2020).

19.80015.5007.232T Implementation of advanced processing technology for rosehip and other dried fruits to obtain powders with increased biological value for use in the food industry (2019-2020).

20.80009.5107.09 Improvement of food quality and safety by biotechnology and food engineering (2020-2023).

18.51.07.01A/PS Reducing the contamination of raw materials and food with pathogenic microorganisms (2018-2020).

COST CA Action CA 15136 European network to advance carotenoid research and applications in agro-food and health (EUROCAROTEN) (2018-2020).

16.80013.5107.22/Ro Substitution of synthetic food additives with bioactive components extracted from renewable natural resources, 2016-2018

”Eugen Ionescu” postdoctoral fellowship. Proiect ”Eco procédés pour la récupération des antioxydants à partir de déchets et produits horticoles”, “Ion Ionescu de la Brad” University of Agricultural Sciences and Veterinary Medicine from Iasi, AUF (01.03.-31.05.2015).

BECO- 2012-53-U-56135FT205. Formation de préparation et de perfectionnement à l’analyse moderne des composés chimiques bioactifs dans les produits agro-alimentaires d’origine végétale, AUF (2013 – 2014).

The applicative value of the work:

- based on the experimental results obtained, processes were developed for obtaining and stabilizing the color of hydroalcoholic extracts from grape marc;
- processes were obtained for obtaining hydroalcoholic and fat-soluble extracts from sea buckthorn, rosehip, hawthorn and chokeberry fruits;
- the manufacturing technologies of the following functional foods have been developed and patented:
  - flour products with fat-soluble extracts from sea buckthorn, rosehip and sorbus fruits;
  - cheese cream with fat-soluble extracts from sea buckthorn, rosehip and hawthorn fruit;
  - cheese dessert with hydroalcoholic extracts from sea buckthorn, rosehip, hawthorn and chokeberry fruits;
  - yogurt with hydroalcoholic extracts from sea buckthorn, rosehip, hawthorn and chokeberry fruits;
  - ice cream with powders and extracts of sea buckthorn, rosehip, hawthorn and chokeberry fruits;
- technologies were developed for the manufacture of sugar products (jellies and confectionery masses) with hydroalcoholic extracts from berries and grape marc;
- the technological and normative documentation for obtaining powder, vegetable extracts and the manufacture of functional foods was elaborated, approved and sent to the enterprises;
- the practical recommendations were developed for the use of powders and extracts for the formulation of functional foods, which were placed on the website of SRL "Rose Line" https://roseline.org/ru/blog/;

- the impact of powders, hydroalcoholic and fat-soluble extracts from berries and grape marc on the quality indicators, sensory parameters, microbiological stability, shelf life and antioxidant activity (*in vitro*) of elaborated functional foods was elucidated.

Approval of the work at national and international scientific forums. The results obtained during the work were presented and discussed at 37 national and international conferences: International Conference ”Modern Technologies in the Food Industry”, Chisinau (2012, 2014, 2016, 2018); XX International Scientific and Technical Conference “Engineering and Technosphere of the XXI century”, 2013, Sevastopol; Scientific Symposium "Horticulture - Science, Quality, Diversity and Harmony", May 24-26, 2013; International Symposium “EuroAliment”, Galati, Romania (2013, 2015, 2019); Work-shop “L’extraction des composés phénoliques à partir de produits horticoles” USAMV, Iași, 8 may 2015; The 9th edition of International Conference of Applied Sciences, CISA-2015, 04-06 June 2015, Bacau, Romania; International Scientific Congress ”Soil and food resources for a healthy life”, 22-24 October 2015, Iasi, Romania; 8th International Congress ”Pigments in Food. Colored Food for Health Benefits”, 28 June-01- July 2016, Cluj-Napoca, Romania; CISA-2016, Conference Proceedings, Vasile Alecsandri University of Bacau, Romania, June 02-04 2016; International Conference “Processes in Isotopes and Molecules”, Cluj-Napoca, Romania, 27-29 September 2017; International Conference Biotechnologies, Present and Perspectives, 24th - 25th November 2017, Suceava, Romania; International Scientific Conference ”Perspectives and Problems of Integration in the European Research and Education Area”, State University ”B.P. Hasdeu” from Cahul, June 7, 2017; Colloque franco-roumain de chimie appliquee, CoFrRoCA-2018, 27-29 Juin 2018; Conference on Applied and Industrial Mathematics, CAIM-2018, 20-23 September 2018; Symposium francophone AUF/UDJG. Y-a-t-il besoin dune nouvelle education nutritionnelle?, Galati, Romania, December 13-14 2018; Conference "Days of the Academy of Technical Sciences of Romania", October 17-19, 2019; VI International Scientific and Technical Conference TC-2020 ”Progressive Directions of Technological Complexes Development”, Lutsk, Ukraine, June 2-4 2020; National Scientific-Practical Conference "Innovation: factor of socio-economic development" 5th edition December 17, 2020; International scientific conference ”Yesterday’s heritage – implications for the development of tomorrow’s sustainable society”, February, 2021; International Exhibition of Research, Innovations and Inventions ”PROINVENT”, Cluj-Napoca, Romania (2018 - 2020); International Exhibition of Inventics “INVENTICA”, Iasi, Romania (2018, 2019); European Exhibition of Creativity and Innovation „EUROINVENT”, Iasi, Romania (2018 – 2021); International Specialized Exhibition

”InfoInvent-2019”, Chisinau, Moldova; Food Inventions and Innovations Fair ”INOVALIMENT 2020”, Bucharest, Romania.

Thesis publications. The research results and issues addressed in the thesis were published in 72 scientific papers, including a monograph, a chapter in the monograph, 20 scientific articles, 8 patents, 11 articles in collections and abstracts at national and international scientific events.

Summary of thesis chapters. The work is presented on 212 typed pages and includes the following chapters: annotations in Romanian and English, introduction, 7 chapters, general conclusions, proposals for using the results obtained in the economic fields, suggestions on potential future research directions, bibliography with 488 sources and 9 annexes. The work is illustrated with 69 tables and 99 figures.

Chapter 1, The use of BAC from horticultural sources for functional foods - current trends, includes the description of the basic components of functional foods; it are described: the modern trends applied in the development of functional foods (flour, sugar and dairy products); the characteristics of BAC from horticultural sources and their impact on health; technologies for optimizing the extraction of compounds (PEF, MAE and UAE) and possibilities of substituting synthetic food additives (dyes, antioxidants and preservatives) with BAC extracted from plant sources in order to obtain functional foods.

Chapter 2, Research materials and methods, describes the types of plant raw material and data on the content of compounds in them, the characteristic of other types of raw materials used and reagents, describes the methods of obtaining plant extracts from horticultural sources, methods of chemical, physico-chemical and microbiological analyzes of hydroalcoholic and fat-soluble extracts. It describes the methodology for determining the quality indices of functional foods developed, as well as the methodology of statistical processing and mathematical modeling of experimental results.

In **Chapter 3, Optimization of BAC extraction conditions from plant matter,** the optimal hydromodules for the extraction of the water-soluble complex from plant matter and empirical mathematical models that describe the extraction process in solid-liquid systems are determined. The influence of solvent composition and temperature on BAC extraction yield is investigated. The extraction conditions of the fat-soluble complex, the physico-chemical quality indices and the CIELab chromatic parameters of the fat-soluble extracts from sea buckthorn and rosehip fruits are determined. The dynamics of the accumulation of primary and secondary products of lipid oxidation, the evolution of the change of carotenoid content, antioxidant activity and chromatic parameters of CIELab in the fat-soluble extracts of sea buckthorn and rosehip fruits during storage were analyzed.

In **Chapter 4, The influence of pretreatment on BAC extraction yield**, the influence of PEF on the extraction yield of phenolic compounds from wine waste is investigated. The influence of MAE, solvent composition and temperature on the extraction yield of the water-soluble complex, antioxidant activity and CIELab chromatic parameters of plant hydroalcoholic extracts were investigated. The UAE conditions and the influence of heat treatments on the BAC yield, antioxidant activity and CIELab parameters for berry and grape marc extract were determined.

In **Chapter 5, Stabilization technologies, antimicrobial activity and BAC bioavailability,** the influence of pH and metal ions present in food media - Na^{+}, K^{+} and Ca^{2+} on color stabilization and antioxidant activity of berry and grape marc extracts are investigated. The microbiostatic activity of plant matter on pathogenic microorganisms was investigated. The carotenoids bioavailability (*in vitro*) in sea buckthorn, rosehip and sorbus fruits was determined.

Chapter 6, Technologies for the manufacture of functional flour and sugar products, describes the technologies for the manufacture of pasta, glazed gingerbread, jelly candies and confectionery masses with the addition of vegetable matter. The optimal technological parameters, quality indicators, microbiological stability, shelf life, CIELab chromatic parameters and antioxidant activity *in vitro* were determined.

Chapter 7, Manufacturing technologies of dairy products with BAC, describes the technologies for the manufacture of functional yogurt and cheese cream with the addition of hydroalcoholic and fat-soluble extracts from berries with the determination of optimal technological parameters, quality indicators, microbiological stability, shelf life, CIELab chromatic parameters and antioxidant activity *in vitro*.

Keywords: extracts, vegetable powders, biologically active compounds, dyes, antioxidants, preservatives, functional foods, quality.

THESIS CONTENT

1. The use of BAC from horticultural sources for functional foods - current trends

Current scientific research shows that food has the role not only to satisfy hunger and provide a supply of nutrients to the consumer, but also have the opportunity to prevent nutrition-related diseases and can improve physical and mental well-being. Consumption of functional foods thus provides an opportunity to maintain the health of consumers.

Today, the manufacture of functional foods is a current goal of the modern food industry. In developed countries, programs aimed at improving the health of the population through the development and manufacture of food ingredients are implemented. Plant origin BAC can be applied in the formulation of functional foods to replace synthetic additives (dyes, antioxidants, preservatives), to increase the shelf life and expand the range of foods.

The main directions for the development and formulation of functional foods (flour, sugar, and dairy) have been elucidated, which are aimed at fortification products with BAC of vegetable origin; dietary fiber enrichment; increasing the bioavailability of BAC or reducing their losses and reducing energy value.

For this work, sea buckthorn, rosehip, sorbus, hawthorn, chokeberry fruits and grape marc were selected, which contain phytonutrients with significant health benefits. These berries and marc contain BAC with different chemical composition, are characterized by a wide range of pharmacological effects, show antioxidant and antimicrobial activity against pathogenic microorganisms.

In order to optimize the BAC extraction from the vegetal matter, the particularities of different "*green extractions*" techniques (PEF, MAE, UAE) were analyzed, which are characterized by minimal sample handling, improved extraction efficiency, shorter operating times and by obtaining extracts of quality.

The characteristics of synthetic additives and their impact on consumer health and the possibilities of substituting synthetic food additives by BAC in order to obtain functional foods were analyzed.

2. Research materials and methods

The main research topics are dried berries: sea buckthorn (*Hippophae rhamnoides* L.), rosehip (*Roza canina* L.), sorbus (*Sorbus aucuparia* L.), hawthorn (*Crataegus monogyna*) and chokeberry (*Aronia melanocarpa*) and grape marc (*Vitis vinifera* L.) from red varieties harvested during 2011-2018, hydroethanolic and fat-soluble extracts of BAC obtained by different methods (conventional (maceration), PEF, MAE, and UAE), as well as food products (flour, sugar and dairy) developed on the basis of them.

The methodology for obtaining water-soluble and fat-soluble extracts from plant matter was determined by maceration, PEF, MAE, UAE, and BAC characterization using new non-trivial instrumental methods.

The research methodology of functional foods developed based on plant matter by point of view of quality (sensory, physico-chemical, and microbiological analysis), determination of their shelf life and antioxidant activity *in vitro* was established.

The methodology for calculating the first order statistical characteristics and the elaboration of mathematical models for describing the kinetics of the extraction process, models based on information analysis, sensitivity, polynomial mathematical models, and fuzzy sets were determined.

3. Optimization of BAC extraction conditions from plant matter

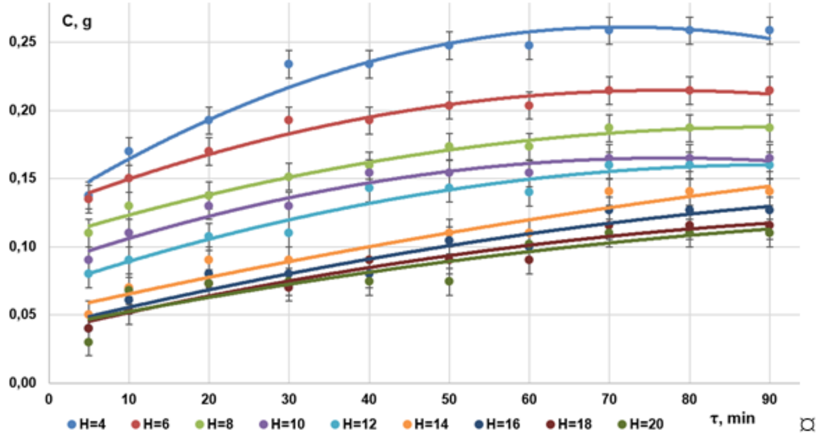
3.1. Determination of the conditions for extraction of the water-soluble complex from plant matter

The BAC extraction from the plant matrix is a complex process, being influenced by extraction conditions, especially by the hydromodule, the composition of the solvent and the extraction temperature. The research results on the extraction of water-soluble complex from plant matter - chokeberry, sea buckthorn, rosehip, sorbus, hawthorn and grape marc are presented.

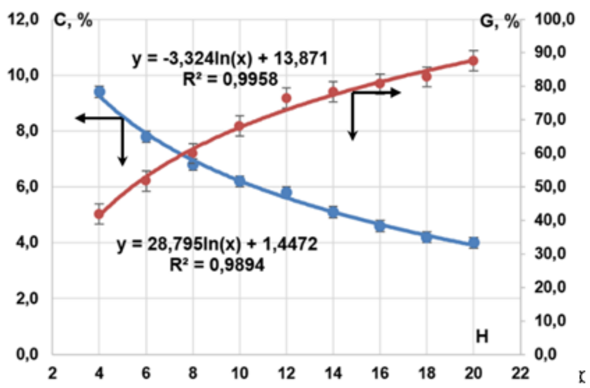
3.1.1. Determination of the optimal hydromodule for the extraction of the water-soluble complex from plant matter

The optimal hydromodule (H_{optim}) is a parameter that ensures an increased extraction efficiency of the water-soluble complex. Distilled water was used as a solvent because it contributes to better tissue separation and rupture of cell walls of plant matter, thus facilitating the diffusion of water-soluble compounds [8]. Fig. 1. shows the kinetics of the process of extracting the water-soluble dry matter content (CSUH) (a), the degree of extraction of the CSUH (b) and the determination of H_{optim} (c) for the extraction of the CSUH from all the powders investigated. It was shown that CSUH in the extract increases over time until the equilibrium concentration is reached, the extraction time being 90 min. The general character of the CSUH extraction kinetics curves in extracts depending on the hydromodule for all powder samples is common (Fig. 1a). Fig. 1b indicates that for all the samples investigated, with the increase of the hydromodule, the CSUH in the extract decreases, due to dilution.

At the modification of the hydromodule from 4 to 20, CSUH in the researched extracts was reduced as follows: for sorbus - 2.4 times, sea buckthorn, rosehip and chokeberry - 2.3 times, grape marc - 2.2 times and for hawthorn powder - 2.1 times in the hydromodule range 8 to 24. As the hydromodule increased from 4 to 20, the extraction degree of CSUH increased as follows: for grape marc - 2.2 times, sea buckthorn, rosehip and chokeberry - 2.1 times and for sorbus - 2.0 times; for hawthorn the increase of the degree of extraction was 2.4 times at the variation of the hydromodule from 8 to 24. The increase in the degree of extraction is probably due to the fact that a larger volume of solvent reacts with the particles of vegetable powders and a higher CSUH could pass into the solvent [9].



a)



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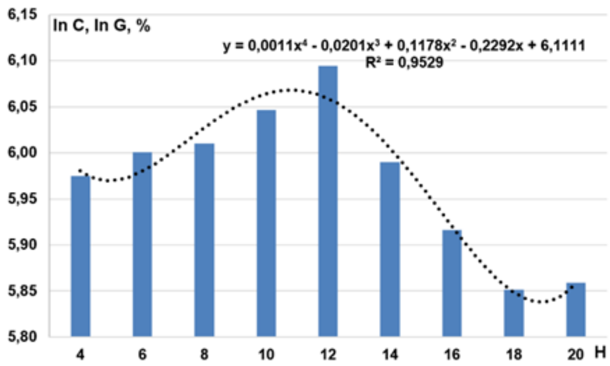


c)

Fig. 1. Determination of the extraction characteristics of CSUH from sea buckthorn powder in water: a) kinetics of the extraction process of CSUH; b) the dependence of the content and the degree of CSUH extraction on the hydromodule; c) dependence of the logarithm of the concentration and the degree of CSUH extraction on the applied hydromodule

Fig. 1c shows the determination of H_{optim} according to the dependence of the logarithms of the concentration and the degree of CSUH extraction in water depending on the applied hydromodule. It was found that H_{optim} for vegetable powders is: 8 for grape marc; 12 - sea buckthorn, rosehip, sorbus; 14 - chokeberry and 20 for hawthorn. The optimal extraction time is 90 minutes, being an important advantage [10].

To describe the kinetics of the extraction process in solid-liquid system, three empirical mathematical models Peleg, Page and model of power were used [11-13]. The values of the coefficient of determination (R^2) and the root mean square error (RMSE), attest that the empirical models demonstrated a good concordance of the experimental data with the approximation data for vegetable powders, tab. 1.

Table 1. Constant values, R^2 and RMSE determined for the Peleg, Page and power empirical models describing the process of extracting CSUH from vegetable powders in water at the optimum hydromodule

Characteristics	Vegetable powders					
	Grape marc	**Sea buckthorn**	**Rosehip**	**Sorbus**	**Chokeberry**	**Hawthorn**
Optimum hydromodule	8	12	14	14	14	20
Peleg empirical model						
K_1	23.3± 7.92	50.18± 22.32	54.57± 2.45	55.33± 25.82	85.57± 41.81	130.46± 68.6
K_2	5.25± 0.30	5.97± 0.65	6.96± 0.70	6.93± 0.77	6.05± 0.99	6.76± 1.48
R^2	0.979	0.952	0.957	0.950	0.940	0.926
RMSE	0.008	0.011	0.009	0.010	0.012	0.012
Page empirical model						
k	2.57± 0.14	3.18± 0.30	3.29± 0.32	3.31± 0.36	3.78± 0.66	4.18± 0.73
n	-0.10± 0.01	-0.12± 0.02	-0.11± 0.03	-0.11± 0.03	-0.15± 0.04	-0.16± 0.04
R^2	0.970	0.950	0.939	0.927	0.908	0.916
RMSE	0.005	0.007	0.007	0.008	0.011	0.009
Empirical model of power						
k	0.08± 0.01	0.05± 0.01	0.04± 0.01	0.04± 0.01	0.03± 0.01	0.02± 0.01
n	-0.18± 0.03	-0.26± 0.05	-0.26± 0.06	-0.26± 0.07	-0.35± 0.10	-0.39± 0.11
R^2	0.970	0.957	0.942	0.930	0.907	0.928
RMSE	0.005	0.006	0.006	0.007	0.011	0.009

The empirical mathematical models Peleg, Page and model of power can be applied to model and optimize extraction in the system solid-liquid of CSUH from powders of sea buckthorn, rosehips, sorbus, hawthorn, chokeberry and grape marc, because there is a high concordance between the data experimental and computational.

3.1.2. The influence of solvent composition on BAC extraction yield

The influence of EtOH concentration on TPC (total polyphenol content) in all plant samples, TAC (total anthocyanin content) in chokeberry and grape marc powder, as well as TC (tannin content) in grape marc was analyzed. The evolution of the antioxidant activity of water-soluble substances (AAWS) determined by the photochemiluminescence (PCL) method was investigated depending on the solvent concentration. Also, the antioxidant activity was evaluated, determined by DPPH and HPSA tests in acidic and alkaline medium depending on the concentration of the EtOH solution.

