Prof. Jelena Vulić
University of Novi Sad

Jointly elaborated by: University of the Aegean

AGRIMA

Fruit and vegetable waste valorization

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Agri-food Waste Management for Sustainable bio-economy through Higher Education curricula and upskilling

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Goals

AGRIMA aims to foster universities' **capacity building** for the **green transition** through **innovative practices and higher education curricula updating in agri-food waste management** for the **circular bioeconomy**.

AGRIMA addresses:

1. **Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
2. **Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.

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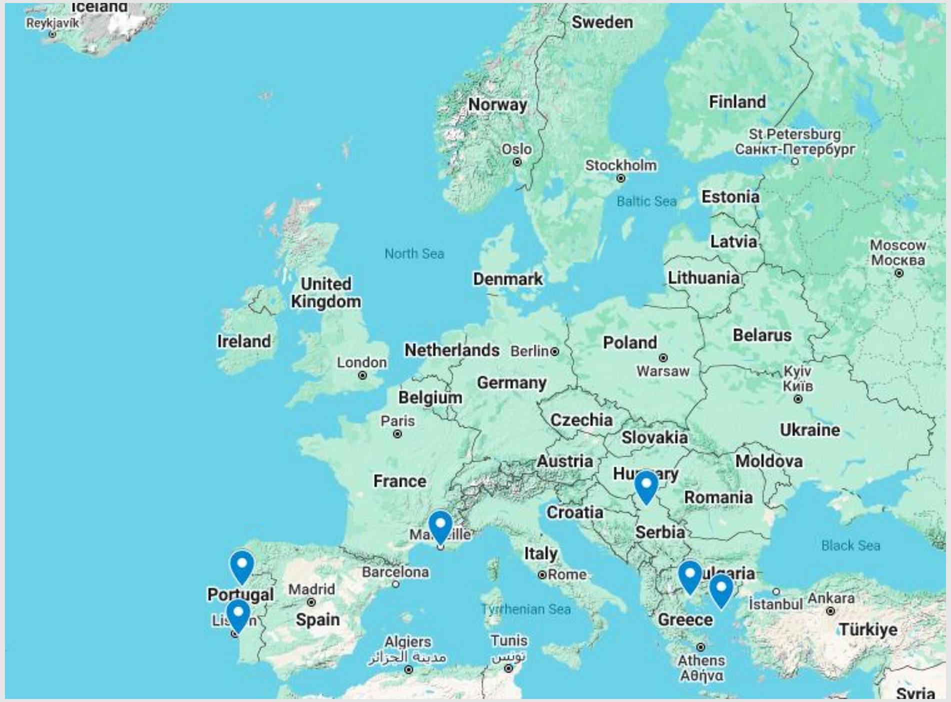
Partners



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The University of Novi Sad, Faculty of Technology (FoTNS) was founded to give academic education in the sphere of food, chemical, and pharmaceutical engineering, as well as biotechnology. High level of academic education, scientific papers, projects, innovations, and patents have ranked FoTNS very high among other institutions. FoTNS attaches great importance to international cooperation, which has been successfully achieved over the years through the numerous scientific projects. Through national and international projects, FoTNS made network with researchers at other scientific institutions abroad, expanded areas of interest and research, but also provided financial support from various international funds.

Prof. Dr. Jelena Vulić was born in 1982 in Novi Sad, Serbia. She graduated at the Department of Food Preservation, and since 2008 is affiliated at Faculty of Technology Novi Sad, University of Novi Sad, where she received PhD on functional and antioxidant activities of beetroot pomace in 2012. Currently, she is Associate Professor at Department of Applied and Engineering Chemistry at Faculty of Technology, Novi Sad.

Her research areas belong to food chemistry, organic chemistry, antioxidants and additives and aroma quality control in food industry. As author and co-author, she has published over 150 scientific papers, out of which 80 were published in SCI journal and 4 as book chapter. Her articles have been cited more than 2500 times and her Hirsch index is 31. She has been coordinating or participating more than 20 national and international research and networking projects, bilateral projects, etc.

- ✓ Fruit and vegetables are consumed every day raw, minimally processed or processed, due to their nutritional and health-promoting compounds.
- ✓ Since the world population is rapidly growing and changing diet habits, also the production and processing of fruits and vegetables is growing.
- ✓ Significant losses and waste are becoming a very serious nutritional, economical, and environmental problem.
- ✓ The United Nations Food and Agriculture Organization (FAO) has estimated that at least one-third of the food produced in the world is lost and wasted every year and losses and waste in fruits and vegetables are the highest among all types of foods (up to 60%).
- ✓ The fruit and vegetables waste is composed mainly of seed, skin, rind, and pomace, containing good sources of potentially valuable bioactive compounds, such as natural pigments, polyphenols, dietary fibers, vitamins, enzymes and oils.
- ✓ These phytochemicals can be utilized in different industries including the food industry, for the development of functional or enriched foods, medicines, pharmaceuticals, and in textile industry.
- ✓ The use of waste is an important step toward sustainable development.

