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DIGITALNI I ZELENI RAZVOJ PRIVREDE

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Beograd, 6 - 7. novembar
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LIST KAO ALTERNATIVNI IZVOR PROTEINA - KA CIRKULARNOJ BIOEKONOMIJI
LEAF AS AN ALTERNATIVE PROTEIN SOURCE - TOWARD CIRCULAR BIOECONOMY

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Abstract: *The projection that the world's population will reach nearly 10 billion people by 2050 serve as a clear signal for the urgent need to find sustainable solutions that can provide enough food for the growing population, along with reducing environmental impact. To achieve this, a global transition towards sustainable nutrition that fulfills all nutritional requirements is essential. Currently, the primary sources of protein, essential macronutrients in our diet, are of animal origin. This presents a significant challenge for the environment, given that the livestock system is responsible for emitting significant amount of greenhouse gases (GHG). As a result, finding alternative protein sources becomes imperative goal of circular bioeconomy in order to meet the demands for this crucial macronutrient while respecting the principles of sustainable development. Leafy biomass presents a good alternative protein source with respect to both nutritive and sustainability demands.*
Key words: *Sustanability, alternative protein, leaf biomass.*

Apstrakt*: Prognoza da će do 2050. godine na planeti biti gotovo 10 milijardi ljudi predstavlja signal za hitno pronalaženje održivih rešenja koja bi omogućila dovoljno hrane za rastuću populaciju uz smanjenje ekološkog otiska. Da bi se ovo postiglo neophodna je globalna tranzicija prema održivoj ishrani koja pritom ispunjava sve nutritivne zahteve. Osnovni izvori proteina, esencijalnih makronutrijenata, u ishrani su životinjskog porekla, što predstavlja ozbiljan problem za životnu sredinu, jer je samo stočarska industrija odgovorna za značajnu emisiju gasova staklene baste. Pronalaženje*

alternativnih izvora proteina tako postaje imperativ cirkularne bioekonomije kako bi se zadovoljile potrebe za ovim makronutrijenom uz istovremeno poštovanje principa održivog razvoja. Lisna biomasa, predstavlja dobar alternativni izvor proteina i sa nutritivnog i održivog aspekta.
Ključne reči: *Održivi razvoj, alternativni protein, lisna biomasa.*

1. INTRODUCTION

In the 2015. United Nations Member States adopted the plan to attain sustainable development: the 2030 Agenda for Sustainable Development (United Nations, 2015). The Agenda comprises 17 Sustainable Development Goals (SDG) that include all dimensions of sustainable development. Unfortunately, according to António Guterres, Secretary-General of the United Nations, people and the planet need an urgent rescue plan since the Special edition of The Sustainable Development Goals (SDG) Report reveals that halfway to the deadline of the 2030 Agenda, SDGs are in a challenging predicament (UN DESA, 2023). Namely, among 140 targets, progress on more than half is poor, while even 30% of targets had no progress or regressed (UN DESA, 2023). The red flag for humanity should be the latest estimate by the Food and Agriculture Organization of the United Nations (FAO), which reveals that food insecurity rose from 25.3% in 2019 to a worrying 29.6% in 2022 (FAO et al., 2023). In order to produce enough food for a continuously growing world population and simultaneously meet the SDGs it is necessary to make the transition towards a circular bioeconomy (Aiking & de Boer, 2020). However, data from the Circularity Gap Report 2020 show that only 8.6% of the economy is circular, meaning that 92.0 Gt of minerals, fossil fuels, metals and biomass are not being reused (de Wit et al., 2020).

Bioeconomy utilizes knowledge of biotechnology to achieve different goals of sustainable development, with sustainable food systems being the predominant niche (International Advisory Council of GBS2020, 2020). Alternative proteins are one of the main interests of bioeconomy since protein supply directly affects several SDGs (2,3,6,13,14,15). The impact on SDG2 (Zero hunger), particularly target 2.2: “end all forms of malnutrition” is the most obvious, because the proteins present essential macronutrients (Chen et al., 2022). Meat production is responsible for 15% of GHG emissions and consequently obstructs the progress of SDG13 (Climate action) (de Moraes et al., 2023; Deprá et al., 2022). Additionally, it is associated with other environmental and sustainability challenges globally: land-use change and consequently loss of biodiversity and freshwater consumption, therefore affecting progress toward SDG 6 (Clean water and sanitation), SDG14 (Life below water), and SDG15 (Life on land). Overconsumption of meat is also associated with several severe health issues, making an impact on SDG3 (Good Health and Well-Being) (de Moraes et al., 2023; Ekmekcioglu et al., 2018).

Soybean presents the primarily utilized meat and milk substitute crop, but use of soy raises other enviornmental issues such as land use change and water pollution (Deroni et al., 2022). Hence, these concerns encouraged the search for alternative and more sustainable protein sources and opened a new perspective market for companies and investors. Analysts predict different scenarios for the growth of the alternative protein market ranging from $555 billion to $1,1 trillion in value by 2050 (The FAIRR Initiative, 2022).