Under the influence of the solvent composition (20% - 80% (v/v)) on the yield of the water-soluble complex, it was found that TPC in plant extracts increases with increasing EtOH concentration in hydroalcoholic solutions from 20% to 60% (v/v) and then decreases when directed to the 80% (v/v) EtOH concentration. Thus, the maximum values of TPC extracted with the hydroalcoholic solution of 60% (v/v) from vegetable powders were (the values are presented in descending order): chokeberry - 17.39 mg GAE/g d.w.; rosehip - 12.31 mg GAE/g d.w.; scorus - 6.44 mg GAE/g d.w.; sea buckthorn - 5.80 mg GAE/g d.w.; hawthorn - 5.57 mg GAE/g d.w. and grape marc - 3.66 mg GAE/g d.w., fig. 2. The efficiency of the extraction process depends on the nature of the polyphenolic compounds present in vegetable powders. The solubility of polyphenols can be explained by their stereochemistry (polar and non-polar fragment of their molecules) and intermolecular forces (mainly hydrogen bonds), which occur between them and the solvent [14]. The concentration of the EtOH solution influences the extraction yield of phenolic compounds, decreases the boiling point of the extract at concentration and reduces the rate of degradation of bioactive compounds [15].

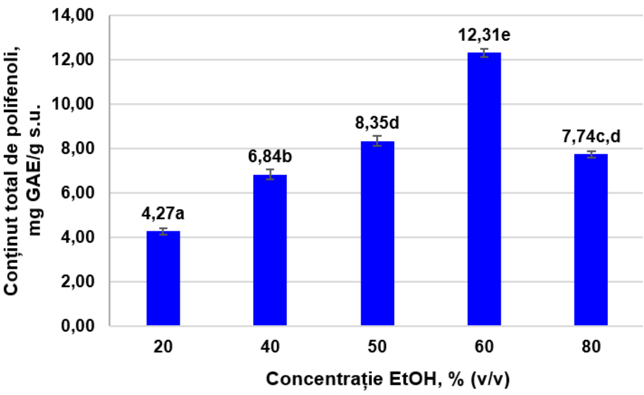


Fig. 2. Influence of EtOH concentration on total polyphenol content in rosehip extracts

Anthocyanins, having in their chemical composition phenolic groups, can be efficiently extracted with hydroethanolic solutions, as in the case of polyphenols. Maximum TAC values in chokeberry and marc extracts were reached at 60% (v/v) EtOH concentration, constituting 1.80 mg ME/g d.w. and 0.38 mg ME/g d.w. respectively. It is attested that pure EtOH is not preferable for the extraction of hydrophilic anthocyanins due to the presence of a small amount of water, necessary for their extraction [16]. Pure EtOH can cause damage to the internal structure of anthocyanins [17].

A significant increase of TAC in chokeberry and marc grape extracts was demonstrated with an increase of EtOH in water to 60% (v/v), and a further increase of alcohol content led to a reduction of the total anthocyanin content, 1.69 mg ME/g d.w. (chokeberry) and 0.27 mg ME/g d.w. (grape marc). The decrease of TAC extracted from 80% (v/v) solutions is due to the fact that hydrophilic anthocyanins are no longer extracted, due to the reduction of water content and degradation of the internal structure of anthocyanins [18].

The evolution of TC in grape marc depending on the concentration of the EtOH solution is shown in fig. 3. Extracted TC increased from 0.25 mg TAE/g d.w. up to 0.45 mg TAE/g d.w. with increasing EtOH concentrations up to 60% (v/v). Subsequently, it decreased with increasing volume of EtOH (96% v/v) to 0.1 mg TAE/g d.w. This phenomenon is due to the different solubility of the components, procyanidins being soluble in the aqueous phase, and catechins - in the organic part of the solvent [19]. An important contribution has the degree of polymerization, due to the increase in the number of hydroxyl groups [20].

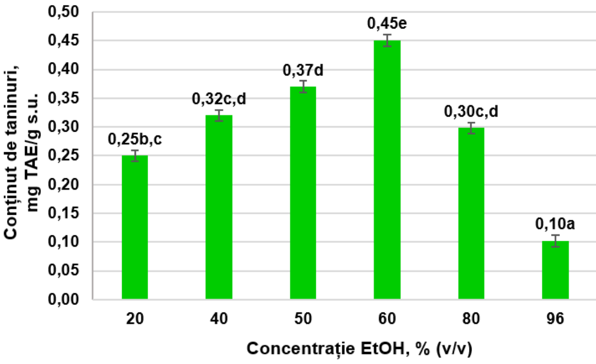


Fig. 3. Influence of EtOH concentration on TC from grape marc

Due to the hydrophilic nature of polyphenols in berries and grape marc, they can influence the AAWS, determined by the PCL test. It was found that in the samples of plant extracts AAWS is in accordance with the TPC values, namely the samples with the highest TPC are in accordance with the samples with the highest AAWS. In hydroalcoholic extracts with a concentration of 60% (v/v) AAWS constitutes (descending order): chokeberry - 119.65 μmol AAE/g d.w., rosehip - 98.67 μmol AAE/g d.w., sorbus- 47.92 μmol AAE/g d.w., sea buckthorn - 39.41 μmol AAE/g d.w., hawthorn - 35.91 μmol AAE/g d.w. and grape marc - 19.67 μmol AAE/g d.w.

The correlation between TPC and AAWS in the studied extracts is very good, and the R^2 values vary between 0.90 - 0.98; in the case of the TAC-AAWS and TC-AAWS correlation this is good, constituting R^2 = 0.67 - 0.81 and R^2 = 0.71 respectively. This correlation can be explained by the chemical composition of the extracted phenolic compounds, which having an enthalpy of osmolytic dissociation of -OH bonds and low ionization potential, positively influenced their antioxidant activity [21, 22].

The influence of pH on the stability of BAC in plant extracts was evaluated by determining the antioxidant activity based on DPPH and HPSA tests. It is attested that all EtOH extracts show important antioxidant activity by neutralizing free radicals DPPH and H_2O_2 in both acidic (pH 2.0 ± 0.1) and alkaline (pH 8.0 ± 0.1) medium, fig. 4. The highest % inhibition of the DPPH radical in acid medium had the extracts with a maximum yield of extraction of polyphenolic compounds from vegetable powders, from the hydroalcoholic solution of 60% (v/v): sea buckthorn - 93.06%; rosehip - 84.38%; sorbus - 83.21%; hawthorn - 81.1%, chokeberry - 87.7% and grape marc - 89.7% inhibited. The low pH value contributed to the stability of polyphenols, especially anthocyanins, because in this pH range = 1.5 - 2 in their chemical structure appears a cation flavylium, being very stable [23]. Also, under these conditions, the content of anthocyanins increases by releasing the monomeric anthocyanins from the polymeric ones, by disturbing the macromolecules, and as a result, the antioxidant activity value increases [24].

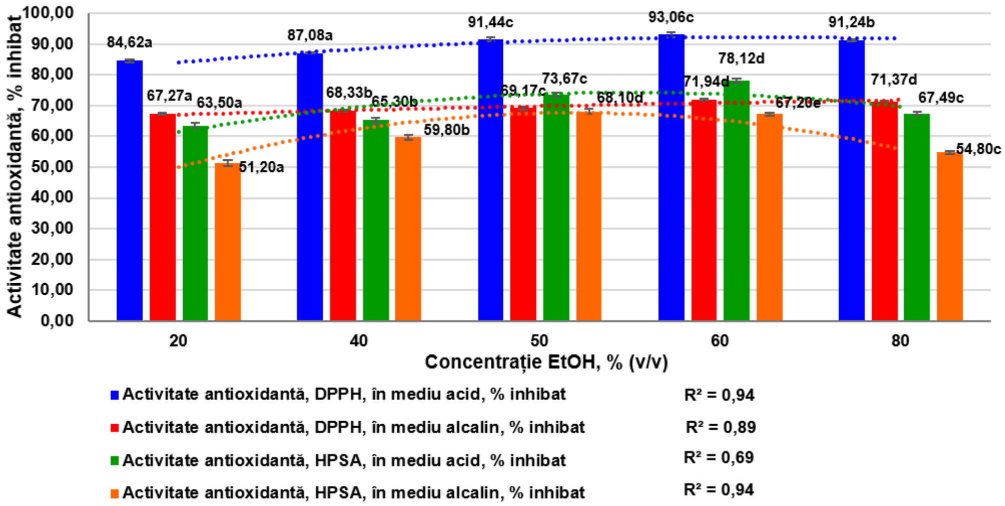


Fig. 4. Evolution of antioxidant activity in acidic and alkaline medium, determined after DPPH and HPSA tests depending on the concentration of EtOH solution in sea buckthorn extracts

In alkaline medium, % inhibited DPPH values were lower compared to the values obtained in acidic medium and consists: sea buckthorn - 71.9%, rosehip - 77.8%, sorbus - 71.7%, hawthorn - 69, 0%, chokeberry 71.9% and grape marc - 76.9% inhibited. This phenomenon is explained by the decrease of polyphenols stability in an alkaline medium. In the case of anthocyanins, the reduction of stability is due to the structural changes of the flavylium cation to a colorless chalcone, which is less stable [23], contributing to the decrease of the antioxidant activity value. The % inhibited H_2O_2 values in acidic and alkaline medium, for all the investigated samples are lower than in the case of the DPPH test. This is explained by the fact that H_2O_2 is a weak oxidizing agent and can directly inactivate some enzymes, by oxidizing the main groups of thiol (-SH) that are contained in the studied plant matter.

The mutual information analysis established the influence of the concentration of the ethanolic solution on the BAC and the antioxidant activity, in the 6 types of vegetal materials. It was shown that the concentration of the ethanolic solution has a different influence on the analyzed parameters. It was found that the interdependencies between parameters are more pronounced than the dependencies between the concentration of ethanol as a target influencing factor and the parameters.

Using the canonical correlation analysis, the mathematical model was established that allows the determination of the dependence between the content of BAC and antioxidant activity in grape marc extracts.

3.1.3. The influence of temperature on BAC extraction yield

Temperature is an important factor influencing the increase of BAC extraction yield. In general, the increase of temperature has a positive effect on the extraction of polyphenolic compounds from plant sources [25, 26], explained by increasing the solubility of polyphenols in the solvent, increasing the diffusivity of extracted molecules, improving mass transfer from plant matter, and reducing solvent viscosity. Also, high extraction temperatures lead to changes in the plant matrix, and heat increases the permeability of cell walls, facilitating the extraction process [9].

The influence of the extraction temperature 30, 45 and 65°C for 90 min on the extraction yield of polyphenolic compounds and antioxidant activity in berry and grape marc extracts was investigated. Water and EtOH solution in concentrations of 40% - 96% (v/v) were used as solvent. It has been shown that in all the researched samples, when the temperature is increased from 30 to 65°C, the BAC content increases with the variation of the EtOH concentration up to 60% (v/v) and then decreases to 96% (v/v).

At 65°C, the maximum values of BAC content were attested for hydroalcoholic solutions of 60% (v/v). In the case of TPC, the values were (in descending order): for rosehip - 73.57 mg GAE/g d.w.; chokeberry - 55.22 mg GAE/g d.w.; sea buckthorn - 32.33 mg GAE/g d.w.; sorbus - 15.23 mg GAE/g d.w.; hawthorn –14.89 mg GAE/g d.w. and grape marc - 11.02 mg GAE/g d.w. For the total flavonoids content (TFC) the order has changed slightly: the first is chokeberry, with 50.71 mg GAE/g d.w., followed by rosehip - 46.22 mg GAE/g d.w., sea buckthorn - 29.65 mg GAE/g d.w.; sorbus –10.79 mg GAE/g d.w.; hawthorn –10.64 mg GAE/g d.w. and grape marc - 7.76 mg GAE/g d.w. The TC values, arranged in descending order, are similar to the order for polyphenols: rosehip - 7.33 mg TAE/g d.w.; chokeberry - 5.49 mg TAE/g d.w.; sea buckthorn - 3.20 mg TAE/g d.w.; sorbus - 1.59 mg TAE/g d.w.; hawthorn –1.45 mg TAE/g d.w. and grape marc - 1.37 mg TAE/g d.w.

The chokeberry extracts had the highest TAC, constituting 4.12 mg ME/g d.w., followed by grape marc extracts, with 0.97 mg ME/g d.w.

At high temperatures, the degradation of anthocyanins is mainly caused by oxidation or rupture of covalent bonds and can lead to the formation of a variety of compounds depending on the strength and nature of heating. Thermal degradation of anthocyanins involves the opening of the pyrylium ring and the formation of calcone, which being unstable, rapidly degrades into phenolic acid and aldehyde, fig. 5.

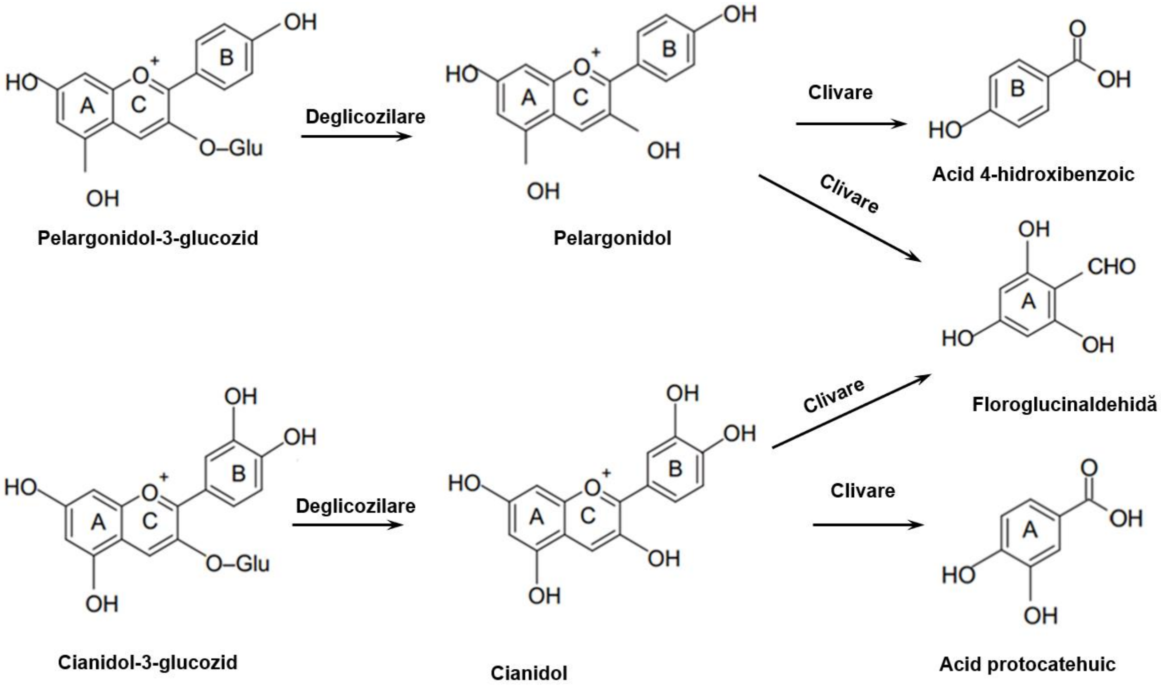


Fig. 5. Possible mechanisms of thermal degradation of anthocyanins [27]

Extraction temperature is one of the most important factors influencing antioxidant activity in plant extracts. Similar trends in the evolution of antioxidant activity were demonstrated, determined by the DPPH test in the researched extracts depending on the extraction temperature. As the temperature increased from 30 to 65°C, the antioxidant activity increased simultaneously with the variation of the EtOH solution concentration up to 60% (v/v), then decreased to the concentration of 96% (v/v). Maximum values of antioxidant activity in plant extracts were obtained for 65°C and the concentration of the 60% (v/v) EtOH solution. The values of antioxidant activity in the extracts are presented in descending order: rosehip - 97.86%; grape marc - 91.55%; hawthorn - 89.23%; chokeberry 88.35%, sorbus - 87.27% and sea buckthorn - 84.46% inhibited. The extraction temperature of 65°C allowed to maintain the antioxidant activity of polyphenolic compounds in berries and grape marc, due to the combined effect of non-enzymatic reactions and the stability of polyphenolic compounds [28].

The correlation between TPC - AA, TFC-AA and TC-AA in the studied extracts is very good, and the R^2 values vary between 0.914 - 0.971, 0.916 - 0.973 and 0.74 - 0.94 respectively. This phenomenon can be explained by the fact that the evolution of antioxidant activity in plant extracts depending on the extraction temperature was similar to the evolution of TPC, TFC and TC, which suggests the idea that these compounds capture free radicals and show antioxidant activity. The correlation between TAC-AA (chokeberry and grape marc extracts) is positively moderate, the values of the coefficient R^2 vary between 0.566 - 0.760. In this case, the correlation was influenced not only by the extraction temperature, but also by the BAC structure.

Individual polyphenols were identified by the HPLC method in the hydroethanolic extracts obtained at a concentration of 60% (v/v) and at an extraction temperature of 65°C. The extracts were found to contain significant amounts of hydroxybenzoic acid, hydroxycinnamic acids, flavones, flavonoids and ferulic acid methyl ester. In the hydroethanolic extracts from chokeberry fruits and grape marc, the composition of individual anthocyanins was identified by HPLC method. In the chokeberry extract, the highest content was cyanidol-3-galactoside (64.0%) and cyanidol-3-arabinoside (30.5%), as the level of cyanidol-3-glucoside (2.8%) and petunidol-3-glucoside (2.7%) was lower.

Malvidol-3-glucoside (53.6%) is the main anthocyanin identified in grape marc extract, followed by malvidol-3-acetylglucoside (12.3%), peonidol-3-glucoside (8.6%), petunidol -3-glucoside (8.2%), delfinidol-3-glucoside (5.3%) and malvidol-3-coumarylglucoside (5.1%). In addition, differences in chemical structure showed a selective extraction of anthocyanins: monoglycosides were better extracted than acetylated glycosides and coumarin glycosides. Also, methoxyl and hydroxyl groups probably affect the polarity and stability of anthocyanins, as malvidol was extracted in larger quantities than peonidol> petunidol> delfinidol> cyanidol, due to the higher number of methoxyl and hydroxyl groups [29].

In the hydroalcoholic extracts from berries and grape marc, organic acids have been identified, which have an essential importance in the elaboration of fortified foods with vegetable matter. The malic, citric, ascorbic and acetic acids was identified in all plant extracts in different amounts. Thus, in significant quantities, malic acid was in the extracts of sorbus (3337.0 mg/L) and sea buckthorn (2151.0 mg/L), citric acid - in the rosehip extract (1684.0 mg/L), ascorbic acid in the extracts rosehip (486.0 mg/L) and sea buckthorn (146.7 mg/L) and acetic acid - in grape marc (92.95 mg/L). The highest succinic acid content was identified in sea buckthorn extract (153.20 mg/L), followed by scorus (128.40 mg/L) and rosehip (56.89 mg/L). Lactic acid was identified only in two hydroalcoholic extracts: sea buckthorn (92.64 mg/L) and rosehip (30.54 mg/L), and tartaric acid was determined only in grape marc (795.90 mg/L).

The color of plant extracts is an extremely important feature. The representation the shade of the colors of the vegetal extracts according to the CIELab system attests that in the extracts of chokeberry and grape marc the shade of red predominates; in the extracts of hawthorn and rosehip - the orange shade, and in the extracts of sorbus and sea buckthorn - the yellow color, fig. 6. Cubic spline functions were used to establish the mathematical model with variable coefficients for the values of the total polyphenol content in grape marc extracts, at different temperatures and concentrations of the EtOH solution. Canonical correlation analysis was used to establish a mathematical model for grape marc extracts that reveals the dependence between BAC, antioxidant activity, extraction temperature and EtOH concentration.