- ✓ Food losses are commonly the result of technical limitations and handling, such as storage, packaging, marketing, while food waste is usually the result of food discard.
- ✓ Losses and waste occur during all phases of the supply and handling chain, like harvesting, transport to packinghouses or markets, classification, storage, marketing, processing, and at home before or after preparation.
- ✓ Fruit and vegetable losses and waste do not represent only the wasting of food, but also wasting of land, water, fertilizers, chemicals and energy. This is big environmental problem as they decompose in landfills and emit harmful greenhouse gases. Followed by household garbage, fruit and vegetable processing units commonly produce the highest wastes into the environment.
- ✓ During fruit processing, solid (peel, seeds, stones, etc.) and liquid waste (juices and wash water) are produced, while their storage in non-appropriate conditions could bring flies and different insects or animals in processing areas.
- ✓ Also, fruit and vegetable waste are subject to microbiological degradation and autooxidation due to high water content and active enzymes and consequently uncontrolled oxygen consumption and green house gas emissions could cause environmental problems.

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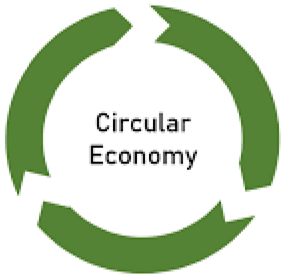
- ✓ **Circular economy** is a term for an industrial economy that is producing no waste and pollution, by design or intention, and in which material flows are of two types: biological nutrients designed to reenter the biosphere safely and technical nutrients designed to circulate at high quality in the production system without entering the biosphere as well as being restorative and regenerative by design.
- ✓ Circular economy in the food industry is based on the valorization of waste, changing markets, technologies and institutions through innovation. Valorization pathways are diverse, depending on the source, resources and the end-user, with the general idea of making a profit by exploiting waste.
- ✓ By-products of industrial processing of fruits and vegetables, such as grape pomace, citrus pulp, apple pomace, carrot peel and pulp, tomato waste and beet waste, are a rich source of dietary fibre, polyphenolic compounds and natural pigments, and after processing are treated as waste.
- ✓ Besides nutrients and bioactive ingredients in functional food, by-products from food processing side streams, managed adequately, can be used as bioabsorbents, additives, animal food, microorganism growth substrate, fertilizer materials after composting or as energy sources, substrates for biofuel production, etc.
- ✓ Moreover, prebiotics, from this waste material in combination with the antioxidants, are often associated with the possible therapeutic applications in formulas that enhance the human health by improving the intestinal environment and metabolic processes with insignificant side effects.



- ✓ Usually, only the fruit and vegetable flesh or pulp is consumed, producing significant amounts of waste, but many studies proved that significant amounts of health beneficial compounds are is in the seeds, peels, and other components of fruits and vegetables which is often not used.
- ✓ Waste from fruit and vegetables is known as rich source of potentially valuable bioactive compounds. Those compounds are natural pigments, phenolic compounds, dietary fibers, sugar derivatives, organic acids, minerals and others. Waste can be used to extract and isolate bioactive compounds that can be used in the food through foods enriched with health-enhancing substances, pharmaceuticals, cosmetics, and textile industries. Bioactive compounds possess beneficial health attributes: antibacterial, antitumor, antiviral, antimutagenic, and cardioprotective activities. Applications of fruit and vegetables by-products could be divided in two categories: those with technical purposes, which include the improvement of shelf life, safety, stability, sensory quality, etc. and those with biological purposes, which aim to enhance health-promoting effects.

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- ✓ **Phenolic compounds** are among the largest classes of bioactive compounds with diverse and important biological functions. Phenolic compounds are secondary metabolites synthetized in plants and possess one or more phenolic rings with (one or more) attached hydroxyl groups. Depending on the strength of phenolic rings, they are classified into several groups, including phenolic acids, flavonoids, stilbenes and lignans.
- ✓ Polyphenols are naturally found in fruits, vegetables, nuts, coffee, tea, seeds and their agro-industrial by-products. These compounds offer numerous health benefits, notably exhibiting antioxidant, anticancer, antimutagenic, antimicrobial, and other bioactive properties. A growing number of research evidences prove that the content of phytochemical compounds is higher in peel and seeds with respect to the edible tissue of fruits and vegetables.
- ✓ Many factors affect the concentration of phenolic compounds. These factors usually include environmental factors, such as soil, sunlight, rainfall, various farming methods, plant varieties, tree fruiting, as well as biochemical factors, such as degree of ripening, storage and cooking methods.
- ✓ The level of antioxidant activity of different phenolic compounds varies according to their chemical structure. The antioxidant property of phenolic compounds stems from their hydroxyl groups and conjugated aromatic system, and it depends on the ability of phenolic compounds to give electrons to trap free radicals by forming stable phenoxyl compounds.