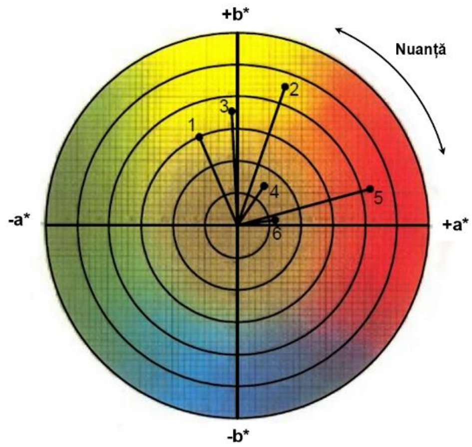
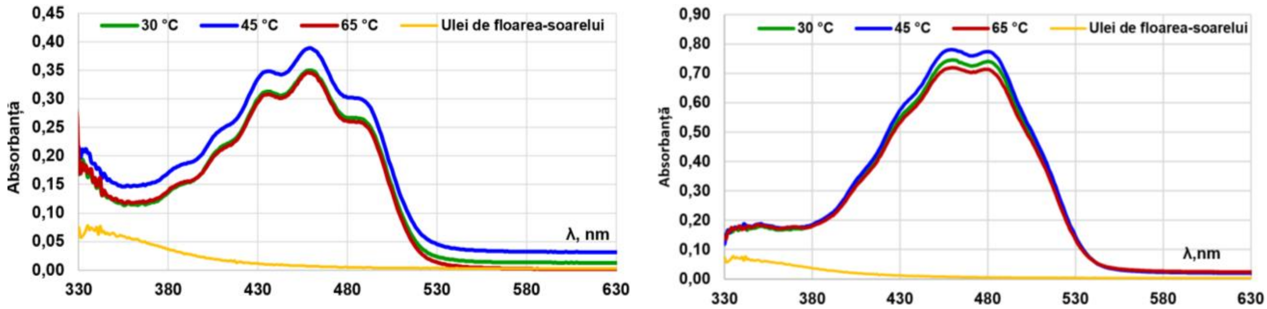


Fig. 6. Representation of the color shade of the extracts according to the CIELab system: 1- sea buckthorn; 2- rosehip; 3-sorbus; 4-hawthorn; 5- chokeberry; 6-grape marc

3.2. Determination of the extraction conditions of the fat-soluble complex from sea buckthorn and rosehip fruits

Sea buckthorn and rosehip fruits have a promising source of lipophilic BAC, which includes carotenoid pigments. Refined deodorized sunflower oil was used as a solvent for the extraction of carotenoids, with a high content of mono- and polyunsaturated acids. It was highlighted that the physico-chemical quality indices of sea buckthorn and rosehip extracts obtained at temperatures 30–65°C vary insignificantly and correspond to the rules established for refined deodorized vegetable oil [30]. UV-Vis absorption spectra have shown that fat-soluble extracts obtained at temperatures of 30-65°C have absorption curves of the same shape but are offset by the vertical axis, indicating a different extraction yield of fat-soluble compounds at the same wavelength, fig. 7.

Carotenoid pigments in the spectra of sea buckthorn extracts have three important absorption bands in the visible range: the first (λ_{max} = 435 - 437nm); the second (λ_{max} = 458 - 460nm) and the third (λ_{max} = 480 - 484nm). In the case of rosehip extracts in the spectra, two essential bands are presented - the first (λ_{max} = 459 - 460nm) and the second (λ_{max} = 479 - 481nm). The absence of maximum absorption in the spectra of sunflower oil attests to the lack of carotenoid pigments, due to the application of refining and deodorization operations in the oil production technology, the pigments being thermally decomposed and removed [31].



a) b)

Fig. 7. UV-Vis absorption spectra of fat-soluble extracts of berries and sunflower oil as a function of temperature: a) sea buckthorn; b) rosehip

The infrared (IR) absorption spectra of the fat-soluble extracts of sea buckthorn and rosehip fruits were analyzed in a function of the extraction temperature, fig. 8. The extraction temperature does not influence the position of the specific absorption maxima. However, with the increase of the extraction temperature, a higher intensity of the spectral lines characteristic of the double bonds is attested. This fact speaks of the favorable influence of the extracted antioxidants on the oxidative stability of sunflower oil

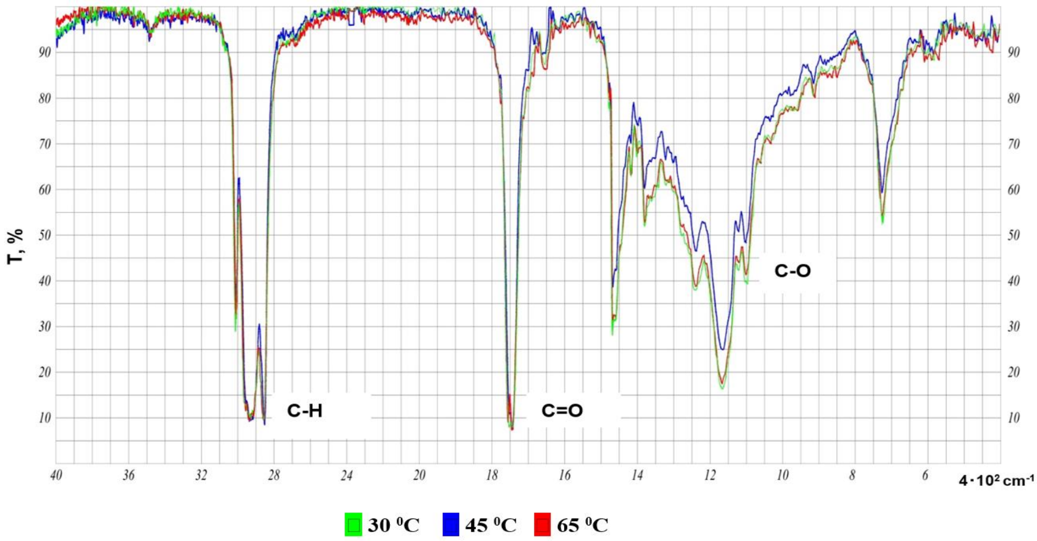


Fig. 8. IR absorption spectra of fat-soluble extracts of sea buckthorn fruit in a function of extraction temperature

It was found that the temperature variation from 30 to 65°C changed the carotenoids extraction yield in the fat-soluble extracts of sea buckthorn and rosehip fruits, showing that the highest rate of pigments was reached at 45°C and then decreased at 65°C. In berry extracts, zeaxanthin and lycopene had the highest yield: for sea buckthorn - 9.55 and 9.40 mg/100g d.w. and for rosehips - 14.62 and 14.41 mg/100g d.w. respectively. Lutein was detected only in sea buckthorn extracts, which was also demonstrated by HPLC analysis, the content being 7.76 mg/100g d.w. at 45°C and 6.84 mg/100 g d.w. at 65°C, fig 9. The reduction in carotenoid extraction efficiency to 65°C is explained by the fact that the heat treatment caused the formation of different *cis* isomers of carotenoids, thus reducing the content of *trans* carotenoids. In addition, carotenoids have different ability to form *cis* isomers.

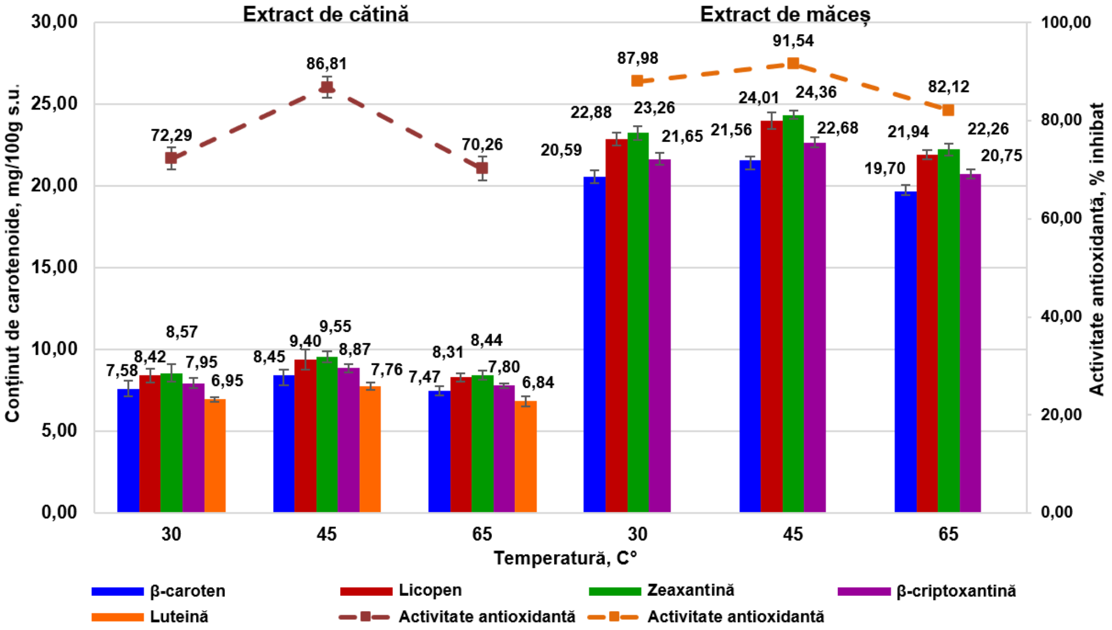


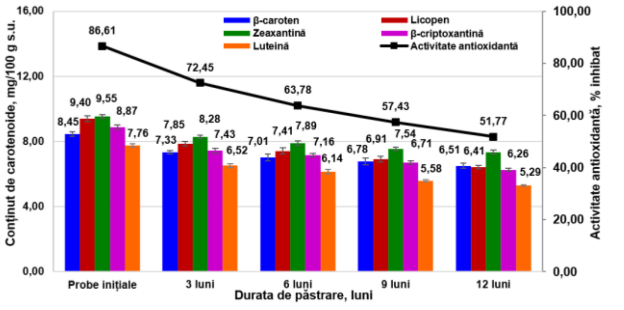
Fig. 9. Influence of extraction temperature on carotenoid content and antioxidant activity in fat-soluble extracts of sea buckthorn and rosehip

The data in fig. 9 attests that carotenoid pigments are responsible for the antioxidant activity in fat-soluble extracts of sea buckthorn and rosehip. High values of DPPH inhibited percentage correspond to carotenoid extraction efficiency at 45°C. Thus, in sea buckthorn extracts the maximum value of antioxidant activity was 86.81% inhibited DPPH, and for rosehip - 91.54% inhibited DPPH. The increased antioxidant activity of the oily extracts was due to the synergistic interaction between the carotenoids in the berries and the tocopherols, which were present in the sunflower oil [32]. The isomers of the cis configuration of carotenoids, which have lower antioxidant properties than trans carotenoids [33], contributed to the decrease of antioxidant activity values at 65°C.

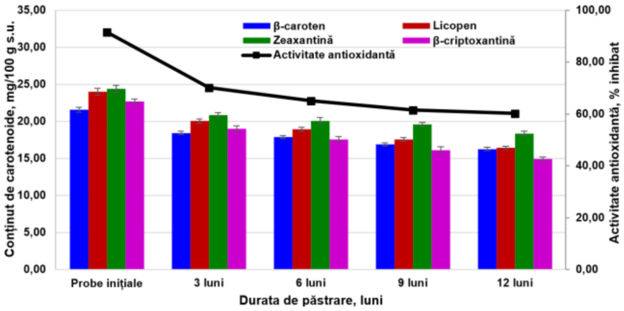
The color of sea buckthorn and rosehip oil extracts is an important feature that can influence consumer perception and food quality. The highest value of component a* (red/green) was detected in the fat-soluble extract rosehip, being 24.90, which is attributed to the increased content of orange carotenoids (lycopene, β-carotene), and in the case of sea buckthorn extract and sunflower oil, a* were shifted to green and are negative -10.52 and -3.84 respectively, indicating the presence of chlorophyll pigments. In all studied samples the values of component b* (yellow/blue) correspond to the presence of yellow pigments (zeaxanthin, β-cryptoxanthin, lutein), being positive. In sea buckthorn and rosehip extracts, the b* component is 69.51 and

50.69, also demonstrating the increased presence of carotenoid pigments, and in the case of vegetable oil the b* value is lower, being 29.08 due to technological operations which contributed to the decrease of the content of yellow pigments [31].

Lipid oxidation is a major factor responsible for reducing the shelf life and nutritional value of foods by limiting the content of polyunsaturated fatty acids and altering sensory properties (taste, smell and color) [34]. Analysis of the change in physico-chemical properties of fat-soluble extracts (acidity index, peroxide index, conjugated dienes and trienes, p-anisidine index) during storage for 12 months at 4°C and in the absence of light demonstrates, that the BAC fat-soluble complex from vegetable powders positively influenced the oxidative stability of the extracts relative to sunflower oil. The monitoring of the individual carotenoid content and the antioxidant activity in the fat-soluble extracts of sea buckthorn and rosehip during storage for 12 months was performed, fig. 10.



a)



b)

Fig. 10. Change in the individual carotenoids content and antioxidant activity in the oily extracts of sea buckthorn (a) and rosehip (b) during storage

Based on the data obtained, it was established that during storage for 12 months at 4°C in the extracts of sea buckthorn and rosehip the content of individual carotenoids was reduced: β-carotene with 23.0% and 29.4%; lycopene with 31.8% and 31.3%; zeaxanthin with 23.0% and 30.3%; β-cryptoxanthin with 29.4 and 29.9% respectively and lutein with 31.9% in sea buckthorn extracts. The total carotenoid content (TCC) decreased with 26.9% in sea buckthorn extracts and with 30.3% in rosehip extracts. It was found that the largest leap in TCC reduction occurred in the first three months of oil extracts storage, accounting for about 15% compared to the initial carotenoid content. The reduction of TCC in oily extracts during storage was most likely due to geometric isomerization and oxidation, fig. 11. The antioxidant activity of carotenoids is based on their individual properties of neutralizing singlet oxygen and the ability to capture peroxyl radicals. It has been shown that the reduction of antioxidant activity is directly related to the decrease in carotenoid content and the chemical composition of the food matrix.

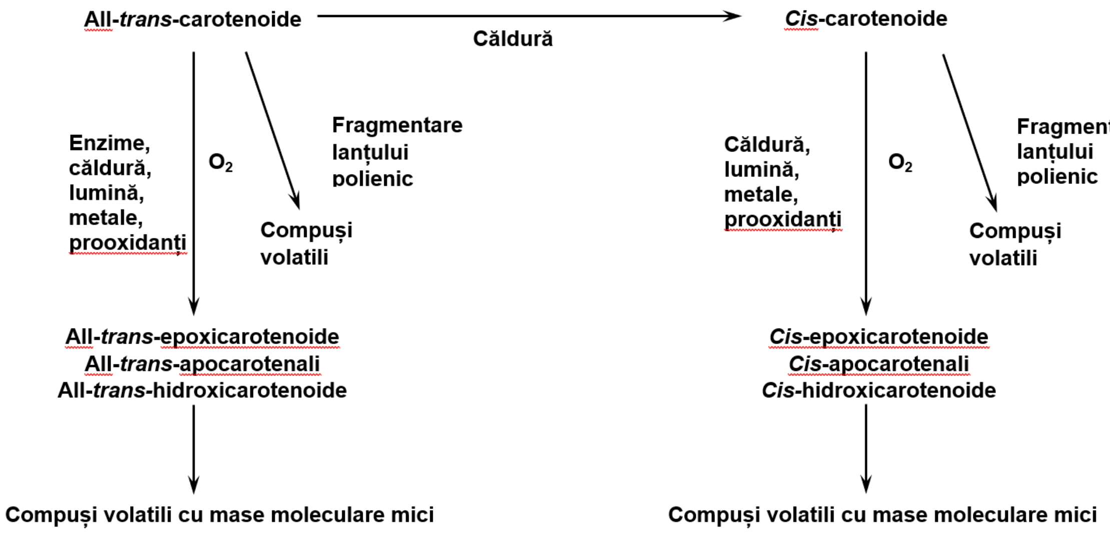


Fig. 11. The probable scheme of carotenoid degradation in food [33]

It is attested that the storage time of the oily extracts from sea buckthorn and rosehip influenced the chromatic parameters CIELab. In the case of sea buckthorn extract, the number of green pigments was reduced, which is justified by increasing the value of a* from -3.84 to -0.56. In the case of rosehip extracts, the content of red pigments was reduced, resulting in a decrease of the value of a* from 24.90 to 9.56. The change in the b* value from 69.51 to 37.11 for sea buckthorn and from 50.69 to 25.94 for rosehip was due to the decrease in the content of yellow carotenoid pigments. The increase of L* value in sea buckthorn and rosehip fruit extracts is in the range of 87.12 - 95.58 and 73.4 - 81.04, respectively. This phenomenon is explained by exposure to the oxidation of extracts during storage, which led to partial discoloration of carotenoids and the appearance of a pale color.

Fuzzy sets were used to establish the generalized mathematical model for determining the influence of temperature on physico-chemical quality indices and antioxidant activity of fat-soluble extracts, fig. 12 and 13.

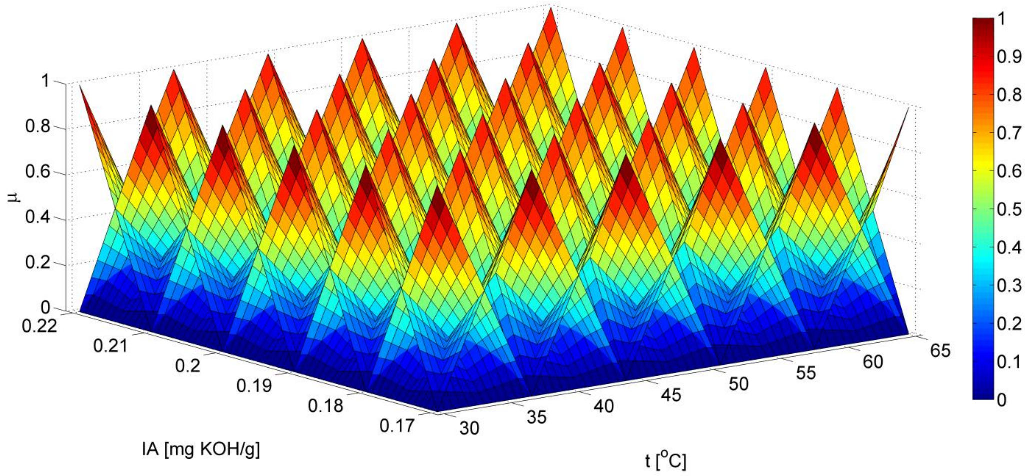


Fig. 12. Generalized fuzzy mathematical model PI= f (t, AI) for sunflower oil, the extracts of sea buckthorn and rosehip: representation of triangular fuzzy sets (6 and 6)

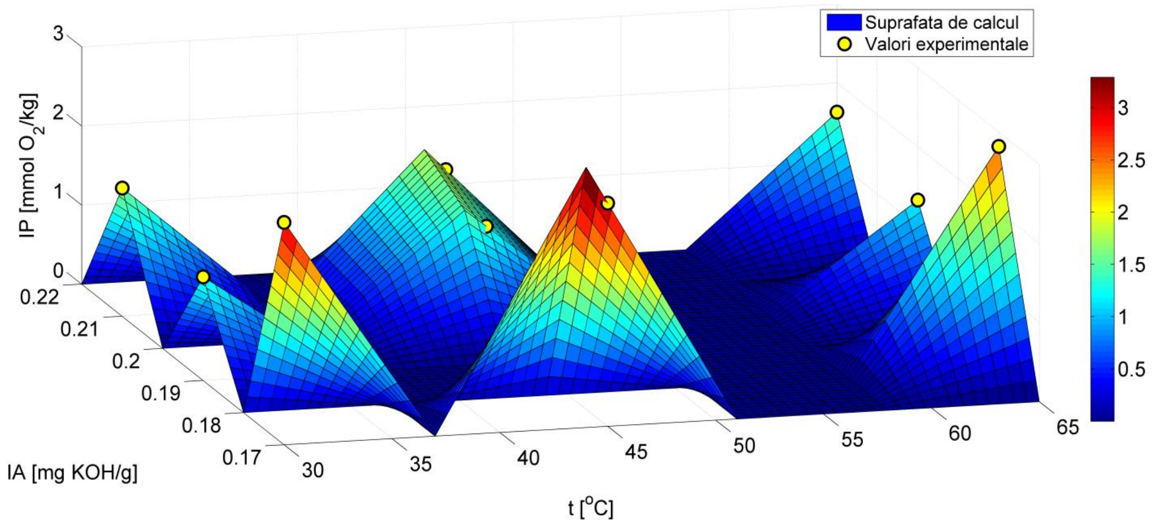


Fig. 13. Generalized fuzzy mathematical model PI = f (t, AI) for sunflower oil, the extracts sea buckthorn and rosehip: experimental values and calculation area

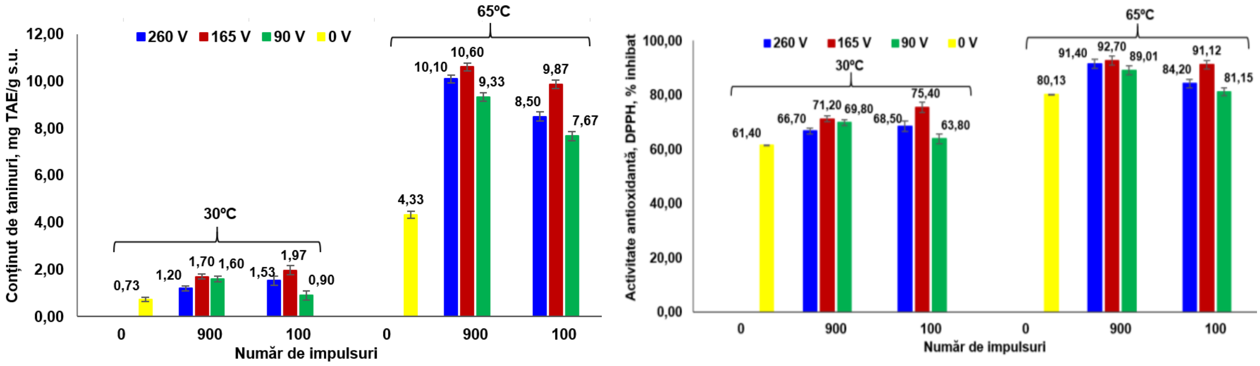
Mutual information analysis was applied to determine the influence of shelf life on physico-chemical quality indices and antioxidant activity of fat-soluble extracts of sea buckthorn and rosehip.

4. The influence of pretreatment on BAC extraction yield

To increase the efficiency of the BAC extraction process from plant matter, three "green extractions" techniques were used, pulsed electric field (PEF), microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE).

4.1. The influence of the pulsating electric field on the extraction efficiency of phenolics from wine-growing wastes

The extraction efficiency of BAC can be increased using PEF due to the phenomenon of electroporation, which influences the permeability and rupture of cell membranes in plant matter [10]. This phenomenon facilitates the extraction of soluble intracellular compounds without significant increase of temperature, without chemical or physical changes in the plant matrix [35]. In fig. 14 presents data on the influence of PEF parameters and extraction temperature on TC (a) and antioxidant activity in red grape seed extracts (b). Assisted extraction of PEF at t = 65ºC, U = 165V, n = 900 pulses allowed to obtain extracts from red grape seeds with high tannin content (10.6 mg TAE/g d.w.) with high antioxidant activity (92.70% inhibited), due to the change of permeability and rupture of cell membranes in the plant matrix. The correlation between TC - AA in grape seed extracts shows that the value of R^2 is 0.928, proving that the evolution of antioxidant activity in plant extracts depending on PEF parameters and extraction temperature is synergistic with the rate of tannin extraction.



a) b)

Fig. 14 Influence of PEF parameters and extraction temperature on TC yield (a) and antioxidant activity (b); grape seed extracts (H = 8; 60% EtOH (v/v))

Phenolic compounds extracted from grape seeds were identified and quantified by HPLC. Experimental data show the presence of 10 phenolic compounds, which have been identified, namely: vanillin, resveratrol, qercetin and acids: cinnamic, *p*-hydroxybenzoic, floretic, vanillic, gallic, *p*-coumaric and caffeic. In order to determine the influence of temperature, number of pulses and electrical voltage on TC and antioxidant activity, sensitivity analysis was used by applying the first-order Sobol index, which showed that electrical voltage has a greater influence than the number of pulses, and increasing the extraction temperature from 30 to 65°C has a synergistic effect with PEF parameters on the tannins yield.

4.2. The influence of microwaves on the extraction efficiency of the water-soluble complex from plant matter

The influence of MAE and extraction temperature (30, 45 and 65ºC) on the yield of the water-soluble complex, especially of phenolic compounds and antioxidant activity in hydroalcoholic extracts from berries and grape marc, was investigated. The MAE demonstrated that when the concentration of the EtOH solution increases from 0% to 96% (v/v), the phenolic compounds yield goes through a maximum at the use of EtOH of 60% (v/v) and subsequently decreases, and the evolution of the extraction temperature from 30 to 65°C contributed to the increase of the phenolic compounds yield. The extraction efficiency of polyphenols is higher compared to conventional extraction (maceration). In the case of polyphenols, the highest efficacy was recorded for sea buckthorn by 1.9 times, and the lowest effect was attested for hawthorn by 1.1 times, tab. 2. Comparing the rate of flavonoid extraction by the conventional and microwave-assisted method, it was shown that microwaves increased flavonoid extraction.

The highest extraction rate was recorded for hawthorn by 1.6 times, and the lowest - for rosehips and marc by 1.2 times. As with polyphenols and flavonoids, the application of microwaves has made it possible to increase the extraction efficiency of tannins compared to the conventional method. The highest efficacy was recorded for grape marc by 2.1 times, and the lowest for hawthorn - 1.1 times. The same trends in increasing of extraction rate were observed in the case of anthocyanins. In grape marc extracts the yield increased by 1.4 times and for chokeberry - 1.1 times.

Table2. Efficiency of CBA extraction extracted from vegetable powders by MAE compared to conventional extraction

CBA extraction efficiency, times	Hydroalcoholic extracts *					
	Sea buckthorn	Rosehip	Sorbus	Hawthorn	Chokeberry	Grape marc
Total polyphenol content	1.9	1.2	1.7	1.1	1.3	1.7
Total flavonoid content	1.3	1.2	1.4	1.6	1.3	1.2
Tannin content	1.3	1.4	1.2	1.1	1.2	2.1
Total anthocyanin content	n.d.	n.d.	n.d.	n.d.	1.1	1.4

* Extraction conditions: C = 60% (v/v); t = 65°C; τ_{MAE} = 5min; P = 700W; ν = 2400MHz. ** n.d. - not determined.

The dielectric properties of water and EtOH were considered in the MAE. It is attested that water, having a high dielectric constant (ε′ = 78.3), absorbs an increased amount of electromagnetic energy and has a reduced capacity to dissipate this energy, as a result to induce a rapid increase of temperature in the sample. EtOH, having a lower dielectric constant (ε′ = 24.3), absorbs less energy compared to water, but has a greater capacity to dissipate this energy [36]. Therefore, different concentrations of ethyl alcohol were used in MAE (40, 60, 80% (v/v)), in which water facilitated the extraction of phenolic compounds, and EtOH increased the extraction efficiency of phenolic compounds [37].

Microwave-assisted extraction has shown that the antioxidant activity in ethanolic extracts is directly correlated with the content of phenolic compounds. There are similar trends with the variation of the antioxidant activity in extracts obtained by the conventional method. It was found that the maximum values of antioxidant activity in plant extracts correspond to the maximum values of TPC, TFC, TC, TAC, obtained at 65°C and the concentration of 60% EtOH solution (v/v). The microwave extraction performed in a very short time (5 min) allowed maintaining the increased values of antioxidant activity in the extracts, due to the stability of phenolic compounds.

Also, individual polyphenols were identified in hydroethanolic extracts under optimal conditions of microwave-assisted extraction at 60% (v/v) EtOH concentration, extraction temperature 65°C, magnetron power 700W and microwave application time 5 min. The extracts were found to contain the same groups of phenolic compounds as in the case of conventional extraction.

The values of the CIELab parameters of the extracts from berries and grape marc obtained by MAE were analyzed, fig. 15. The high values of component a* were determined in the extracts of chokeberry and grape marc, 23,58 and 14,21 respectively. In the rosehip and hawthorn extracts, a small amount of red pigments was attested, so the values of a* were 1.6 and 0.17 respectively. In the sea buckthorn and sorbus extracts, green pigments predominated, the values of the component a* being negative, -10.59 and -1.04 respectively. In the extracts of rosehips, sorbus, sea buckthorn and hawthorn, yellow pigments predominate, the b* values being 56.38; 38.02; 29.55 and 15.00 respectively. Chokeberry and marc extracts contain small amounts of yellow pigments and the b* values were 1.03 and 0.08. Sensitivity analysis demonstrated the low influence of microwave-assisted extraction temperature on phenolic substances and antioxidant activity in the extracted extracts in relation to the influence of EtOH solution concentration. The use of the high frequency electromagnetic field intensifies technological processes of BAC extraction, contributing to the increase of the efficiency and efficiency of polyphenolic compounds extraction from vegetable powders.

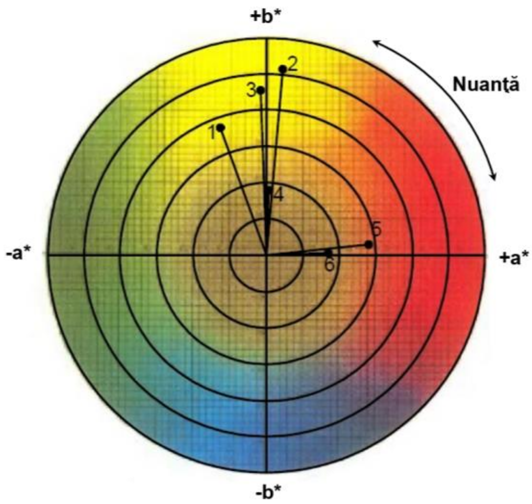
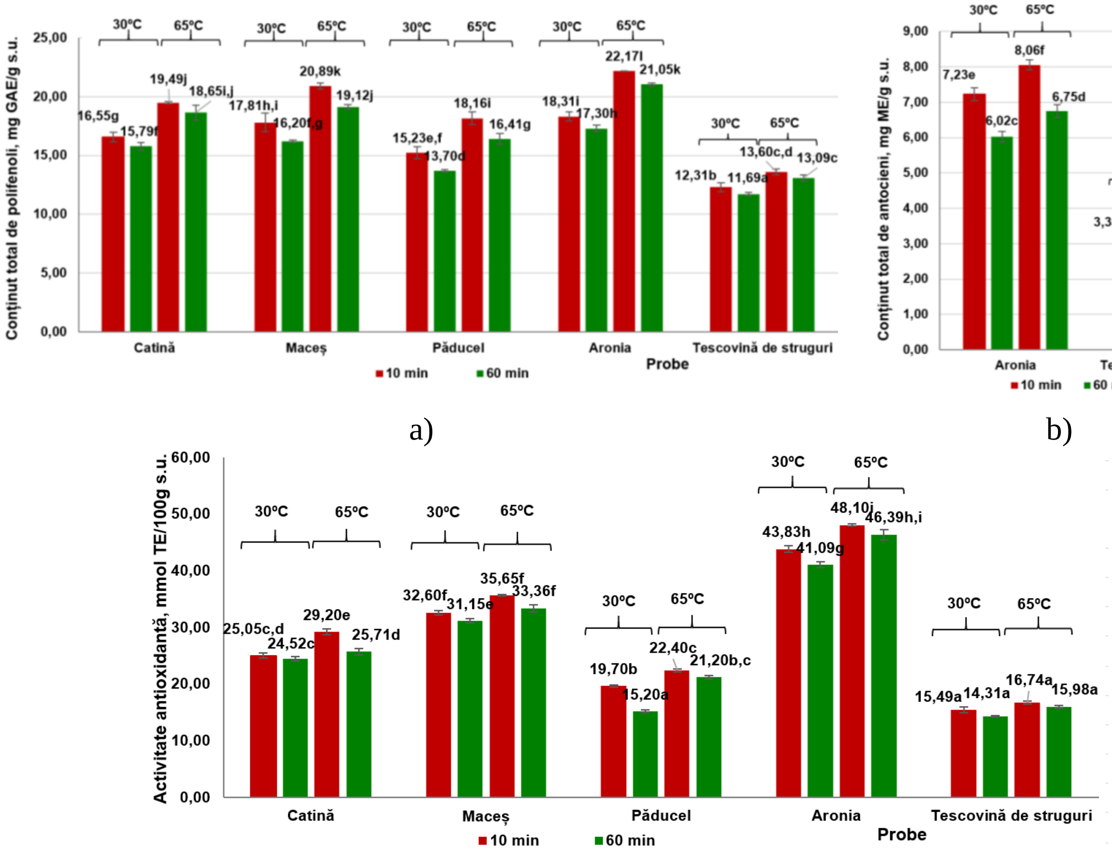


Fig. 15. The color shade of the extracts obtained by MAE at the concentration of EtOH 60% (v/v), extraction temperature 65°C according to the CIELab system: 1-sea buckthorn; 2-rosehip; 3-sorbus; 4-hawthorn; 5-chokeberry; 6-grape marc

4.3. The influence of ultrasound on the BAC extraction efficiency from plant matter

The research of the UAE influence on the BAC extraction yield was performed in two stages. In the first stage, the influence of the ultrasound application time (10 and 60 min) and the extraction temperature of 30 and 65°C on the yield of TPC, TAC and antioxidant activity, determined by the DPPH test, in the ethanolic extracts of 60% (v/v) of sea buckthorn, rosehip, hawthorn, chokeberry and grape marc were studied. In the second stage, the heat treatments influence of the fruits of sea buckthorn, rosehip and sorbus on the BAC yield (total carotenoids and polyphenols) and antioxidant activity, determined by the DPPH test and with nanoparticles in extracts obtained by UAE, was investigated.

The results of fig. 16a and b attest that temperature has a pronounced influence on the ultrasonic extraction efficiency of TPC and TAC.



a) b) c)

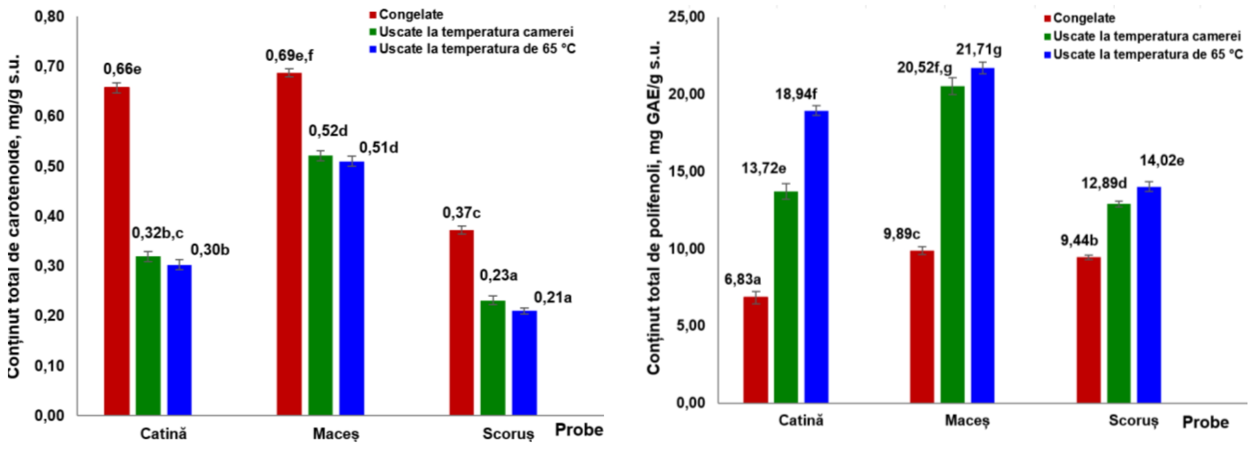
Fig. 16. Variation of BAC and antioxidant activity of extracts from berries and grape marc depending on the duration of the UAE and the extraction temperature: a) total polyphenol content; b) total anthocyanin content; c) antioxidant activity

It was shown that by increasing the extraction temperature from 30 to 65°C through UAE for 10 min, the extraction of polyphenols increased for sea buckthorn with 17.8%; rosehips with 17.4%, hawthorn with 19.2%; chokeberry with 21.1% and marc with 10.6%, and in the case of anthocyanins in chokeberry and pomegranate extracts the extraction rate increased with 11.5% and 13.4% respectively. In case of application of UAE for 60 min the extraction yield of polyphenols increased for sea buckthorn with 18.1%; rosehips with 18.0%, hawthorn with 19.8%; chokeberry with 21.7%, marc by 11.9% and in the case of anthocyanins in the extracts of chokeberry and grape marc increased with 12.1% and 14.3% respectively. The increase of the extraction efficiency of phenolic compounds is due to the production of matrix bonds ruptures, the increase of phenolic compounds solubility, solvent diffusion rate and mass transfer, reduction of viscosity and surface tension of the solvent.

The polyphenols recovered under different extraction conditions revealed similar antioxidant activities, which suggests a similar composition, fig. 16c. The higher values of antioxidant activity correspond to the extraction rate of phenolic compounds at 65°C and the duration of UAE 10 min. Thus, the values of antioxidant activity in plant extracts are arranged in descending order: chokeberry 48.10 mmol TE/100 g d.w.; rosehip 35.65 mmol TE/100 g d.w.; sea buckthorn 29.20 mmol TE/100 g d.w.; hawthorn 22.40 mmol TE/100 g d.w. and marc 16.74 mmol TE/100 g d.w. Linear relationships were established between BAC content and antioxidant activity. In the case of TPC-AA, the R^2 values are in the range 0.732 (sea buckthorn) - 0.978 (rosehip) and TAC-AA in the range 0.685 (chokeberry) - 0.980 (grape marc).

Sensitivity analysis was applied, which showed that the extraction temperature more significantly influences the phenolic compounds yield and antioxidant activity in plant extracts than the duration of ultrasound.