- ✓ **Antioxidants** can be categorized in many different ways: natural and synthetic; polar and non-polar; enzymatic and non-enzymatic; endogenous and exogenous; and also by the mechanisms in which they are involved. Antioxidants primarily exhibit activities based on three mechanisms, hydrogen atom transfer, single electron transfer, and metal chelation.
- ✓ Endogenous antioxidants are primarily enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), while non-enzymatic endogenous antioxidants, such as glutathione and lipoic acid, are products of the body's metabolism. The first-line defense antioxidants (enzymatic) convert reactive superoxide and hydrogen peroxide into water and oxygen. The non-enzymatic antioxidants can act as a second-line defense against ROS by rapidly inactivating radicals and oxidants. The enzymatic antioxidants further act as the third-line defense involved in the detoxifying and removal. Dietary antioxidants, such as vitamins, carotenoids, polyphenols, flavonoids, and bioflavonoids are exogenous antioxidants that have *in vivo* activity.
- ✓ **Oxidative stress** is defined as the imbalance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and cellular antioxidant defenses. Oxidative stress is a result of excess ROS/RNS, which occurs due to a lack of counteraction by cellular antioxidant systems. Increased oxidative stress can have severe consequences in biological systems, including molecular damage (such as nucleic acids, lipids, and proteins), which can severely impact health. Damage to biomolecules or the induction of several secondary reactive species due to oxidative stress ultimately leads to cell death (apoptosis or necrosis). It was proved that oxidative stress is conected with more than 100 diseases, including cardiovascular disease, cancer, hypertension, diabetes, neurogenerative diseases, aging, etc.

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- ✓ Contrary to their harmful effects on health, ROS/RNS can have beneficial effects depending on their function, location, and amount. For instance, superoxide and nitric oxide radicals at low or medium concentrations are involved in cellular responses and participate in signaling pathways. Hydrogen peroxide (H2O2), formed by various oxidase enzymes, and the action of superoxide dismutase (SOD), allows its use as an important signaling molecule, also it is substrate for generating further reactive species such as hypochlorous acid (HOCl). ROS are also involved in immunological responses, degrading xeno compounds and organisms through phagocytosis.
- ✓ ROS are oxygen-containing molecules, including radicals (like the superoxide anion) and non-radicals (like H2O2) that greatly vary in their chemical abilities, such as diffusion in living cells and chemical reactivity with biomolecules. ROS examples include singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals.
- ✓ Singlet oxygen is the highest energy spin state of molecular oxygen. In contrast to molecular oxygen in ground state, the two valence electrons are paired in an anti-bonding orbital. Singlet oxygen is therefore only generated, when molecular oxygen is energized via radiation. In contrast to other ROS subspecies, no electron transfer does occur during this process. Singlet oxygen is very reactive towards organic compounds and plays a damaging role in biological systems, for instance, by involving in the oxidation of LDL cholesterol, which can lead to cardiovascular diseases. Moreover, increased ROS can trigger mitochondrial DNA mutations as well as promote uncontrolled proliferation and carcinogenesis. The balance of harmful and beneficial effects of free radicals is crucial for life processes, and antioxidants play an essential role in achieving this balance.

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Antioxidant assays mechanistic pathways:

1. Electron transfer-based assays: In these assays, a single electron transfer occurs between the antioxidant and substrate, which is measured to assess the potential of the plant's secondary metabolites. Assays like cupric ion reducing antioxidant capacity (CUPRAC), N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD), ferric reducing-antioxidant power (FRAP), ABTS radical cation decolorization assay, etc.

2. Hydrogen atom transfer-based assays: In these assays, a hydrogen atom transfers from the antioxidant to the substrate. Assays such as oxygen radical absorbance capacity (ORAC) and total radical-trapping antioxidant parameter (TRAP)methods.

3. Electron/hydrogen atom transfer (mixed) based assays: In these assays, the hydrogen atom transfer occurs via two-step mechanisms.

4. Metal chelation-based assays. In thesse assays, antioxidants chelate with transition metals. Ferrous ion and cuprous ion chelating activity are examples.

5. Lipid oxidation and ROS/RNS scavenging activity assays: These assays are based on the ability of antioxidants in reducing/preventing lipid oxidation and scavenging ROS and RNS. β-carotene linoleic acid method/conjugated diene assay, ferric thiocyanate method (FTC), thiobarbituric acid method (TBA), hydrogen peroxide (H_2O_2) scavenging assay, hydroxyl radical averting capacity method (HORAC), nitric oxide scavenging activity, peroxynitrile radical scavenging activity, superoxide radical scavenging activity (SRSA/SOD), and xanthine oxidase methods.

6. Enzymatic antioxidant assays: Antioxidant enzyme systems that catalyze reactions to counterbalance free radicals and reactive oxygen species include superoxide dismutase and catalase. Catalase, ferric reducing ability of plasma, γ-glutamyl transpeptidase , glutathione peroxidase estimation, glutathione (reduced) GSH estimation, glutathione-S-transferase, LDL assay, lipid peroxidation assay and superoxide dismutase method.