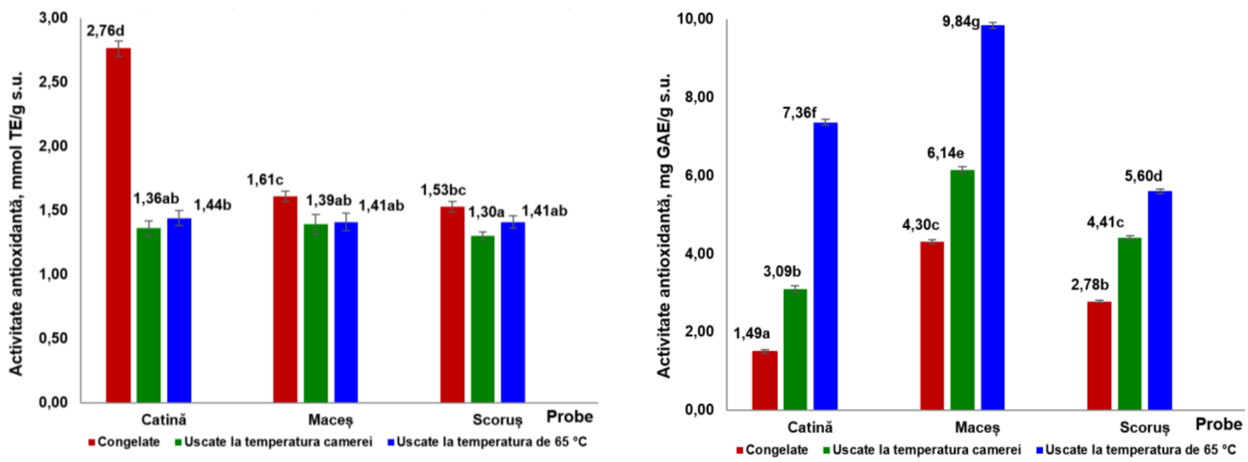
In the second stage, the influence of heat treatments was studied, especially of freezing at -18°C and drying at room temperature and at 65°C of sea buckthorn, rosehip and sorbus fruits on the extraction yield of TCC (total content of carotenoids) and TPC, as well as antioxidant activity, determined by DPPH tests and with the stabilization of Ag nanoparticles in ultrasound-assisted extracts, fig. 17-18.



a) b)

Fig. 17. Influence of treatment temperature on TCC (a) and TPC (b) extracted through the UAE

The results of fig. 17a demonstrates that the highest TCC was determined in the frozen pulp samples, so the rosehip contains 0.69 mg/g d.w., sea buckthorn - 0.66 mg/g d.w. and sorbus - 0.37 mg/g d.w., and drying conditions of the berries pulp contributed to the reduction of TCC. Dry pulp is considered more susceptible to carotenoid degradation due to increased contact surface with the thermal agent and the formation of porosity. As a result, in the dry samples at room temperature and at 65°C, compared to the frozen samples, the TCC was considerably reduced: in the case of sea buckthorn by 2.1 times, rosehips by 1.3 times and scorches by 1.7 times. It was shown that the TCC values in the dry samples at different temperatures were not significantly different: 0.32 - 0.30 mg/g d.w. in sea buckthorn powder; 0.52 - 0.51 mg/g d.w. in rosehip and 0.23-0.21 mg/g d.w. in the sorbus. This phenomenon can be explained by the fact that dehydration with the air exposed the carotenoids to the action of oxygen, causing their extensive degradation, transforming β-carotene *trans* into various *cis* isomers, which is one of the mechanisms of carotenoid degradation in food. The activity of lipoxygenase and peroxidase enzymes, responsible for oxidative degradation, has also contributed to the reduction of carotenoid content [38].



a) b)

Fig. 18. Influence of treatment temperature on the antioxidant activity in hydroethanolic extracts, determined by DPPH test (a) and by stabilization of Ag nanoparticles (b)

The diagram in fig. 17b demonstrates the TPC evolution in samples treated by freezing and drying at different temperatures. It was found that the maximum extraction yield was obtained in the dry samples at 65°C, the samples being arranged in descending order: rosehip - 21.71 mg GAE/g d.w.; sea buckthorn - 18.94 mg GAE/g d.w. and sorbus - 14.02 mg GAE/g d.w., and the lowest TPC was obtained in the case of frozen samples: rosehip - 9.89 mg GAE/g d.w.; sorbus 9.44 mg GAE/g d.w., and sea buckthorn 6.83 mg GAE/g d.w. Pulp dehydration at 65°C influenced the integrity of the berries cell walls and led to skin rupture. The structural changes inside the cells are irreversible, causing the release of compounds related to the plant matrix and increasing their availability. These processes are responsible for the more intense diffusion of compounds, especially polyphenols, from the skin to the pulp [39]. Damage of cell wall triggered the release of polyphenol oxidase and peroxidase enzymes, but the drying temperature 65°C deactivated these enzymes, preventing the loss of polyphenolic compounds and, consequently, TPC values are increased, fig. 17b.

The antioxidant activity values were measured by two methods, by the DPPH test and by the stabilization of the Ag nanoparticles, fig. 18. [40]. The highest values of antioxidant activity determined by the DPPH test were obtained in hydroethanolic extracts from frozen berries: sea buckthorn - 2.76 mmol TE/g d.w.; rosehip - 1.61 mmol TE/g d.w. and sorbus - 1.53 mmol TE/g d.w., and in the case of stabilization of Ag nanoparticles, the highest values were obtained in extracts from dried pulp at 65°C: sea buckthorn - 7.36 mg GAE/g d.w.; rosehip - 9.84 mg GAE/g d.w. and sorbus - 5.60 mg GAE/g d.w. The correlations between TPC-AA (DPPH) in sea buckthorn, rosehip and sorbus extracts are inversely proportional and is very good for rosehip extracts (R^2 = 0.97), good for sea buckthorn extracts (R^2 = 0.77) and moderate - for sorbus (R^2 = 0.55). In the case of antioxidant activity determined by the stabilization of Ag nanoparticles, the correlation with total polyphenols is directly proportional and is very good for sea buckthorn extracts (R^2 = 0.89) and sorbus (R^2 = 0.96) and moderately positive for rosehip (R^2 = 0.66).

The identification and quantification of carotenoids in unsaponified and saponified extracts from the frozen pulps of sea buckthorn, rosehip and sorbus obtained by UAE was performed by reverse phase liquid chromatography (RP-HPLC). It was shown that in the unsaponified extracts increased amounts of carotenoid esters were identified, and in the saponified extracts, these were not identified because they were hydrolyzed with the formation of free carotenoids.

5. Stabilization technologies, antimicrobial activity, and BAC bioavailability

5.1. The influence of pH on antioxidant activity and color stabilization of plant extracts

The pigments stability in hydroethanolic extracts depends on their chemical structure and their interaction with other food components and may be affected by pH, light, storage temperature, the presence of enzymes, oxygen, etc. The influence of the pH evolution on the antioxidant activity, determined by the ABTS test, and of the CIELab chromatic parameters in the hydroethanolic extracts from rosehip, sea buckthorn, chokeberry and grape marc was investigated, fig. 19-20.

The changes of antioxidant activity and chromatic parameters in extracts at different pH ranges: 2.3-2.6; 3.5-3.8; 5.4-5.6; 7.0–7.4, 8.0–8.8 were studied [41]. It was found that the pH value shows specific influences on the antioxidant activity of hydroethanolic extracts. In the rosehip extract, the essential changes of the antioxidant activity occurred in the strong acid environment, pH 2.5 and in the alkaline environment, at pH 8.7 with 29% and with 21% respectively, in relation to the control, fig. 19a. In sea buckthorn extract, the highest leap of antioxidant activity occurred at pH 3.6, constituting 13.27 mmol TE/L, being by 1.7 times higher than in the case of control, and at pH 7.0 and pH 8.5 increased by 1.5 times and by 1.4 times, respectively, fig. 19b. In chokeberry extracts, the antioxidant activity values in the acidic medium decreased significantly compared to the control: at pH 2.3 with 48.9%, pH 3.5 with 23.0% and at pH 5.6 with 17.2 %. On the other hand, in the alkaline medium the antioxidant activity in the chokeberry extract did not change significantly compared to the reference sample, fig. 19c. The data shown in fig. 19d shows that the evolution of pH from 2.6 to 8.8 did not substantially alter the antioxidant activity values in grape marc EtOH extract compared to the control sample, except of pH 3.5, at which the antioxidant activity increased with 15%.

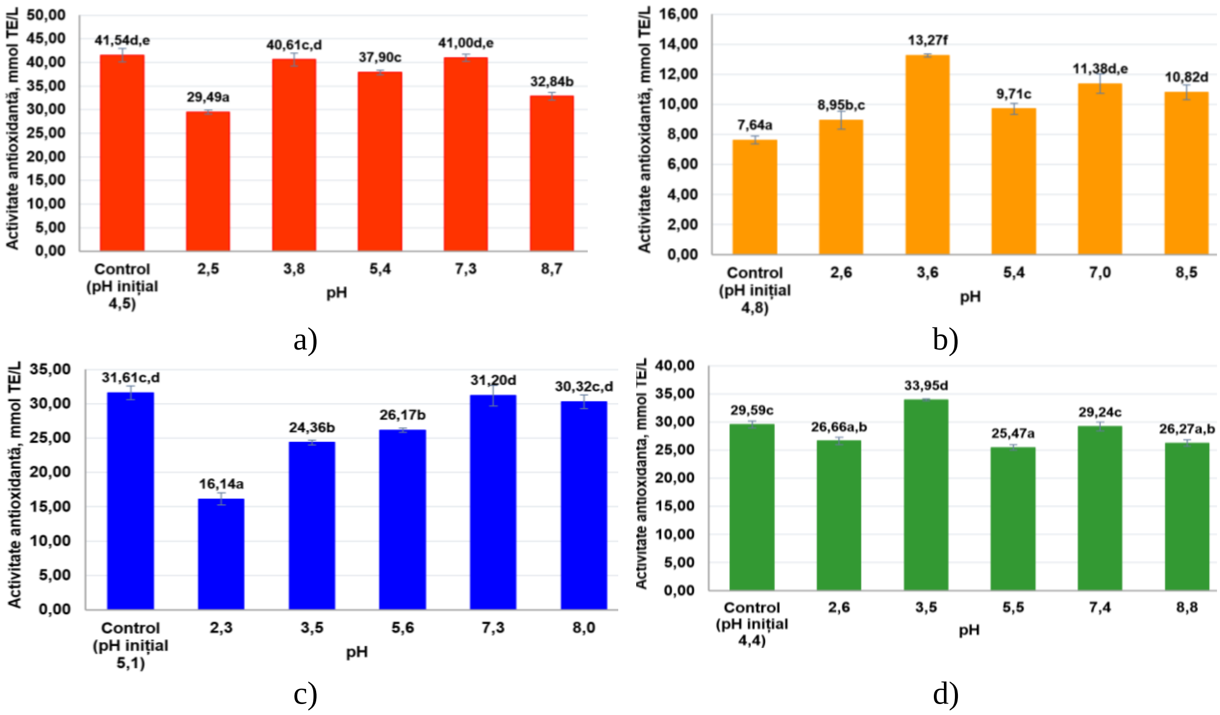
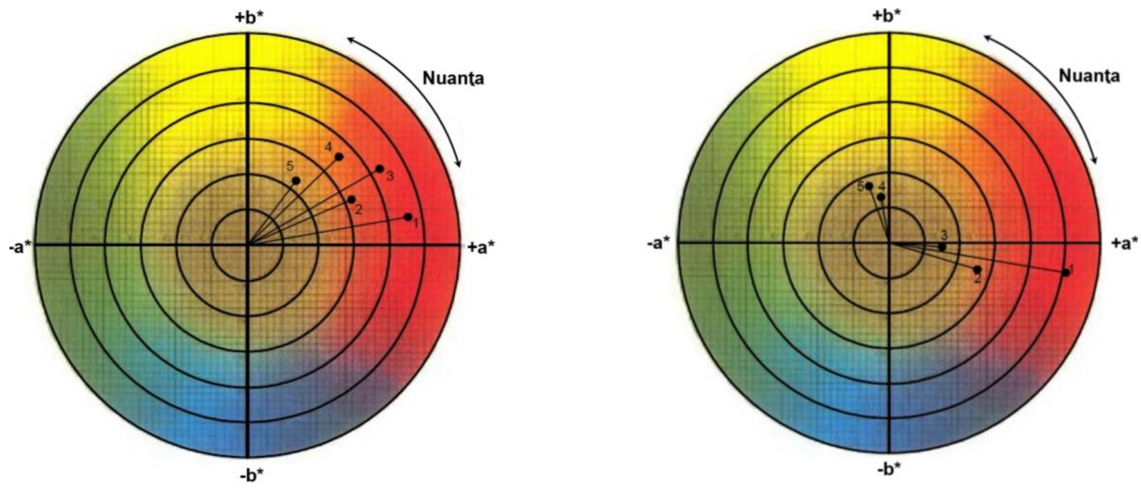


Fig. 19. Antioxidant activity variation in depending of the pH change in hydroethanolic extracts: a) rosehip; b) sea buckthorn; c) chokeberry; d) grape marc

The antioxidant activity of polyphenols in plant extracts is related to the presence of phenolic groups and the ability to donate electrons to deprotonation. At pH 7.0-7.4, the polyphenols are stabilized due to polymerization reactions. In these polymerization reactions polyphenols can form new oxidizable parts -OH in their polymeric products, resulting in a higher activity of inhibition of free radicals. In the case of flavonoids, increasing the number of free -OH groups contribute to the increase of antioxidant activity [42], and polymerized tannins have higher antioxidant properties than monomeric tannins [43]. The low values of antioxidant activity under strong acid conditions in rosehip and chokeberry extracts were due to the fact that the hydrolysis of chlorogenic acid led to a decrease in the number of caffeoyl. In the case of grape marc extract, the low pH value did not essentially influence the antioxidant activity, but contributed to the stability of anthocyanins, due to the appearance in their chemical structure of the cation flavylium, being very stable [23]. The antioxidant activity of various plant extracts indicates a strong dependence of the pH system.

The pH influence on the chromatic parameters of plant extracts after pH adjustment was analyzed. The pH evolution from 2.5 to 8.7 did not essentially change the color of rosehip extract, except for the weakly acidic (pH 5.4) and alkaline (pH 8.7) medium. In the first case, the luminosity reduction approx. with 10 units, led to darkening the color of rosehip extract. In the second case, a* (2,4) and b* (14,2) were influenced, demonstrating the presence of the red and yellow pigments respectively. In the case of sea buckthorn, the L* was not influenced by the pH evolution, except for the alkaline medium which contributed to the decrease of a* values, the tones being shifted to green (-7.8) and to the increase of b* values, demonstrating the presence of yellow pigments (26.9).

The slightly acidic medium pH 5.6 and neutral pH 7.3 reduced the L* values of the chokeberry extract to 55.4 and 45.5 respectively, as a result the extracts color darkened. Under the same conditions, the a* values decreased to 31.5 and 12.6, respectively, due to the reduction of red pigments, and b* improved to 17.7 and 20.8, respectively, demonstrating the shift of color to yellow. In the strongly acidic medium pH 2,3, there is an essential improvement of the a * component, the value being 37.8, due to the stabilization of the flavylium cation which is red. Another parameter that has been improved under these conditions is the chromaticity C* 38.4, which demonstrates the increase of the color saturation of the extract, fig. 20a.

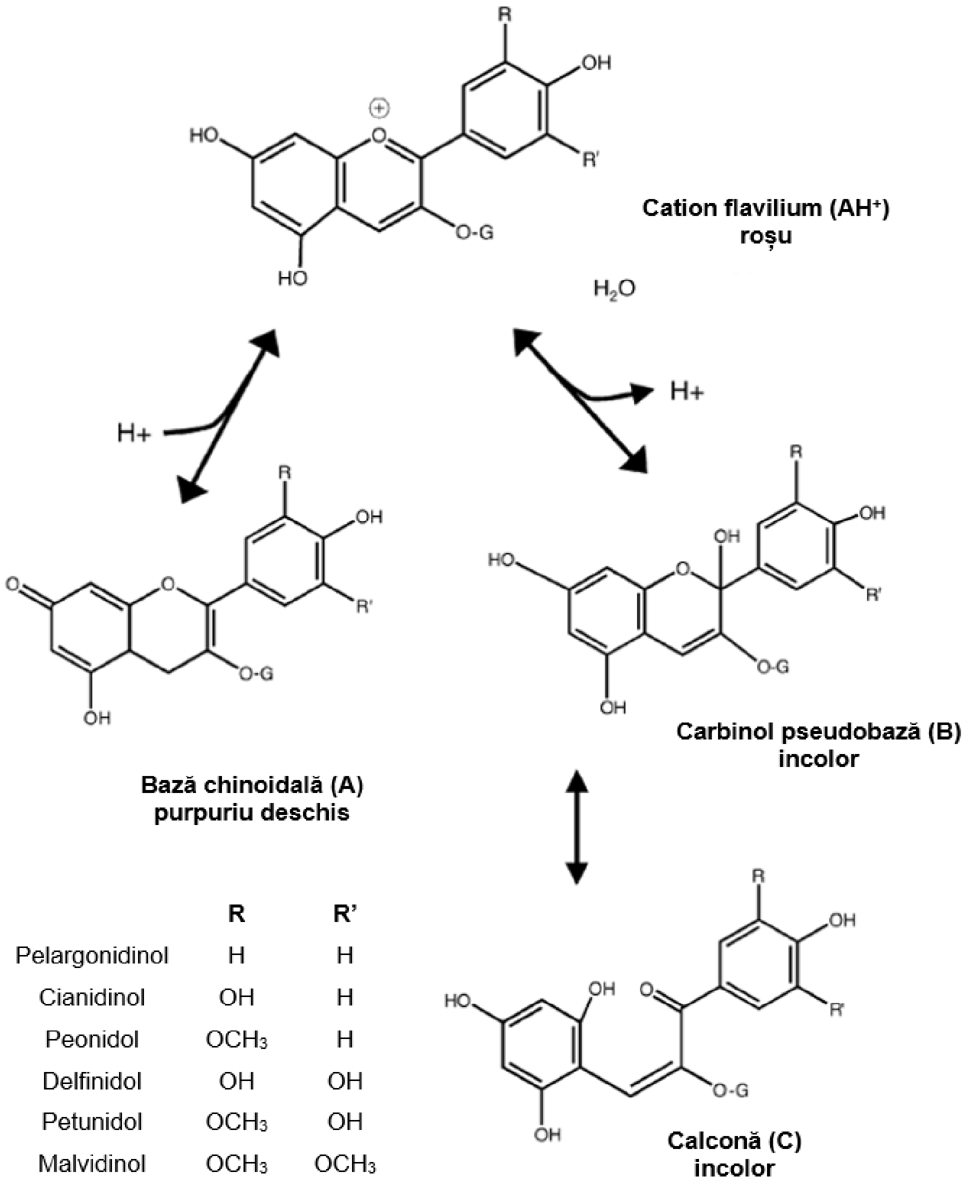


1- pH 2,3; 2 - pH 3,5; 3 pH – 5,6; 4 – pH 7,3; 5 – pH 8
a)

1- pH 2,6; 2 - pH 3,5; 3 pH – 5,6; 4 – pH 7,4; 5 – pH 8,8
b)

Fig. 20. Representation of the color shade of plant extracts from chokeberry fruit (a) and grape marc (b) when the pH changes

In the alkaline environment pH 8, in the chokeberry extract there is a reduction of the red tone a* (11.5) and the increase of the yellow pigments b* (15.0). The modification of these chromatic parameters led to the decrease of the C* value (19.0), the reduction of the color intensity was observed, fig. 20a. At pH 5.6 and neutral pH 7.3 reduced the L* values of the chokeberry extract 55.4 and 45.5 respectively, as a result the extracts color darkened. Under the same conditions, the a* values decreased, 31.5 and 12.6, respectively, due to the reduction of red pigments, and b* improved 17.7 and 20.8, respectively, demonstrating the shift of color to yellow. In the strongly acidic medium pH 2,3, there is an essential improvement of the a* component, the value being 37.8, due to the stabilization of the flavylium cation which is red. Another parameter that has been improved under these conditions is the C* chromaticity 38.4, which demonstrates the increase of the extract color saturation. In the alkaline environment pH 8, in the chokeberry extract there is a reduction of the red tone a* (11.5) and the increase of the yellow pigments b* (15.0). The modification of these chromatic parameters led to the decrease of the C* value (19.0), the reduction of the color intensity was observed, fig. 20a, 21.



	R	R'
Pelargonidinol	H	H
Cianidinol	OH	H
Peonidol	OCH_3	H
Delfinidol	OH	OH
Petunidol	OCH_3	OH
Malvidinol	OCH_3	OCH_3

Fig. 21. The pH influence on the structure and anthocyanins color

In the case of marc extract, pH 2.5 contributed to a decrease of L* 72.1 and significantly improved the red hue of a* 48.1 due to anthocyanins having a cationic flavylium structure with a charged oxygen atom are stabilized in the presence of H^+ cations. Also, the b* component values decreased (-5.3), demonstrating the presence of blue pigments. Under these conditions the C* has an increased value 48.4, demonstrating the increased color saturation, fig. 20b. With increasing pH in alkaline medium, L* changed non-essential. Under these conditions, the a* component value has decreased and the values are already negative, due to the shift of tones to green, and the b* component value has increased 14.7, demonstrating the dominance of yellow, due to the degradation of anthocyanins. The C* values attest to the presence of the gray shade and the reduced saturation of the extract color, fig. 20b.

As mentioned above, at low pH (2.3-2.6), the color of anthocyanins is red (AH^+), as pH values increase, anthocyanins can undergo changes in two possible ways: deprotonation with formation of the quinoidal compound of purple color (A) or hydration, obtaining the colorless calcone (C), fig. 21. Increasing the pH from 2.6 to 8.8 decreases the probability of π - π* transitions and the *p* orbitals from anthocyanin molecules expand, forming a conjugate system. Changing the color of anthocyanins from red in acidic medium to colorless in alkaline medium is associated with both an increase in the resonant structure and the formation of a planar structure, which allows π electrons to spread more evenly over the molecule.