- ✓ In addition, natural pigments present in fruit and vegetables by-products, can be used as **natural colorants** in the mentioned industries. Carotenoids, betalains, anthocyanins remain in fruit and vegetables by-products in large amounts. In recent years, there has been an increased interest in the development of **non-toxic and environmentally friendly natural dyes**. Awareness of healthy lifestyle is growing day by day, which is why consumers are turning their attention to natural and safe foods. Frequent discoveries of harmful effects of synthetic pigments on human health have led to public interest in natural pigments as alternatives in the food industry, resulting in increased research and development of natural colorants.
- ✓ Beetroot (Beta vulgaris L.) contains a number of bioactive compounds, including **betalains** (**red-violet betacyanins** and **yellow-orange betaxanthins**) and phenolic compounds (flavonoids and phenolic acids), that can exhibit health-promoting effects mostly due to their antioxidant properties. Betalains are a commonly used food colorant and additive (E162) in various food products (e.g., juice, candy, and yogurt). Betalain pigments, derived from beetroot fruits, are a natural alternative to synthetic red dyes. In this context, betalains from beetroot are the most promising, not only as colorants, but also because of their free radical scavenging and reducing properties. Other reported health benefits consist of antimicrobial, anticancer, anti-lipidemic and anti-inflammatory activity as well as hepato- and neuro-protective effects. Many scientific studies, including studies in our laboratory proved very high amounts of betalains remaining in beetroot by-products!

- ✓ Carotenoids stand as the major group of compounds used as colouring agents, which can provide better sensorial, nutritional and antioxidant quality of the final product. In general, carotenoids in foods are classified into carotenes and xanthophylls. They are widely distributed fat-soluble pigments, which naturally exhibit the red, orange, and yellow color of fruits and vegetables. The biological functions of carotenoids are influenced by their chemical structure. The basic molecular structure of most carotenoids consists of a polyisoprenoid carbon chain with a series of conjugated double bonds located in the central portion of the molecule. This feature permits effective delocalization of electrons along the entire length of the polyene chain and provides carotenoids their pigmentation, light-harvesting potential during photosynthesis, and chemical reactivity. Another structural feature of most carotenoid molecules is the presence of cyclic end groups. Recent interest in carotenoids mostly arises from their health benefits. Some carotenoids serve as precursors of vitamin A. Vitamin A is essential for vision, reproduction, and bone health. In addition, vitamin A plays an important role in the immune system function.
- ✓ However, the use of betalains and phenolic compounds in food products has been restricted, because these bioactive compounds are very unstable under different conditions such as pH, light, temperature, and presence of oxygen. Changes in the chemical structure of bioactive compounds could happen and lead to the loss of their activity and potential health benefits. Also, carotenoids are very susceptible to degradation. In fact, the high number of conjugated double bonds makes carotenoids prone to autooxidation and isomerization during food processing and storage.

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- **Encapsulation technique** has been intensively studied as a promising method for improving stability, to preserve the biological activity and to ensure controlled release of the active compounds. In the food sector, encapsulation is a useful tool to improve delivery of bioactive molecules (e.g. antioxidants, minerals, vitamins, carotenoids) and living cells (e.g. probiotics) into foods, keeping the bioactives fully functional. In the process of encapsulation one material or a mixture of materials (core) is coated with or entrapped within another material or system called the wall material. Many encapsulation technologies are available (spray drying, spray cooling, spray chilling, coacervation, extrusion, fluidized bed coating, molecular inclusion, and freeze drying). Also, a range of edible materials can be used as wall materials for encapsulates (polysaccharides, proteins, fats, and waxes). Accordingly, by encapsulation, bioactive compounds as core material can be stabilised using different matrices. The selection of appropriate carrier material, encapsulation technique and encapsulation conditions are critical issues, which require consideration for the process of encapsulation.
- Encapsulation efficiency, also known as loading efficiency, is a numerical measure of the amount of incorporated core material in microparticles, which is generally expressed as the percentage of core material encapsulated relative to the amount of initially added core material. Encapsulation efficiency is one of the critical properties of microencapsulation that have important influence on subsequent applications. Microparticles with high encapsulation efficiencies are often desired as less carrier materials are required, and less net amount of encapsulated products could deliver the required amount of core material.

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Representative projects:

1. Inovation Fund, TECHNOLOGY TRANSFER PROGRAM (2023-2024)- Title: Nutritionally and Functionally Enhanced Dairy Products using Fruit and Vegetable By-products (Principal Investigator).
2. Bilateral project between Serbia and Slovenia (2020-2022) – Title: Isolation and stabilization of betalains from natural sources (Principal Investigator).
3. Project funded by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Serbia (2019-2020) – Title: Hemometric modeling of functional characteristics of corn-enriched zeaxanthin foods (Principle investigator).
3. Project funded by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Serbia (2021-ongoing) – Title: New ecological phytopreparation for antifungal and antioxidant treatment of selected fruits and vegetables – EcoPhyt (Researcher).
3. Project funded by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Serbia Title: A new concept of enriching food products with Vojvodina cereal sprouts (2016-2019) (Researcher).
4. Project funded by Ministry of Education, Science and Technological Development of Republic of Serbia (2011-2019) - Title: Fruit and vegetable processing by-products – an important source of phytonutrients, 23011 (Researcher).
5. Project funded by Ministry of Education, Science and Technological Development of Republic of Serbia (2011-2019) – Title: Functional physiologically active plant materials with additional values for application in pharmaceutical and food industry, 45017 (Researcher).

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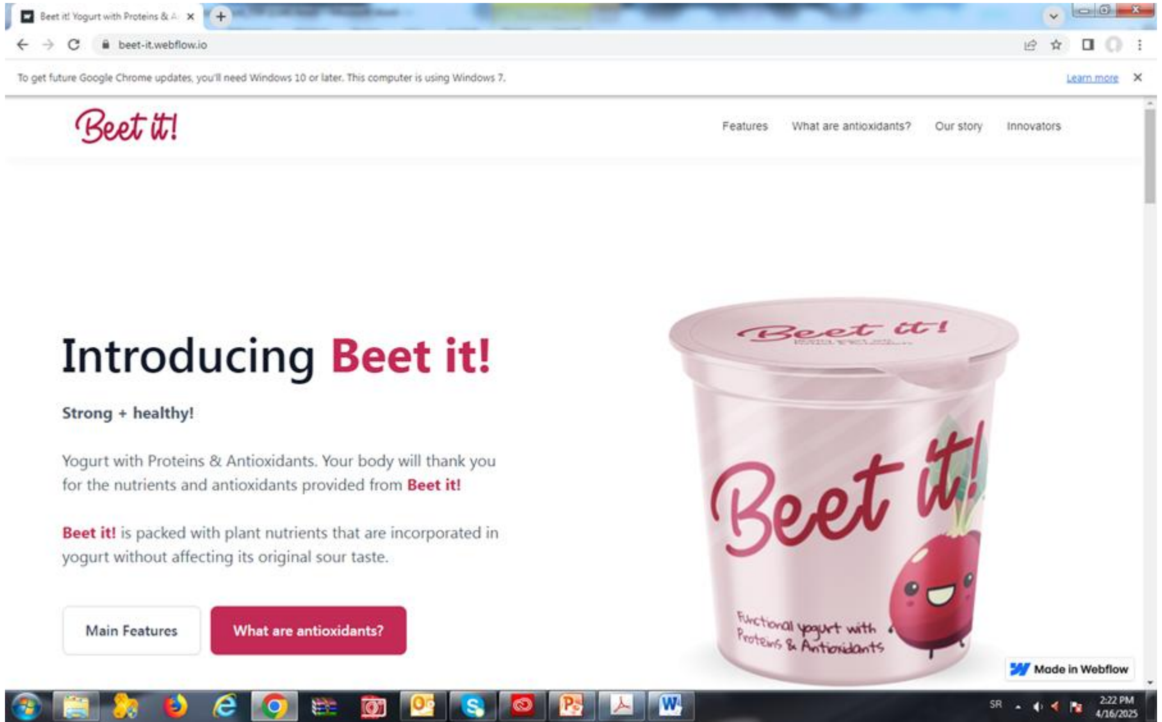
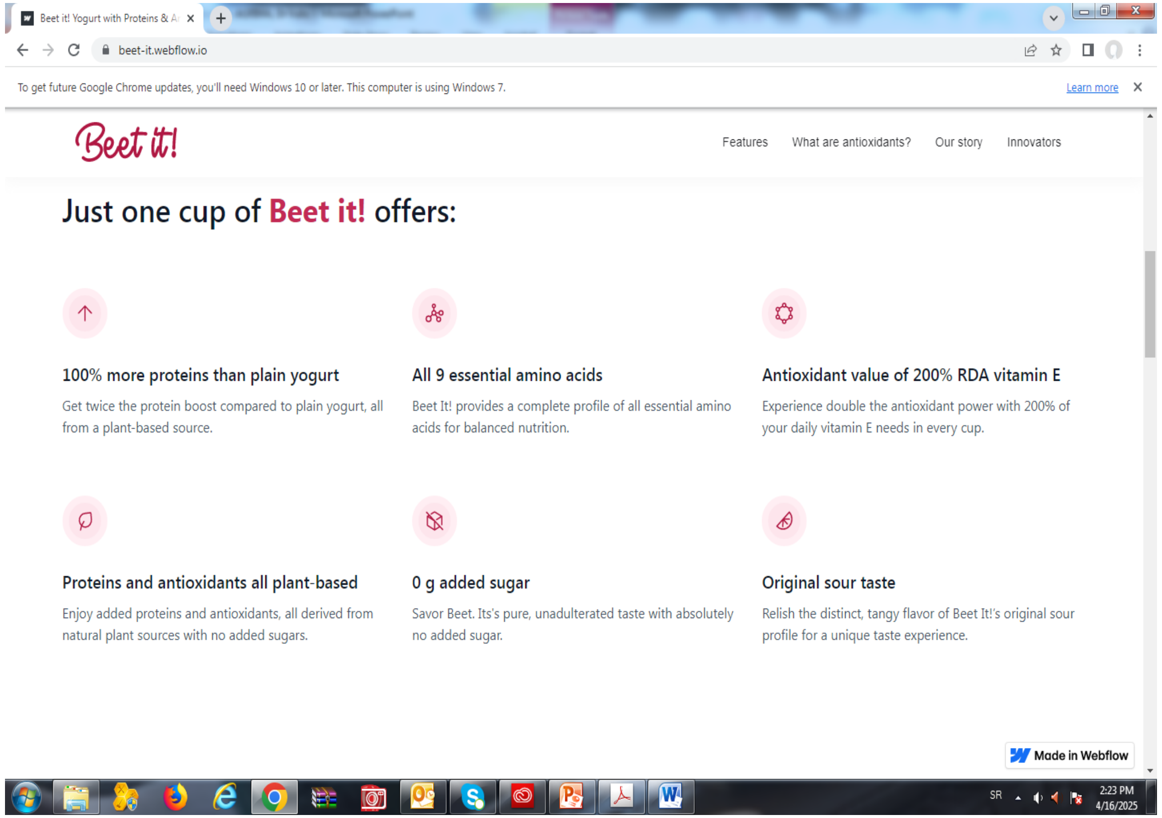
PROJECT TITLE: **NUTRITIONALLY AND FUNCTIONALLY ENHANCED DAIRY PRODUCTS USING FRUIT AND VEGETABLE BY-PRODUCTS**