The results of this research show that it is possible to predict the color changes of extracts depending on the medium pH, knowing the chemical structure of pigments, but color changes must be taken into account during food processing to determine complex interactions inside the food matrix.

5.2 Effect of metal ions from food on color stabilization and antioxidant activity in plant extracts from chokeberry and grape marc

The effect of Na^+, K^+ and Ca^{2+} metal ions from food at concentrations 0.001M, 0.01M and 0.1M on color stabilization, valuated by CIELab chromatic parameters, and on antioxidant activity in chokeberry and marc extracts was investigated. It have been shown, that metal ions Na^+, K^+ and Ca^{2+} contribute to change in the chromatic parameters in chokeberry and grape marc extracts.

In the case of chokeberry extracts, the presence of K^+ ions in the range of concentrations from 0.001M to 0.1M led to the improvement of L*, the maximum value being 46.16 (0.1M) and to the lightening of the extract color. The a* values decreased due to the reduction of the red pigments content 38.40 (0.01M), and at concentrations 0.001M and 0.01M the b* values show a decrease of yellow pigments, 21.01 and 21.05 respectively. Also, the low concentrations of K^+ had an impact on C*, the values decreased being 43.98 (0.001M) and 43.80 (0.01M), thus reducing the extracts color saturation. In the case of Na^+ ions in concentrations 0.001M - 0.1M, the L* values increased (43.91 - 44.05) and the b* values decreased (22.52 - 22.93). Ca^{2+} ions at a concentration 0.1M had the most significant effect on the chromatic parameters of chokeberry extracts by increasing the a* (45.01) and C* (50.77) values. However, $Ca2^+$ ions did not visibly change the extracts color relative to the control.

In the case of grape marc extracts, the significant influence of K^+ ions on chromatic parameters was demonstrated at a concentration 0.001 M. It was found that K^+ ions (0.001M) contributed to the lightening of the marc extract color, the L* value being 68.96. Under these conditions, there is a reduction of the a* (27.89), the b* (-5.75) and the C* values (28.48). The visible changes of the marc extract color were obtained in the presence of Na^+ ions at a concentration 0.1M, being ameliorated the red shade a* 35.99, the blue shade b* (-9.18) and C* 37.14. Ca^{2+} ions at concentrations 0.001M, 0.01M and 0.1M had the most important effect on the chromatic parameters CIELab in grape marc extracts, fig. 22. There was a direct dependence between increasing the concentration of Ca^{2+} ions and the effect of improving the color of the marc extracts. There is a decrease of L* values from 59.82 (0.001M) to 47.46 (0.1M) and darkening of the color of the marc extracts. The a* red tone improved from 45.55 to 69.00, and the b* blue tone decreased from (-9.80) to (-6.25). The C* values attest the essential influence on the color saturation of the marc extracts, fig. 22.

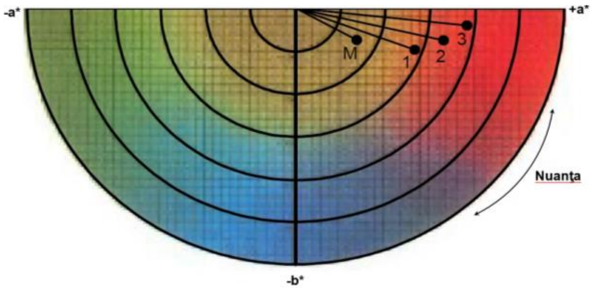
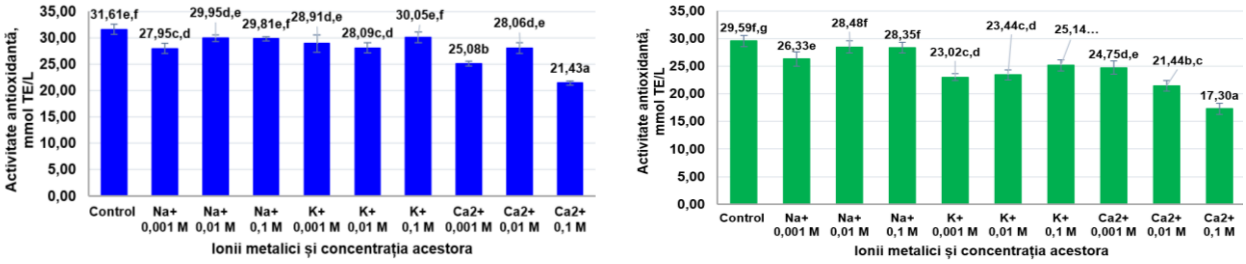


Fig. 22. Representation of the color shade of grape marc extracts at different concentrations of Ca^{2+} metal ions: M - sample-control; 1 – Ca^{2+} 0,001 M; 2 - Ca^{2+} 0,01 M; 3 - Ca^{2+} 0,1 M

The improvement of the marc extract color was probably due to the polymerization process of the phenolic compounds and the anthocyanins complexation with the metal ions [44]. It was also noted that increasing the concentration of Ca^{2+} ions from 0.001M to 0.1M led to a gradual decrease of pH in tescovine extracts from 4.1 to 3.2, contributing to the stabilization of the flavylium ion in acidic medium [45].

Fig. 23 demonstrates the influence of different concentrations of metal ions Na^+, K^+ and Ca^{2+} on the antioxidant activity, determined by the ABTS test, in extracts from chokeberry and grape marc. In the chokeberry extracts, the addition of metal ions in concentrations of 0.001M, 0.01M and 0.1M did not significantly influence the antioxidant activity, except for Ca^{2+} ions. At the highest concentration of Ca^{2+} ions (0.1M), the antioxidant activity decreased with 32.2% compared to the control. In grape marc extracts, there is a direct dependence between the decrease of antioxidant activity and the increase of the Ca^{2+} metal ions concentration, the values being 24.75 mmol TE/L (0.001M), 21.44 mmol TE/L (0.01M) and 17, 30 mmol TE/L (0.1M). In the case of Na^{+}, K^{+} ions such dependence was not noticed.



a) b)

Fig. 23 The effect of Na^{+}, K^{+} and Ca^{2+} metal ions at different concentrations on the antioxidant activity in hydroalcoholic extracts: a) chokeberry; b) grape marc

Probably, the complexation between anthocyanins or derivatives of flavonoids with metal ions contributes to the reduction of the BAC antioxidant activity, because oxygen atoms being bound to the metal ion are not available for oxidation reactions [23]. Chemical stabilization of natural red pigments in chokeberry and grape marc extracts is necessary for their application in food formulation as an alternative to the substitution of synthetic dyes.

5.3. The microbiostatic activity of plant matter on pathogenic microorganisms

The identification and evaluation of antimicrobial agents to inhibit the growth of pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*) in food, in order to provide consumers with safe and healthy food, is relevant. The antimicrobial activity (AMA) was investigated and minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and bactericidal effect of sea buckthorn, rosehip, hawthorn, chokeberry and grape marc powders on Gram-positive pathogenic microorganisms - *Staphylocus aureus* and Gram-negative: *Escherichia coli* and *Klebsiella pneumoniae* were determined.

Following the tests performed, it was found that the vegetable powders achieved a different level of AMA compared to all the pathogenic microorganisms investigated. Sea buckthorn powders had AMA against Gram-positive and Gram-negative microorganisms, compared to other vegetable powders. The diameter of the inhibition zone was 22 mm for *S. aureus*, 18 mm for *E. coli* and 17 mm for *K. pneumoniae*. Rosehip powders made AMA against *S. aureus*, the diameter of the inhibition zone being 16 mm, on *E. coli* and *K. pneumoniae*, the diameters of the inhibition zones being 10 mm and 9 mm respectively. Vegetable powders from chokeberry, hawthorn and grape marc showed poor AMA on the tested microorganisms [46, 47]. The antimicrobial potential of vegetable powders is probably attributed to the polyphenolic compounds content, especially quercetin and gallic acid derivatives. AMA of polyphenols may involve various mechanisms, namely destabilization and permeabilization of the cytoplasmic membrane and the enzyme inhibition by oxidized products, possibly by reaction with sulfhydryl groups or by more non-specific interactions with proteins, with the formation of reactive quinones that can react with amino acids and proteins, inhibiting the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria [48]. Gallic acid can change bacterial hydrophobicity, while quercetin leads to bacteriostasis by damaging of cell walls and membranes [49].

MIC, MBC and the bactericidal effect of the analyzed vegetable powders on pathogenic microorganisms, capable of causing food contamination are presented in table 3.

Table 3. MIC, MBC and the bactericidal effect of vegetable powders on pathogenic microorganisms*

Pathogenic microorganisms	MIC, mg/mL	MBC, mg/mL	MBC/ MIC	Bactericidal effect
Sea buckthorn powder				
Staphylococcus aureus* ATCC *25923	1,95±0,12	3,90±0,23	2	+
Escherichia coli* ATCC *25922	7,81±0,37	15,6±0,7	2	+
***Klebsiella pneumoniae* ATCC 13883**	15,60±0,50	31,25+1,25	2	+
Rosehip powder				
Staphylococcus aureus* ATCC *25923	3,91±0,15	7,81±0,21	2	+
Escherichia coli* ATCC *25922	31,25±0,98	62,50±1,80	2	+
***Klebsiella pneumoniae* ATCC 13883**	62,50+2,10	125±5	2	+
Chokeberry powder				
Staphylococcus aureus* ATCC *25923	15,63±0,37	31,25±0,62	2	+
Escherichia coli* ATCC *25922	-**	-	-	-
***Klebsiella pneumoniae* ATCC 13883**	-	-	-	-
Hawthorn powder				
Staphylococcus aureus* ATCC *25923	41,66±1,35	83,33±2,47	2	+
Escherichia coli* ATCC *25922	62,50±2,20	125±5	2	+
***Klebsiella pneumoniae* ATCC 13883**	-	-	-	-
Grape marc powder				
Staphylococcus aureus* ATCC *25923	7,81±0,19	15,62±0,41	2	+
Escherichia coli* ATCC *25922	62,50±1,57	125±5	2	+
***Klebsiella pneumoniae* ATCC 13883**	-	-	-	-

*results are presented as mean ± standard deviation; **”-” did not show bactericidal effect

Sea buckthorn powder had the lowest MIC and MBC, the values being 1.95 mg/mL and 3.90 mg/mL respectively, followed by rosehip powder with the values MIC and MBC - 3.91 mg/mL and 7.81 mg/mL, respectively. Hawthorn powder, compared to other samples, had the highest MIC and MBC, 41.66 mg/mL and 83.88 mg/mL respectively. Sea buckthorn and rosehip powders showed bactericidal effect against all pathogenic microorganisms studied; hawthorn and grape marc powders against *S. aureus* and *E. coli*, and chokeberry powder only against *S. aureus*.

Vegetable powders from sea buckthorn, rosehip, hawthorn, chokeberry and grape marc have demonstrated an important antimicrobial potential on the studied pathogenic microorganisms and can be used in the food industry to reduce the degree of microbial contamination of raw materials and food.

5.4 Carotenoids bioavailability from sea buckthorn, rosehip and sorbus fruits

The health benefits of BAC, especially carotenoids from sea buckthorn, rosehip and cinnamon, are well known. There is particular interest in determining the bioavailability of these compounds, because which are used in the formulation of functional foods. Bioaccessibility refers to the amount of compound ingested, which is released from the food matrix during the digestion process and becomes available for intestinal absorption [33]. Minekus et al. (2014) developed a simulated digestion model *in vitro*, which includes oral, gastric and intestinal phases, imitating physiological conditions *in vivo*, taking into account the presence of digestive enzymes and their concentrations, pH, digestion time and salt concentrations [50].

Figure 24 shows the carotenoids bioavailability values from sea buckthorn, rosehip and sorbus powders. It was found that sorbus powders have the highest bioavailability of carotenoids - 15.3%, followed by sea buckthorn - 9.1% and rosehip -7.5%. The obtained results attest that the availability of carotenoids depends on the structure of the plant matrix and the presence of the certain matrix components [51].

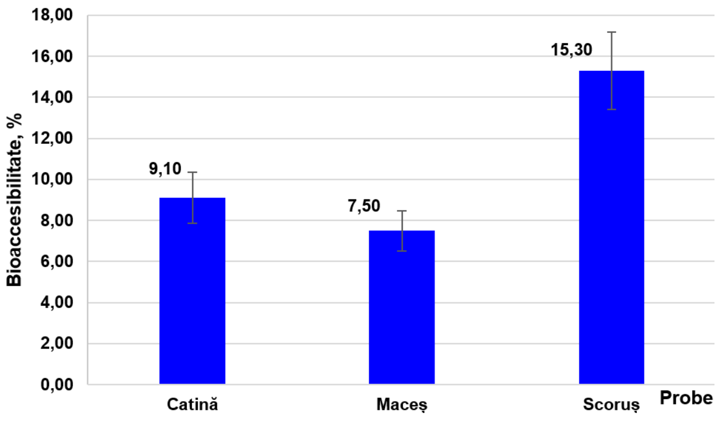


Fig. 24. Carotenoids bioaccessibility from sea buckthorn, rosehip and sorbus fruits

In berries, carotenoids are associated with proteins: carotenes and lycopene form complexes with proteins embedded in chromoplasts, while lutein is localized in chloroplasts [52]. The formation of complexes between carotenoids and protein compounds, but also the carotenoids crystalline state reduce their bioavailability [53].

Processing operations, especially drying and grinding, by reducing particle size, as well as enzymatic processes during digestion, which soften and destroy cell walls, disrupting protein-carotenoid complexes, promote the release of carotenoids, increasing their bioavailability [54]. The individual carotenoids were identified by the RP-HPLC method in unsaponified extracts of sea buckthorn, rosehip and sorbus after digestion, performed *in vitro*. In the sea buckthorn pulp extract were identified: zeaxanthin, lutein, β-cryptoxanthin, all-*trans*-β-carotene, cis-β-carotene and esters of β-cryptoxanthin, zeaxanthin and mutatoxanthin. Lycopene, zeaxanthin, rubixanthin, α- and β-cryptoxanthin were identified in the unsaponified rosehip extract. Esters of β-cryptoxanthin and zeaxanthin, free zeaxanthin, β-cryptoxanthin, all-*trans*-β-carotene and *cis*-β-carotene were identified in the sorbus extract. The effect of food processing on carotenoid bioavailability positively correlates with in vivo studies on carotenoid bioavailability, confirming that the consumption of processed plant foods improves carotenoid bioavailability [55].

6. Technologies for the manufacture of functional flour and sugar products

6.1. Development of pasta manufacturing technology made from wheat flour with the addition of vegetable powders from rosehip and chokeberry

The main objective of the research presented is the development of technology for the pasta manufacture made from wheat flour with rosehip and chokeberry powders, which are used to strengthen gluten, as natural dyes and to diversify of functional products range.

6.1.1. The influence of vegetable addition on the biochemical and rheological processes of wheat flour dough

Superior quality wheat flour, rosehip and chokeberry powders, in quantities of 1.5%, 3% and 5% were used for the manufacture of pasta. The influence of rosehip and chokeberry powder concentrations on the quality of wet gluten and the dry gluten content in wheat flour was studied, tab. 4.

The analysis of experimental data shows that the introduction of vegetable powders leads to a reduction in the amount of wet gluten, in the case of rosehip with 9.6% and chokeberry with 2.2% compared to control sample. This can be explained by the increase of the total mass of the dough due to the addition of vegetable powders and the reduction of the gluten hydrating capacity. Also, the dry gluten content was reduced with 2.7% for samples with chokeberry and with 6.3% for samples with rosehip. This reduction was due to the fact that vegetable powders have a higher hydrating capacity than wheat flour. This led to insufficient water to hydrate the gluten proteins, as the unhydrated gluten was partially washed during the experiment.

Table 4. Influence of rosehip and chokeberry powder concentration on physicochemical indicators of gluten and dough*

Indicator	CS	Concentration of rosehip powder in the dough,%			Concentration of chokeberry powder in the dough,%		
		R1.5	R3	R5	C1.5	C3	C5
Wet gluten content,%	32.4±0.1	31.4±0.1	29.8±0.1	29.3±0.2	31.9±0.1	31.2±0.1	30.7±0.2
Dry gluten content,%	11.1±0.1	10.9±0.1	10.5±0.2	10.4±0.1	10.9±0.1	10.9±0.1	10.8±0.1
Quality on the IDK device, u.c.	84±1	81±1	79±1	77±1	82±1	80±1	78±1
Active acidity, pH	5.85± 0.01	5.45± 0.01	5.18± 0.01	4.97± 0.01	5.72± 0.01	5.54± 0.01	5.39± 0.01
Water activity, aw, u.c.	0.734± 0.001	0.730± 0.002	0.728± 0.001	0.725± 0.001	0.732± 0.002	0.729± 0.001	0.726± 0.001

*results are presented as mean ± standard deviation; CS-control sample; R- rosehip; C - chokeberry

The elastic properties of gluten changed significantly: initially the gluten quality of CS corresponded to 84 u.c., belonging to the “poorly satisfactory” group, at the dosage of the vegetable powder concentration of 5% the gluten quality values at the IDK device constituted 77 u.c. (rosehip) and 78 u.c. (chokeberry), which corresponds to the “good” gluten and the group of first quality. This phenomenon is due to ascorbic acid from berries, which in the presence of oxygen included in the dough during kneading, oxidizes the groups of hydrophilic sulfur -SH, which belonging to two protein molecules form disulfide bridges -SS- strengthening the gluten in wheat flour [56], fig. 25.

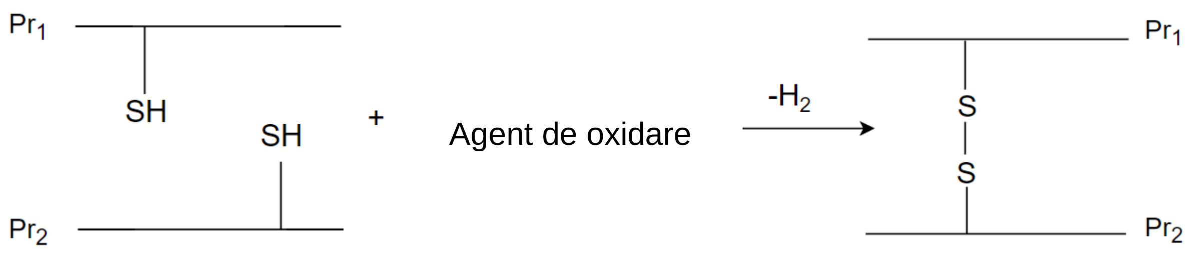


Fig. 25. The hypothetical oxidation reaction of proteins (Pr1 and Pr2) in wheat flour, which leads to changes in the dough rheological properties [28]

The sensitivity analysis showed that the concentration of powders has the greatest and equal influence on the gluten quality determined at the IDK apparatus. In addition, the influence of rosehip powder is more pronounced than chokeberry powder.

Table 5 presents the rheological characteristics of the doughs with rosehip and chokeberry powders at concentrations 1.5%, 3% and 5% compared to the control sample (without powder).

Increasing of vegetable powders concentration led to an increase of the dough tenacity and extensibility, index of swelling, baking strength and reduced the configuration ratio, except for the rosehip sample with a concentration 5%, where configuration ratio increased by 2.61 times compared to CS - 2.41 times. This was due to the presence of organic acids, dietary fiber, pectic substances in rosehip and chokeberry powder.

Tabelul 5. Rheological characteristics of the dough with rosehip and chokeberry powder at concentrations 1.5%, 3% and 5% relative to the control sample *

Rheological characteristic	CS	Concentration of rosehip powder in the dough,%			Concentration of chokeberry powder in the dough,%		
		R1.5	R3	R5	C1.5	C3	C5
Tenacity, mm	77±2	82±1	90±1	107±1	86±1	90±1	93±1
Extensibility, mm	32±0.5	36±0.5	39±1.0	41.0±0.5	39±0.5	42±0.5	45±0.5
Index of swelling, $cm^{3/2}$	12.5±0.2	13.4±0.1	13.8±0.1	14.2±0.2	13.8±0.1	14.4±0.1	14.9±0.1
Baking strength, 10^{-4} J	92±2	124±2	143±2	167±2	129±2	151±2	160±2
Configuration ratio	2.41± 0.09	2.28± 0.10	2.31± 0.16	2.61± 0.21	2.21± 0.14	2.14± 0.10	2.07± 0.12

* results are presented as mean ± standard deviation; CS-control sample; R- rosehip; C - chokeberry

The sensitivity analysis finds that in the case of chokeberry powder the influences of the concentration on the rheological properties of the dough are generally higher than in the case of rosehip powder.

6.1.2. The influence of the concentration of rosehip and chokeberry powders on the pasta quality indicators

The technology for manufacturing pasta made from wheat flour fortified with vegetable powders was developed. Previously, chokeberry and rosehip powders were macerated at a temperature 65±1°C, in order to intensify the extraction process of natural pigments. It has been shown that the macerated powders used during kneading, color the dough with increased intensity and uniformity, and the culinary properties of cooked pasta are superior. The pasta quality with vegetable addition determined by sensory analysis (taste, smell, color, appearance) and physico-chemical (mass fraction of moisture, evolution of acidity over time, mass fraction of broken products and culinary properties), is accordingly with the data from technical regulations for pasta [57].

The antioxidant activity was determined in conditions of gastric digestion in vitro, fig. 26. CS did not show antioxidant activity, the values being negative (-9.47) % inhibited. This is explained by the fact that under conditions f gastric digestion (acid pH) *in vitro*, wheat flour starch decomposes into glucose, which in turn has a prooxidant effect [58]. The antioxidant activity of the pasta was improved by adding chokeberry and rosehip powder in concentrations 1.5% - 5% and was 2.42 - 18.42 % inhibited and 4.15-22.71 % inhibited respectively. Thus, at high concentrations of vegetable powder from chokeberry and rosehip fruits the antioxidant effects of BAC exceed the prooxidant ones of glucose, and as a result, the antioxidant activity has positive values.

Sensitivity analysis showed that rosehip and chokeberry powders influence the physico-chemical quality indicators the most, in relation to sensory indices. The antioxidant activity is influenced to a greater extent by the concentration of chokeberry powder than by the concentration of rosehip powder.

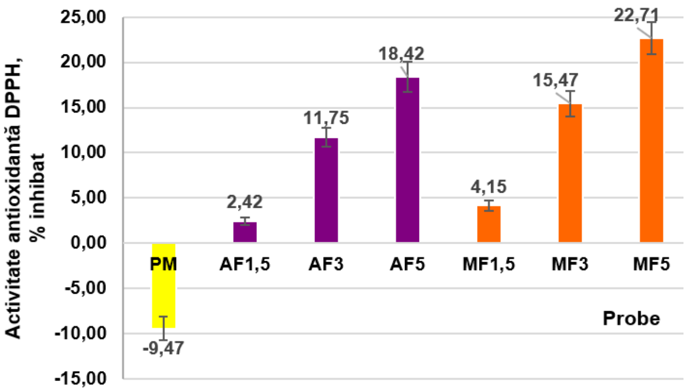


Fig. 26. Antioxidant activity (% DPPH inhibited) of cooked pasta determined under gastric digestion *in vitro*

The CIELab analysis results of raw and cooked pasta with chokeberry and rosehip powders showed that the operation of boiling the pasta contributed to the decrease of the values of all chromatic parameters L*, a* and b*, due to the thermal degradation process of pigments and their elimination in boiling water. As a result, in pasta with chokeberry powder the color difference ΔE* reached the maximum values up to 19.07, and in rosehip samples - up to 17.87. In food matrices, anthocyanins and carotenoids are sensitive to heat, so processes involving heat treatment must be maintained for a short time [59]. The concentration 3% of vegetable powder is optimal for the pasta manufacture and the analysis of the evolution of acidity for 150 days allowed to establish the shelf life of up to 5 months.

6.2. Functional flour confectionery with added vegetable matter from sea buckthorn, rosehip and hawthorn fruits

The technology of manufacturing glazed gingerbreads was developed with the use of sea buckthorn, rosehip and hawthorn powder additives to diversify the range of functional products. Addition of vegetable powder in concentrations 2 and 4% and vegetable extracts in concentration 2% with a dry matter content of 85±1% was used to prepare the syrup for glazing. The influence of the addition of vegetable powders, extracts and the shelf life on organoleptic indices, physico-chemical indicators, microbiological stability and antioxidant activity, determined *in vitro* in glazed gingerbreads on the first day, on the 25th and 45th day after baking, was investigated.

The addition of vegetable powders has a positive influence on sensory indices, especially taste, smell and color. It is also attested to an improvement of the gingerbreads consistency and the maintenance of freshness, microbiological stability and antioxidant activity are increased. Sensitivity analysis showed different influences of vegetable powder concentrations and shelf life on quality, microbiological stability and antioxidant activity depending on the type of berries used.

Vegetable addition positively influenced the quality indicators and established the optimal powder concentration and shelf life of glazed gingerbreads: with sea buckthorn and rosehip - 2% and 25 days, and with hawthorn - 4% and 45 days respectively [60]. The research results showed that sea buckthorn, rosehip and hawthorn powders and their extracts can be recommended in the manufacture of glazed gingerbread, to increase biological value, to improve the nutritional properties and organoleptic characteristics, to increase the shelf life of the product, avoiding the use of synthesis additives

6.3. Functional sugar confectionery with added vegetable matter

Sugar confectionery is requested by children of different ages [7]. Usually, synthetic dyes are used in the technology of manufacturing these foods, which can affect the health of consumers during long consumption. Thus, there is a need in the development of technology for the manufacture of sugar products with natural dyes, which will be beneficial to the health of the human body [61].

6.3.1. Development of manufacturing technology of functional candies of type jelly

The technology of making jelly candies with concentrated hydroalcoholic extracts from sea buckthorn and rosehip fruits, chokeberry and grape marc was developed to diversify the range of candies [62, 63]. It has been shown that there is a direct dependence between the concentration of extract added to the preparation of jellies and increasing the values of physicochemical indicators, microbiological stability, chromatic parameters and antioxidant activity of candies. Sensitivity analysis demonstrated the different influence of plant extract concentrations and shelf life on quality indicators. For sea buckthorn and rosehip jellies the optimal concentration of extract is 2%, for candies with chokeberry and grape marc - 1.5% and the shelf life is 2 months.

6.3.2. Development of manufacturing technology of functional confectionery masses

Confectionery masses serve to diversify the candies range with or without filling. A technology for the manufacture of confectionery masses with extracts and powders from chokeberry and sea buckthorn was developed. The addition of 5% chokeberry powders (PEPA), 4% sea buckthorn powders (PEPC) and the respective extracts positively influences the quality indicators and the microbiological stability of the confectionery masses during storage. It is recommended that the shelf life be up to 60 days in packaging and storage conditions at 20±1°C and φ max. 75%. The chromatic parameters analysis demonstrates a positive influence of the vegetable powders on the color saturation of the confectionery masses, and the storage time contributed to the reduction of all the chromatic parameters of the confectionery masses, fig. 27.

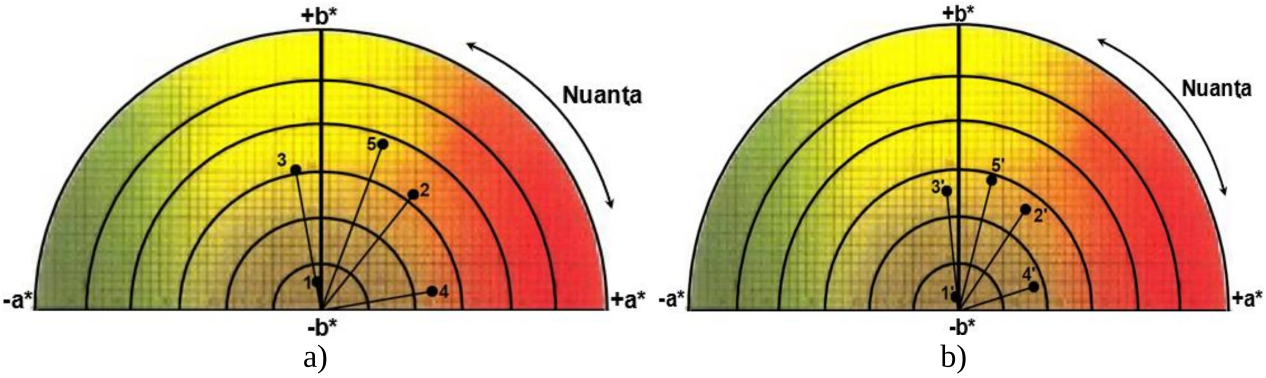


Fig. 27. Changing the colors shade of the confectionery masses during storage: a) on the first day after production; b) after 50 days from production. 1 and 1′ - CS ; 2 and 2′ - PEA; 3 and 3′ - PEC; 4 and 4′ - PEPA; 5 and 5′ - PEPC

The samples with extracts and vegetable powders showed antioxidant activity, the values being considerably higher: 32.20% (PEPA) and 21.42% inhibited (PEPC) compared to the control sample (without plant matter). During storage, the reduction of antioxidant activity is attested, but still the values remained positive, showing the increased antioxidant capacity of the candies compared to the control sample. The extracts in combination with vegetable powders can be successfully used in the technology of manufacturing confectionery masses as natural dyes, helping to increase the biological value of sugar products and allow expanding the range of candies and fillings.

7. Manufacturing technologies of dairy products with BAC

7.1. The study on the influence of the BAC addition to the yogurt manufacture

The technology for manufacturing functional yogurt with increased biological value by adding concentrated hydroalcoholic extracts from berries: sea buckthorn, rosehip, hawthorn and chokeberry was developed. Fortified yogurt was obtained by adding concentrated hydroalcoholic extracts with a dry matter content of 85±1% in a proportion 1% [64]. The evolution of sensory characteristics and physico-chemical indicators during the storage of eighteen days attests that the sea buckthorn yogurt can be kept for a maximum of thirteen days, and the yogurt with rosehip, hawthorn and chokeberry - fifteen days, due to the chemical composition of berries. The CIELab chromatic parameters analysis of yogurt demonstrated the presence of color pigments depending on the type of extract used. It is attested that all yogurt samples show positive and high values of antioxidant activity determined in vitro, due to the phytochemical content of the extracts, but also to the metabolic activity of lactic acid bacteria of the starter culture.

The antimicrobial activity investigation of the extracts on the development of L. *monocytogenes* and L. *monocytogenes* EGDe showed that sea buckthorn and rosehip extracts show AMA against both strains of Listeria pathogenic bacteria, the chokeberry extract showed AMA only on L. *monocytogenes* EGDe, and the extract of hawthorn did not manifest AMA against Listeria. The bactericidal effect evaluation of chokeberry extracts in concentrations 0.5%; 0.75% and 1% in yogurt samples which were infected with L. *monicytogenes*, showed that during storage in all yogurt samples the number of pathogenic bacteria was reduced, including CS, and in samples fortified with chokeberry extracts, the reduction rate was much higher [65], fig. 28.

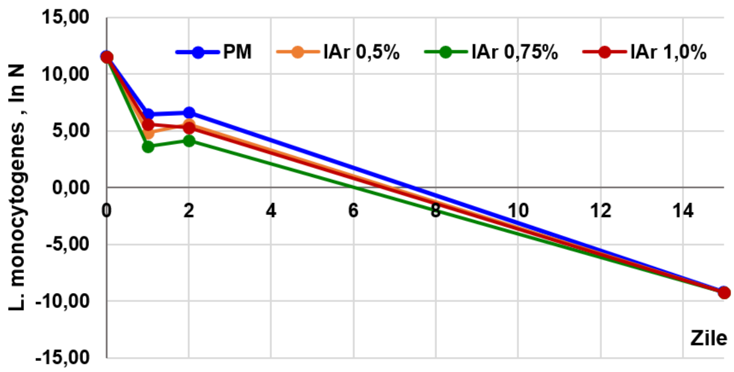


Fig. 28. Influence of different concentrations of chokeberry extract on the bactericidal effect of L. *monocytogenes* in yogurt

7.2. The study on the influence of the BAC addition to the cheese creams manufacture

Cheese-making technologies with the addition of concentrated hydroalcoholic extracts and fat-soluble extracts from berries have been developed [66-68]. Additions of concentrated extracts of sea buckthorn and rosehip (2.5%), hawthorn and chokeberry (1.5%) and fat-soluble extracts of sea buckthorn, rosehip, hawthorn and sorbus (2.5%) ensure an attractive color of the product and increased antioxidant activity.

The physico-chemical indicators analysis of the tested samples demonstrated the increase of the dry matter content and the fat content in the fortified cheese cream due to the high dry matter content in the extracts used. In the samples with fat-soluble extracts, the dry matter content and the fat content increased on average by 11.8% and 19.9% respectively, compared to CSt. This fact positively influenced the sensory characteristics of cheese cream, tab. 6.

The quality indicators evolution of cream cheeses with berry extracts in during storage has shown that they correspond to the regulated allowed values.

Table 6. Influence of berries fat-soluble extracts on the quality of cheese cream samples in relation to CSt*

Indicator**	Cheese cream samples				
	CSt	CrSb	CrR	CrH	CrS
Average total score	19.2±0.1	20.0±0.0	20.0±0.0	20.0±0.0	19.5±0.1
Dry matter content,%	17.00± 0.05	19.20± 0.05	19.00± 0.05	19.00± 0.05	18.85± 0.05
Fat content,%	9.90±0.05	12.00±0.05	11.90±0.05	11.80±0.05	11.80±0.05
Acidity, °T	116.0±0.5	119.0±0.5	118.0±0.5	117.0±0.5	117.0±0.5
Active acidity, pH	5.10±0.01	5.08±0.01	5.09±0.01	5.10±0.01	5.10±0.01
Dynamic viscosity, mPa·s ***	13399±123	11258±145	11250±102	11087±98	11020±106

*results are presented as mean ± standard deviation; **it was determined on the first day of production; *** it was determined by the speed of the spindle 75 min^{-1}. Note: CSt-control sample with tartrazine; CrSb – cheese cream with sea buckthorn extract; CrR - cheese cream with rosehip extract; CrH - cheese cream with hawthorn extract; CrS - cheese cream with scorch extract.

In the case of samples with hydroalcoholic extracts, the shelf life of sea buckthorn cream shall be a maximum of ten days and of creams with rosehip, hawthorn and chokeberry - thirteen days, and in the case of all samples with fat-soluble extracts - a maximum of thirteen days to maintain sensory characteristics , in particular the color, smell and specific taste of the berries. The values of the antioxidant activity were found to be positive, especially in the samples of cream cheese with berry extracts, being an essential argument in favor of fortified foods.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The problems addressed in the thesis are devoted to obtaining and stabilizing dyes, antioxidants and preservatives of natural origin, by elucidating chemical, physico-chemical and biochemical transformations, which take place under extraction conditions and the addition of plant matter in the formulation of functional foods. The main results of the research were formulated by the following conclusions:

1. The methodology for determining the optimal hydromodule for the extraction of the water-soluble BAC complex with a minimum consumption of solvents was developed, which was: 8 for grape marc; 12 - sea buckthorn; 14 - rosehip, sorbus and chokeberry and 20 for hawthorn. Three empirical mathematical models were applied: Peleg, Page, and the power model to describe the kinetics of the extracting process of soluble dry matter content from vegetable powders, demonstrating a high concordance between experimental and computational data, R^2 = 0.907-0.979, subchapter 3.1.1, [10, 69].

2. The BAC extraction yield is influenced by the polarity and viscosity of the EtOH extragent, which increasing in the range of 20-60% (v/v) and decreasing to 96% (v/v). There is a positive correlation between BAC and AAWS, which depends on the chemical composition of the extracted polyphenols, with an enthalpy of osmolytic dissociation of -OH bonds and low ionization potential, which positively influences their antioxidant activity. The mutual information analysis established the degree of the EtOH concentration influence on TPC TAC, TC and antioxidant activity, determined by PCL, DPPH and HPSA tests in different medeum. Canonical correlation analysis established the interdependence between BAC and antioxidant activity, subchapter 3.1.2, [70].

3. Increasing the temperature from 30 to 65°C led to an increase of the extraction yield of TPC, TFC, TC in all water-soluble extracts and TAC in grape marc and chokeberry extracts.The maximum values of antioxidant activity were attested for 65°C and the EtOH solution concentration 60% (v/v). The individual profile of polyphenols, anthocyanins, organic acids and CIElab chromatic parameters were analyzed. A mathematical model of the type TPC=f(t, C) was established, using cubic spline functions and it was found that factorial interdependencies exceed the influence of unique factors, such as temperature, subchapter 3.1.3, [60, 70, 71].

4. The change in the extraction temperature from 30 to 65°C influenced the extraction efficiency of the carotenoids and the antioxidant activity in the fat-soluble extracts, the highest rate of pigments and antioxidant activity being reached at 45°C. The accumulation dynamics of

primary and secondary products of lipid oxidation, the evolution of carotenoid pigment content, antioxidant activity values and CIELab chromatic parameters have shown that fat-soluble extracts can be stored at 4 °C in the absence of light for 12 months. Fuzzy sets were used to develop the generalized mathematical model of type PI = f(t, AI) to determine the influence of temperature on quality physico-chemical indices and antioxidant activity of fat-soluble extracts of sea buckthorn and rosehip, subchapter 3.2, [72].

5. Assisted extraction of PEF at t = 65ºC and U = 165V, n = 900 pulses allowed to obtain extracts from grape seeds with high tannin content - 10.6 mg TAE/g d.w. and with antioxidant activity - 92.70% inhibited, this is due to the change in permeability and rupture of cell membranes in the plant matrix. It was found that voltage has a more important influence than the number of pulses, and increasing the extraction temperature from 30 to 65°C has a synergistic effect with PEF parameters on tannin content efficiency. The extraction process was optimized by sensitivity analysis, determined by the first-order Sobol index and was patented, subchapter 4.1, [73].

6. The MAE increased the phenolic compounds yield and the antioxidant activity of hydroalcoholic extracts compared to conventional extraction. In the case of TPC, the highest efficacy was recorded for sea buckthorn extracts - 1.89 times; for TFC - in hawthorn extracts, 1.6 times; for TC and TAC - in grape marc extracts by 2.3 and 1.4 times respectively.The maximum values of antioxidant activity in plant extracts correspond to the maximum values of TPC, TFC, TC, TAC, obtained at 65°C in 60% (v/v) EtOH solutions, demonstrating a direct correlation, subchapter 4.2.

7. The UAE influenced the BAC extraction yield in 60% (v/v) EtOH extracts from berries and grape marc. Increasing the temperature from 30 to 65°C has a pronounced influence on the extraction efficiency of TPC and TAC by UAE, due to the occurrence of matrix bond breaks, increased solubility of phenolic compounds and solvent diffusion rate, reduced viscosity and surface tension of the solvent; increasing the duration of UAE application from 10 to 60 min has led to an insignificant increase of the BAC extraction efficiency due to cavitation; higher values of antioxidant activity correspond to the extraction rate of phenolic compounds at 65°C and the duration of UAE 10 min. Sensitivity analysis showed that the extraction temperature more significantly influences the TPC yield and antioxidant activity in plant extracts than the duration of UAE, subchapter 4.3, [74].

8. Heat treatments (freezing, drying at room temperature and at 65°C) of sea buckthorn, rosehip and sorbus pulps influence the yield of TCC, TPC and antioxidant activity, determined by DPPH tests and by the stabilization of Ag nanoparticles in extracts obtained by UAE. The

highest TCC was determined in the frozen pulps extracts: rosehip - 0.69 mg/g d.w., sea buckthorn - 0.66 mg/g d.w. and sorbus - 0.37 mg/g d.w. In the dry samples the carotenoids were exposed to oxidation, which led to the transformation of *trans* β-carotene into various *cis* isomers, one of the carotenoid degradation mechanisms in food. The maximum TPC yield was obtained in the extracts from the dry samples at 65 °C. Increased values of antioxidant activity (DPPH) were obtained in frozen pulps extracts, and in the case of Ag nanoparticles stabilization, the highest values were obtained in extracts from dried pulps at 65°C. The sorbus powders have the highest bioavailability of carotenoids - 15.3%, followed by sea buckthorn - 9.1% and rosehip -7.5%. The individual carotenoids were identified and quantified in unsaponified and saponified extracts obtained from frozen pulps by the RP-HPLC method, subchapters 4.3, 5.4.