Beetroot pomace extract and pumpkin pomace were mixed under the optimal experimental conditions in terms of the best encapsulation efficiency to scale-up the process and translate it to the industrial production.

Encapsulate obtained at pilot scale showed very good antioxidant, antihyperglycemic and anti-inflammatory activities, as well as bioavailability after in vitro digestion tests. Also, tested antimicrobial and physicochemical properties showed beneficial potentials for applications in food industry.

After that, dairy functional products were processed first in laboratory and after that at industrial level. Bioactivity and product quality (microbial, sensory and rheological testing) of yoghurt enriched with optimal beetroot encapsulates were tested. Also, stability, safety and bioactivity of functional dairy products during their standard shelf life were investigated. Produced yoghurt with bioactive compounds at industrial settings proved beneficial potential for application in food industry. The product prepared for commercialization is presented at the following web page **http://beet-it.webflow.io/**

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Just one cup of Beet it! offers:
100% more proteins than plain yogurt
Get twice the protein boost compared to plain yogurt, all from a plant-based source.
All 9 essential amino acids
Beet It! provides a complete profile of all essential amino acids for balanced nutrition.
Antioxidant value of 200% RDA vitamin E
Experience double the antioxidant power with 200% of your daily vitamin E needs in every cup.
Proteins and antioxidants all plant-based
Enjoy added proteins and antioxidants, all derived from natural plant sources with no added sugars.
0 g added sugar
Savor Beet. It's pure, unadulterated taste with absolutely no added sugar.
Original sour taste
Relish the distinct, tangy flavor of Beet It!'s original sour profile for a unique taste experience.
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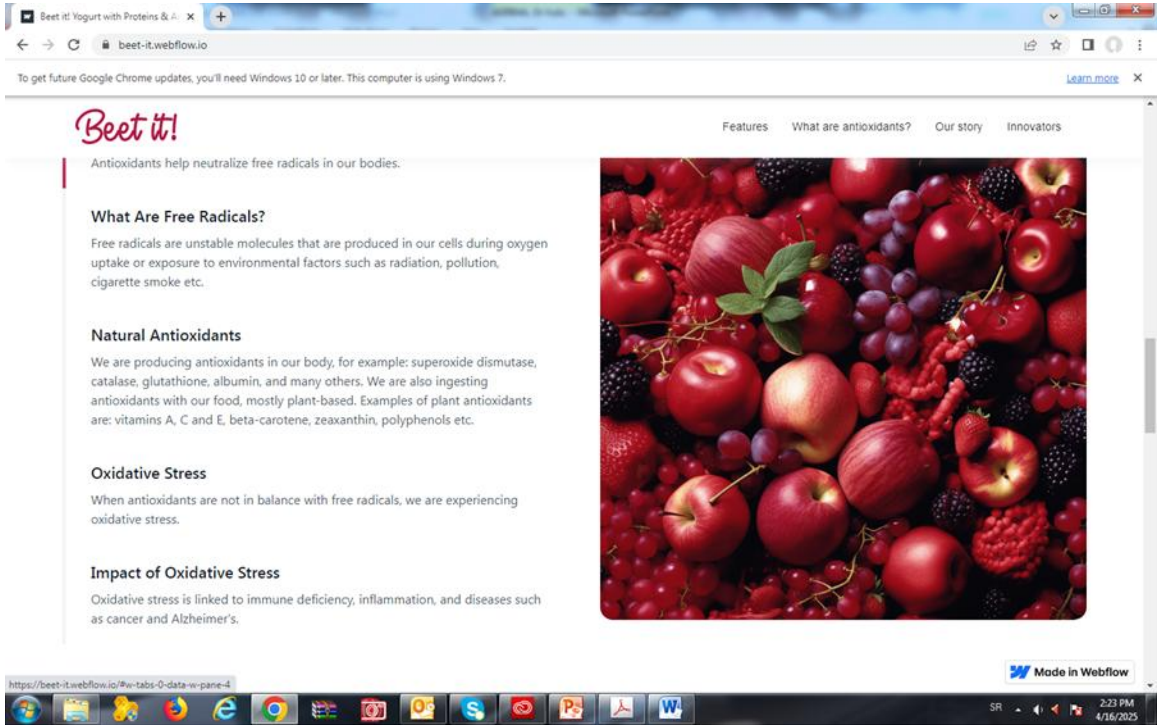
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Features
What are antioxidants?