9. The pH value shows specific influences on the antioxidant activity of hydroethanolic extracts from rosehip, sea buckthorn, chokeberry and grape marc: for rosehip the essential changes occurred at pH 2.5 and pH 8.7; in sea buckthorn extracts, the highest leap of antioxidant activity occurred at pH 3.6, and at pH 7.0 and pH 8.5, antioxidant activity increased by 1.5 times and 1.4 times, respectively; for chokeberry, in the acidic medium the antioxidant activity values decreased significantly, and in the alkaline medium the antioxidant activity did not change significantly. In marc extracts, the antioxidant activity did not change essentially in the pH range 2.6 - 8.8 in relation to the control. Increasing the pH from 2.6 to 8.8 decreases the probability of π - π^* transitions and the *p* orbitals from anthocyanin molecules expand, forming a conjugate system. Changing the color of anthocyanins from red in acidic medium to colorless in alkaline environment is associated with both the increase of the resonant structure and the formation of a plane structure, which allows π electrons to spread more evenly on the molecule, subchapter 5.1, [41].

10. Ca^{2+} ions have the most important effect on the chromatic parameters of tescovine extracts, which is due to the polymerization process of polyphenols and anthocyanin complexation. In the chokeberry extracts, Ca^{2+} ions reduced antioxidant activity with 32.2% compared to the control sample, and in the marc extracts a direct dependence between antioxidant activity reduction and Ca^{2+} ion concentration was attested. The most likely cause is the complexation of anthocyanins or flavonoid derivatives with metal ions, which contribute to the antioxidant activity reduction of BAC by making unavailable oxygen atoms bound to the metal ion, subchapter 5.2, [45].

11. Vegetable powders have of varying level AMA against pathogenic microorganisms investigated, due to the different content of polyphenols, especially of the quercetin and gallic acid derivatives. Sea buckthorn powder had the lowest MIC and MBC, followed by rosehip,

marc, chokeberry and hawthorn. *E. coli* showed resistance to chokeberry, and *K. pneumoniae* showed resistance to chokeberry, hawthorn and grape marc powders. Sea buckthorn and rosehip powders showed bactericidal effect against all pathogenic microorganisms studied; hawthorn and grape marc powders - against *S. aureus* and *E. coli*, and chokeberry powder - only against *S. aureus*, subchapter 5.3. [46].

12. The 7 technologies for the manufacture of flour, sugar and dairy products with various additions of water-soluble, fat-soluble extracts, berry and grape marc powders have been developed. The developed technologies were optimized based on the following criteria: sensory analysis, in order to accept the products by consumers; the conformity of the quality indices of the normative documents towards each product category; microbiological stability, in order to assess the shelf life of the products; antioxidant activity and bioavailability of BAC in situ and in conditions of gastric digestion, *in vitro*, chapters 6-7.

PROPOSALS TO USE THE RESULTS OBTAINED IN THE ECONOMIC FIELDS

The thesis developed a series of processes for the functional products manufacture with vegetable additives, berries and grape marc extracts, which are recommended for their implementation in the food industry:

I. The extraction process of the water-soluble BAC complex from berry powders and grape marc, according to the patents [45, 73].

II. The extraction process of fat-soluble BAC complex from rosehip, sea buckthorn and sorbus fruits, according to the patent [66, 67].

III. Technology for manufacturing pasta with rosehip and chokeberry powders.

Addition of rosehip and chokeberry powders in concentrations of 1.5%; 3% and 5% highlighted the following:

- the addition of vegetable powders led to a reduction in the amount of wet gluten and dry gluten, in the case of rosehip with 9.6% and 6.35% and in the case of chokeberry with 2.2% and 2.7% respectively in relation to CS, due to higher hydration capacity of powders than wheat flour;

- the elastic properties of gluten were positively influenced, IDK values were reduced from 84 u.c. (CS), belonging to the “poorly satisfactory” group, at 77 u.c. (rosehip) and 78 u.c. (chokeberry), which corresponds to the “good” gluten and the group of first quality, due to the action of organic acids from vegetable powders on wheat flour proteins with the formation of disulfide bonds -S-S- inside of gluten matrix;

- the rheological comportment of the dough empirical properties of with and without rosehip and chokeberry powders was influenced. Increasing of vegetable powders concentration led to an increase of the dough tenacity and extensibility, index of swelling, baking strength and reduced the configuration ratio, except for the rosehip sample with a concentration 5%, where configuration ratio increased by 2.61 times compared to CS - 2.41 times. This was due to the presence of organic acids, dietary fiber, pectic substances in rosehip and chokeberry powder.The sensitivity analysis finds that in the case of chokeberry powder the influences of the concentration on the rheological properties of the dough are generally higher than in the case of rosehip powder;

- the pasta quality with vegetable addition determined by sensory analysis (taste, smell, color, appearance) and physico-chemical (mass fraction of moisture, evolution of acidity over time, mass fraction of broken products and culinary properties), is accordingly with the data from technical regulations for pasta;

- it was demonstrated that pasta cooked with vegetable powders in concentrations of 1.5-5% showed actioxidant activity determined in conditions of gastric digestion in vitro, the values being in the range 2.42 - 22.71% inhibited in relation to CS, which did not show actioxidant activity;

- the CIELab analysis results of raw and cooked pasta with chokeberry and rosehip powders showed that the operation of boiling the pasta contributed to the decrease of the values of all chromatic parameters L*, a* and b*, due to the thermal degradation process of pigments and their elimination in boiling water;

- the concentration 3% of vegetable powder is optimal for the pasta manufacture and the analysis of the evolution of acidity for 150 days allowed to establish the shelf life of up to 5 months.

IV. Technology for the manufacturing of glazed gingerbreads fortified with powders and extracts of sea buckthorn, rosehip and hawthorn [60].

The addition of vegetable powders has a positive influence on sensory indices, especially taste, smell and color. It is also attested to an improvement of the gingerbreads consistency and the maintenance of freshness, microbiological stability and antioxidant activity are increased. Sensitivity analysis showed different influences of vegetable powder concentrations and shelf life on quality, microbiological stability and antioxidant activity depending on the type of berries used. Vegetable addition positively influenced the quality indicators and established the optimal powder concentration and shelf life of glazed gingerbreads: with sea buckthorn and rosehip - 2% and 25 days, and with hawthorn - 4% and 45 days respectively.

V. Technology for the manufacturing of jelly-type candies with concentrated hydroalcoholic extracts from sea buckthorn, rosehip, chokeberry and grape marc [62, 63].

It has been shown that there is a direct dependence between the concentration of extract added to the preparation of jellies and increasing the values of physicochemical indicators, microbiological stability, chromatic parameters and antioxidant activity of candies. Sensitivity analysis demonstrated the different influence of plant extract concentrations and shelf life on quality indicators. For sea buckthorn and rosehip jellies the optimal concentration of extract is 2%, for candies with chokeberry and grape marc - 1.5% and the shelf life is 2 months.

VI. Technology for the manufacturing of confectionery masses with extracts and powders from chokeberry and sea buckthorn fruits.

The addition of 5% chokeberry powders (PEPA), 4% sea buckthorn powders (PEPC) and the respective extracts positively influences the quality indicators and the microbiological stability of the confectionery masses during storage. It is recommended that the shelf life be up to 60 days in packaging and storage conditions at 20±1°C and φ max. 75%. The chromatic parameters analysis demonstrates a positive influence of the vegetable powders on the color saturation of the confectionery masses. The samples with extracts and vegetable powders showed antioxidant activity, the values being considerably higher: 32.20% (PEPA) and 21.42% inhibited (PEPC) compared to the control sample (without plant matter). During storage, the reduction of antioxidant activity is attested, but still the values remained positive, showing the increased antioxidant capacity of the candies compared to the control sample.

VII. Technology for the manufacturing of yoghurt fortified with concentrated extracts of sea buckthorn, rosehip, hawthorn and chokeberry [64].

It is recommended to add concentrated extracts in a proportion 1%. The evolution of sensory characteristics and physico-chemical indicators during the storage of eighteen days attests that the sea buckthorn yogurt can be kept for a maximum of thirteen days, and the yogurt with rosehip, hawthorn and chokeberry - fifteen days, due to the chemical composition of berries. The CIELab chromatic parameters analysis of yogurt demonstrated the presence of color pigments depending on the type of extract used. It is attested that all yogurt samples show positive and high values of antioxidant activity determined in vitro, due to the phytochemical content of the extracts, but also to the metabolic activity of lactic acid bacteria of the starter culture. The antimicrobial activity investigation of the extracts on the development of *L. monocytogenes* and *L. monocytogenes* EGDe showed that sea buckthorn and rosehip extracts show AMA against both strains of Listeria pathogenic bacteria, the chokeberry extract showed AMA only on *L. monocytogenes* EGDe, and the extract of hawthorn did not manifest AMA against Listeria. The bactericidal effect evaluation of chokeberry extracts in concentrations 0.5%; 0.75% and 1% in yogurt samples which were infected with L. monicytogenes, showed that during storage in all yogurt samples the number of pathogenic bacteria was reduced, including CS, and in samples fortified with chokeberry extracts, the reduction rate was much higher.

VIII. Technologies for manufacturing cheese cream with extracts from sea buckthorn, rosehip, sorbus, hawthorn and chokeberry [66-68].

Additions of concentrated extracts of sea buckthorn and rosehip (2.5%), hawthorn and chokeberry (1.5%) and fat-soluble extracts of sea buckthorn, rosehip, hawthorn and sorbus (2.5%) ensure an attractive color of the product and increased antioxidant activity. The quality indicators evolution of cream cheeses with berry extracts in during storage has shown that they correspond to the regulated allowed values. In the case of samples with hydroalcoholic extracts, the shelf life of sea buckthorn cream shall be a maximum of ten days and of creams with rosehip, hawthorn and chokeberry - thirteen days, and in the case of all samples with fat-soluble extracts - a maximum of thirteen days to maintain sensory characteristics , in particular the color, smell and specific taste of the berries. The values of the antioxidant activity were found to be positive, especially in the samples of cream cheese with berry extracts, being an essential argument in favor of fortified foods.

SUGGESTIONS ON POTENTIAL FUTURE RESEARCH DIRECTIONS

1. Obtaining and stabilizing carotenoid extracts from other plant sources.

2. Reducing the contamination of bakery products with pathogenic microorganisms that cause ropiness disease by using BAC of plant origin.

3. Technologies for obtaining dietary fiber from horticultural sources.

4. Obtaining foaming and emulsifying substances of vegetable origin for use in food

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1. Specialty books

1.1. single-author specialty books

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2. Articles in scientific journals

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- **GHENDOV-MOSANU, A.,** CRISTEA, E., PATRAS, A., STURZA, R., PADUREANU, S., DESEATNICOVA, O., TURCULET, N., BOESTEAN, O., NICULAUA, M. Potential Application of *Hippophae Rhamnoides* in Wheat Bread Production. *Molecules,* 2020, 25, 1272, doi:10.3390/molecules25061272 (**I.F. 3.06**)
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- **GHENDOV-MOȘANU, A.,** CARAMAN, S., TURCULEȚ, N., RUBȚOV, S., STURZA, R. Technologies de protection des gâteaux sucrés glacés. *9th International Symposium EuroAliment-2019*, 5-6 September 2019, Galati, Romania, 133.
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ANNOTATION

Ghendov-Moşanu Aliona: Obtaining and stabilizing dyes, antioxidants and preservatives of plant origin for functional foods, dissertation of PhD habilitatus of engineering sciences, Chisinau, 2021.

Thesis structure: The thesis consists of an introduction, 7 chapters, general conclusions and recommendations, bibliography - 488 sources, 9 annexes, 212 pages of the main text, 99 figures, 69 tables. The results are published in 72 scientific articles.

Keywords: extracts, vegetable powders, biologically active compounds, dyes, antioxidants, preservatives, functional foods, quality.

The purpose of the work: Establishing the theoretical and practical principles of obtaining and stabilizing dyes, antioxidants and preservatives of natural origin by elucidating chemical, physico-chemical and biochemical transformations that take place under conditions of extraction, storage and addition of plant matter in food, with the formulation of technology of some functional foods.

Objectives: Determination of the optimal hydromodule for the extraction of the BAC complex and application of empirical mathematical models to describe the kinetics of the extraction process; the influence of conventional extraction conditions, of different "*green extractions*" techniques and heat treatments on the yield of BAC from berries and grape marc, of antioxidant activity and chromatic parameters; the influence of pH and the effect of metal ions present in food on the color stabilization of extracts and their antioxidant activity; determination of the microbiostatic activity of the vegetal matter, of the bioavailability BAC *in vitro*; elaboration of functional food manufacturing technologies, determination of quality and food safety indicators, of chromatic parameters and antioxidant activity and their evolution during storage.

Scientific novelty and originality: For the first time, all stages of obtaining and stabilizing BAC from berries and grape marc with the use of secondary metabolites of plant matter with coloring, antioxidant and antimicrobial properties were examined in order to replace synthetic dyes, antioxidants and preservatives in the formulation of functional foods.

Main results: The optimal hydromodule for the extraction of the water-soluble complex from berries and grape marc was determined, which ensures the obtaining of an important BAC content and an optimal solvent consumption; the influence of conventional extraction conditions, various "*green extractions*" techniques and heat treatments on the water-soluble and fat-soluble BAC yield of berries and grape marc, antioxidant activity and CIELab chromatic parameters was elucidated; the influence of pH and the effect of metal ions present in food on the color stabilization of extracts and antioxidant activity has been demonstrated; the microbiostatic activity of the vegetal matter on the pathogenic microorganisms was determined; the bioavailability of carotenoids was determined *in vitro*; technologies for the manufacture of functional foods have been developed with the determination of quality indicators, chromatic parameters, *in vitro* antioxidant activity and their evolution during storage.

Theoretical significance: For the first time, the methodology for determining the optimal hydromodule for the extraction of the water-soluble complex in solid-liquid system was elaborated; for the first time various Informatics methods such as analysis of mutual information and sensitivity, canonical correlation, mathematical models as cubic spleen function and fuzzy sets were applied to determine the influence of extraction conditions and technological parameters on the yield of BAC, quality, food safety and on biological value of functional foods.

Applicative value: Processes for obtaining and stabilizing the color of plant extracts were proposed and performed; technologies for manufacturing functional foods, including flour, sugar, dairy products. 8 patents have been obtained.

Implementation of scientific results: The research results were implemented at "Rose Line" LTD and at "Ungar" LTD. Certain chapters of the thesis are included in the curricula for the training of specialists in bachelor's and master's degree programs: Food Technology, Technology and Management in Public Catering, Food and Nutrition, Food Quality and Safety, Restaurant Management and Catering Services and Oenology, Wine Tourism and Wine Markets.

ADNOTARE

Ghendov-Moșanu Aliona: Obținerea și stabilizarea unor coloranți, antioxidanți și conservanți de origine vegetală pentru alimente funcționale, teză de doctor habilitat în științe inginerești, Chișinău, 2021.

Structura tezei: constă din introducere, 7 capitole, concluzii şi recomandări, bibliografie din 488 de titluri, 9 anexe, 212 de pagini de text de bază, 99 de figuri, 69 tabele. Rezultatele obţinute sunt publicate în 72 de lucrări ştiinţifice.

Cuvinte-cheie: extracte, pudre vegetale, compuși biologic activi, coloranți, antioxidanți, conservanți, alimente funcționale, calitate.

Scopul lucrării: Stabilirea principiilor teoretice și practice de obținere și stabilizare a unor coloranți, antioxidanți și conservanți de origine naturală prin elucidarea transformărilor chimice, fizico-chimice și biochimice care au loc în condiții de extracție, păstrare și la adiționarea materiei vegetale în produse alimentare, cu formularea tehnologiei unor alimente funcționale.

Obiectivele lucrării: Determinarea hidromodulului optim pentru extragerea complexului de CBA și aplicarea modelelor matematice empirice pentru descrierea cineticii procesului de extracție; influența condițiilor de extracție convențională, a diferitor tehnici de „*extracții verzi*” și tratărilor termice asupra randamentului CBA din fructe de pădure și tescovina de struguri, a activității antioxidante și parametrilor cromatici; influența pH și efectul ionilor metalici prezenți în alimente asupra stabilizării culorii extractelor și activității lor antioxidante; determinarea activității microbiostatice a materiei vegetale, a bioaccesibilității CBA *in vitro*; elaborarea tehnologiilor de fabricare a unor alimente funcționale, determinarea indicatorilor de calitate și inofensivitate, a parametrilor cromatici și activității antioxidante și evoluţia lor în timpul pastrarii.

Noutatea și originalitatea științifică: În premieră au fost examinate toate etapele de obținere și stabilizare a CBA din fructe de pădure și tescovină de struguri cu utilizarea metaboliților secundari din materia vegetală cu proprietăți de colorare, antioxidante și antimicrobiene pentru substituirea coloranților, antioxidanților și conservanților de origine sintetică în formularea unor alimente funcționale.

Rezultatele principale: S-a determinat hidromodulul optim pentru extragerea complexului hidrosolubil din fructe de pădure și tescovina de struguri, care asigură obținerea unui conținut important de CBA și un consum optim de solvent; a fost elucidată influența condițiilor de extracție convențională, a diferitor tehnici de „*extracții verzi*” și tratărilor termice asupra randamentului CBA hidrosolubili și liposolubili din fructe de pădure și tescovina de struguri, activității antioxidante și parametrilor cromatici CIELab; s-a demonstrat influența pH și efectul ionilor metalici prezenți în alimente asupra stabilizării culorii extractelor și activității antioxidante; s-a determinat activitatea microbiostatică a materiei vegetale asupra microorganismelor patogene; bioaccesibilitatea carotenoidelor a fost determinată *in vitro*; au fost elaborate tehnologii de fabricare a unor alimente funcționale cu determinarea indicatorilor de calitate, parametrilor cromatici, activității antioxidante *in vitro* și evoluţia lor în timpul pastrarii.

Semnificaţia teoretică: În premieră a fost elaborată metodologia de determinare a hidromodulului optim pentru extracția complexului hidrosolubil în sistem solid-lichid; în premieră pentru prima data au fost aplicate diferite metode informatice precum analiza informației mutuale și sensibilitate, de corelație canonică, modele matematice ca funcție splină cubică și mulțimi fuzzy pentru stabilirea influenței condițiilor de extracție și a parametrilor tehnologici asupra randamentului CBA, a calității, inofensivității și valorii biologice a alimentelor funcționale.

Valoarea aplicativă: Au fost propuse și realizate procedee de obținere și stabilizare a culorii extractelor vegetale; tehnologii de fabricare a unor alimente fucționale, inclusiv produse făinoase, zaharoase, produse lactate. Au fost obținute 8 brevete de invenții.

Implementarea rezultatelor ştiinţifice: Rezultatele cercetărilor au fost implementate la SRL ”Rose Line” și la FPC ”Ungar”. Anumite capitole ale tezei sunt incluse în programele de învăţământ pentru pregătirea specialiştilor la programele de studii de licență și master: Tehnologia Produselor Alimentare, **Tehnologie si Management în Alimentația Publică, Alimentație și Nutriție,** Calitatea și Securitatea Produselor Alimentare, Managementul Restaurantelor și Servicii de Caterings și Oenologie, Enoturism și Piețe Vitivinicole.

GHENDOV-MOȘANU ALIONA

OBTAINING AND STABILIZING DYES, ANTIOXIDANTS AND PRESERVATIVES OF PLANT ORIGIN FOR FUNCTIONAL FOODS

253.01 - Technology of food products of vegetable origin

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