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Innovators
Antioxidants help neutralize free radicals in our bodies.
What Are Free Radicals?
Free radicals are unstable molecules that are produced in our cells during oxygen uptake or exposure to environmental factors such as radiation, pollution, cigarette smoke etc.
Natural Antioxidants
We are producing antioxidants in our body, for example: superoxide dismutase, catalase, glutathione, albumin, and many others. We are also ingesting antioxidants with our food, mostly plant-based. Examples of plant antioxidants are: vitamins A, C and E, beta-carotene, zeaxanthin, polyphenols etc.
Oxidative Stress
When antioxidants are not in balance with free radicals, we are experiencing oxidative stress.
Impact of Oxidative Stress
Oxidative stress is linked to immune deficiency, inflammation, and diseases such as cancer and Alzheimer's.
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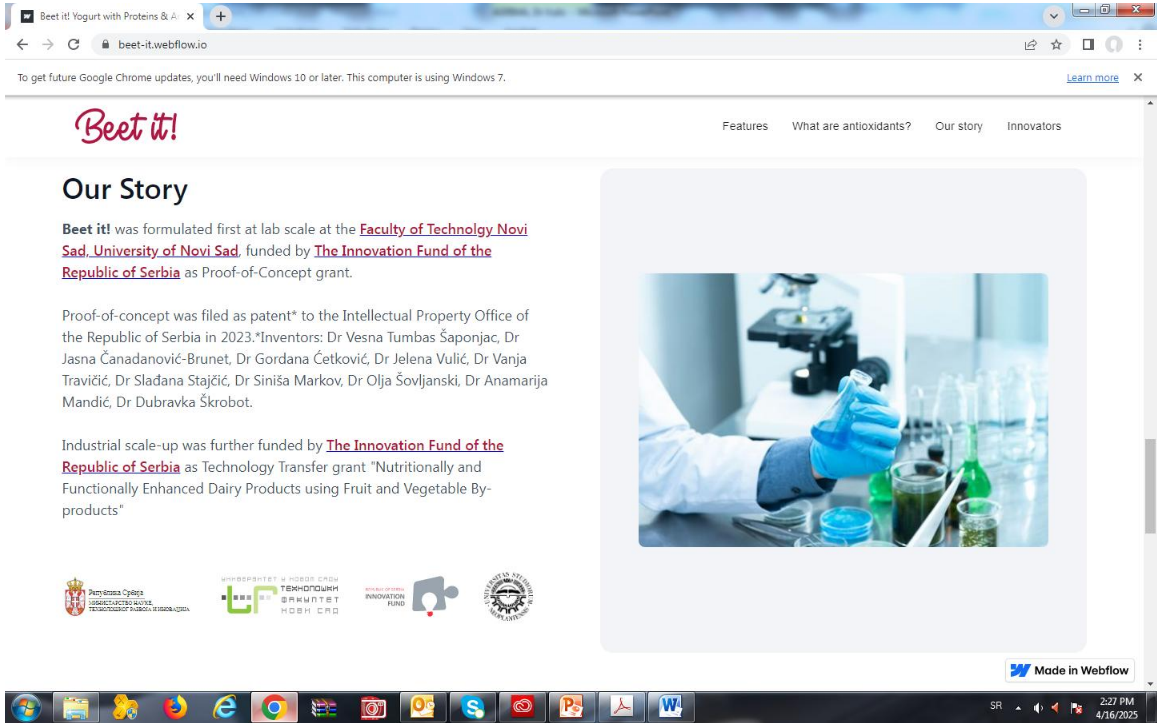
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Our Story
Beet it! was formulated first at lab scale at the Faculty of Technolgy Novi Sad, University of Novi Sad, funded by The Innovation Fund of the Republic of Serbia as Proof-of-Concept grant.
Proof-of-concept was filed as patent* to the Intellectual Property Office of the Republic of Serbia in 2023.*Inventors: Dr Vesna Tumbas Šaponjac, Dr Jasna Čanadanović-Brunet, Dr Gordana Ćetković, Dr Jelena Vulić, Dr Vanja Travičić, Dr Slađana Stajčić, Dr Siniša Markov, Dr Olja Šovljanski, Dr Anamarija Mandić, Dr Dubravka Škrobot.
Industrial scale-up was further funded by The Innovation Fund of the Republic of Serbia as Technology Transfer grant "Nutritionally and Functionally Enhanced Dairy Products using Fruit and Vegetable By-products"
INNOVATION FUND
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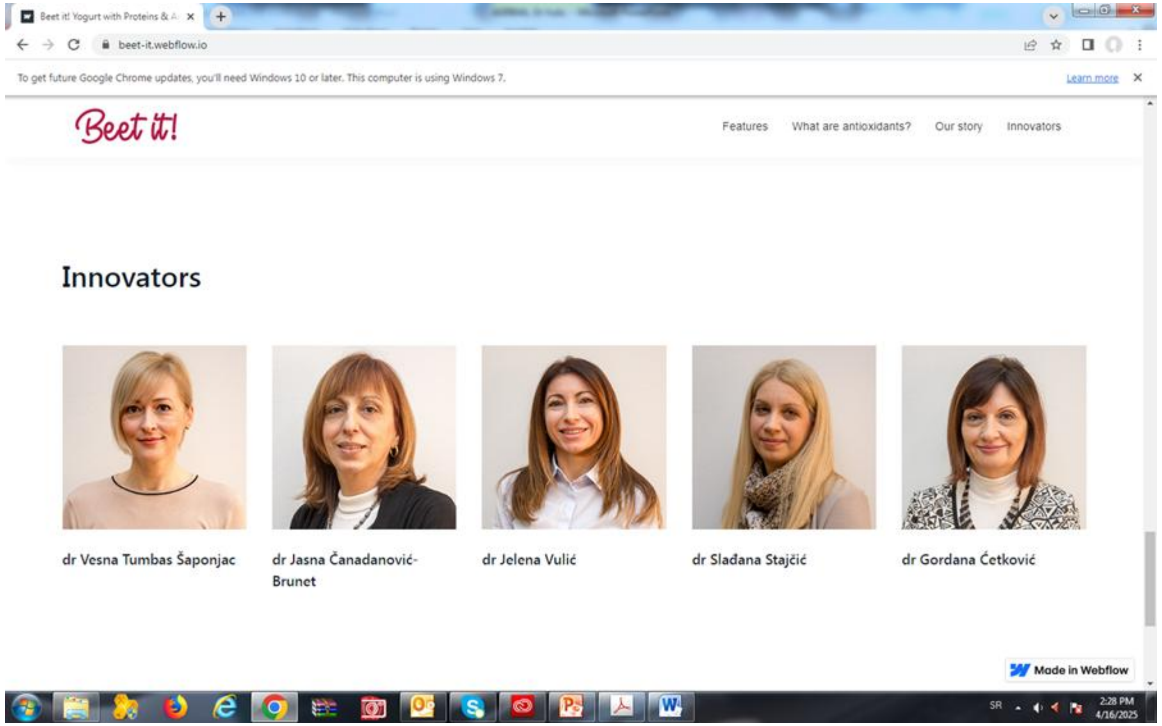
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dr Jasna Čanadanović-Brunet
dr Jelena Vulić
dr Slađana Stajčić
dr Gordana Ćetković
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PROJECT TITLE: **NEW CONCEPT OF YOGURT FORMULATION WITH PUMPKIN BY-PRODUCT EXTRACT**

The aim of project was valorisation of pumpkin by-products through their application, and creating a new value-added product - yogurt with pumpkin by-product extract. Using solvents with different polarities proved the most efficient extraction solvent for targeted compounds (polyphenols and carotenoids). In order to protect targeted compounds from degradation factors, encapsulation by freeze-drying technique was established. Encapsulation was proposed as a successful method to improve the bioaccessibility and bioavailability of the bioactive compounds. High-quality, competitive and economically acceptable functional yogurt was prepared. Stability of targeted compounds, colour parameters, and microbiological safety were evaluated as critical parameters to provide a high-quality yogurt during the storage period. We made a microbiologically, sensory and medically valuable functional product which could easily reach the market and meet consumer demands.

1. EXTRACTION OPTIMIZATION
2. ENCAPSULATION OPTIMIZATION
3. ENCAPSULATE TESTING
4. TESTING THE VIABILITY OF MAKING FUNCTIONAL YOGURT-optimal encapsulate was added to yogurt after fermentation at five different concentrations (0.5, 1.0, 2.0, 4.0 and 10.0%) in order to make the final decision on the level of yogurt supplementation the following criteria were used:

1. sensorial characteristics (appearance, smell, flavor, aroma, texture and aftertaste);
2. level of supplementation with bioactive compounds (% of RDA);
3. cost-effectiveness of yogurt supplementation.

5. FINAL PRODUCT (FUNCTIONAL YOGURT) TESTING

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PROJECT TITLE: **HEMOMETRIC MODELING OF FUNCTIONAL CHARACTERISTICS OF CORN-ENRICHED ZEAXANTHIN FOODS**

Within this project carotenoid-rich encapsulates based on sweet corn by-product extracts that was rich in carotenoid pigments, especially zeaxanthin, was obtained by the freeze-drying and spray-drying techniques, using four different wall materials (soy and pea proteins, maltodextrin and inulin). For the assessment of the stability of the obtained encapsulates, the physicochemical characteristics of all eight types were determined. The other aim of this study was to use classification and ranking tools in order to detect grouping among the investigated encapsulates and to detect the most suitable encapsulates for the assessment of conceivable applications as food additives. The most suitable wall material and encapsulation technique for the assessment of sustainability in food products was produced by freeze-drying pea protein as a wall material. In laboratory conditions, tagliatelle enriched with corn encapsulates were prepared. The preparation of tagliatelle involved the substitution of part of the flour (10%) with a functional additive. The content of total carotenoids was determined before and after cooking the tagliatelle. The highest carotenoid retention was shown by tagliatelle with freeze-dried encapsulated on pea protein. Substituting part of the flour with encapsulated zeaxanthin from corn in the production of tamales creates the possibility of improving nutritional, functional and sensory properties, as well as the possibility of further improving the quality of the raw material and the production process.

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Representative publications:

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Thank you for your attention!

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