Serbian diet ranks at the top of food production-related environmental footprint per capita (>11 kg CO2 eq. per capita), along with Uruguay, Montenegro, New Zeland, and Australia, probably due to consumption of ruminant meat (Chen et al., 2022). This alarming data shows the necessity to transform our dietary habits from the viewpoint of both sustanability and well-being and obey the global trend of alternative protein search.

2. LEAF PROTEINS

Even though the leaves have been recognized as potentially good protein source, their application has still been scarce due to several technological challenges. The green leaf proteins were first isolated in the 18th century but real interest in their application in the human diet stems from Pirie (Pirie N.W., 1942) who saw them as a possible solution for the food shortage in Great Britain during World War II. Similarly, nowadays interest in these proteins is imposed by the need for a sustainable and nutritionally balanced diet for the ever-expanding population. The root of the problem concerning the exploitation of the green leaf proteins is in their concentration on the fresh weight basis, which varies from 1.2 to 8.6% depending on the species and cultivation settings (Balfany et al., 2023). Translated to the dry matter protein content some of the leaves are comparable to the soybean, which is the golden standard in plant-based proteins. However, the moisture content of the leaves is in the range of 77 to 94%, making their large-scale processing difficult due to the large volumes and heavyweight when compared to the quantity of wanted compounds (Tamayo Tenorio et al., 2018; Tamayo Tenorio et al., 2017). This corresponds to low efficiency compared to the protein crops (50-60% yield), even though a protein-rich product is obtained at the end as presented in Table 1 (Tamayo Tenorio et al., 2018). The methods applied for the extraction of proteins from traditional protein crops can not be translated to leafy biomass. The reason is the nature of the proteins and their location in the plant cells. Seeds and protein crops contain storage proteins, while leaf proteins consist of structural and enzymatic proteins (Tamayo Tenorio et al., 2018).

Table 1. Protein content, yield and plant source of leaf protein concentrate (LPC)

Plant source	LPC yield, %	Protein content of LPC, %	Reference
Cauliflower leaf	6.83 ± 0.24	53.33 ± 0.80	(Sedlar et al., 2021)
Cauliflower by-products	53.07	77.6	(Xu et al., 2017)

Broccoli leaf	4.38 ± 0.17	42.22 ± 0.66	(Sedlar et al., 2021)
Cabbage leaf	14.21 ± 0.07	48.17 ± 1.21	(Sedlar et al., 2021)
Beetroot leaf	6.17 ± 0.24	39.76 ± 0.65	(Sedlar et al., 2021)
Dried alfalfa leaves	36	60	(Hojilla-Evangelista et al., 2017)
Moringa Oleifera leaves	14.2	55.7	(Benhammouche et al., 2021)
Amaranth leaf	17.11	96.63	(Famuwagun et al., 2020)
Eggplant leaf	18.26	96.85	(Famuwagun et al., 2020)
Fluted pumpkin leaf	15.85	95.81	(Famuwagun et al., 2020)
Sugar beet leaves	6	90	(Tamayo Tenorio et al., 2016)

So why further consider leaf proteins? The answer lies in their nutritional quality and circularity. Namely, proteins can be extracted from the leaves that are crop by-products and remain on the soil after the crop harvest. In the trend of making agroindustry more sustainable, the usage of these agro-industrial wastes as the source of value-added products is in accordance with the bioeconomy concepts (Contreras et al., 2019). However, the removal of these by-products can affect the soil quality in two ways. Firstly, it can be beneficial from the viewpoint of avoiding specific nutrient excess, which can further pollute ground waters. On the other hand, carbon and other nutrients provided by the leftover biomass need to be compensated from other sources. One of the ways to overcome this problem is the decentralised scenario, where the beginning of leaf processing occurs on the farms (Tamayo Tenorio et al., 2017b). This is beneficial from several viewpoints, namely as mentioned, leaves have high moisture content and therefore their processing needs quick action due to their perishability. Additionally, the extraction of leaf proteins from invasive plants could also present a sustainable solution (Lyer et al., 2021).

3. EXTRACTION OF LEAF PROTEINS

Leaf proteins exhibit considerable diversity in terms of their charge, hydrophobicity and interactions with other leaf components, and they are usually classified with respect to their solubility into two major fractions: soluble and insoluble. Insoluble fraction is also known as the green fraction due to its association with leaf pigments and it mostly consists of membrane proteins located in the thylakoid membranes of the chloroplasts (Tamayo Tenorio et al., 2018). Besides the colour, this fraction is associated with a grassy odour and taste. On the other hand soluble fractions, also known as white proteins, are located in the cytoplasm and comprise mostly of the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo)(Anoop et al., 2023, Chiesa et al., 2011). RuBisCo has one of the major roles in CO_2 assimilation; however, RuBisCo has a very low turnover rate and specificity towards CO_2. As a consequence of its low efficiency, RuBisCo amounts to 50% of the soluble leaf proteins, and for this reason back in 1979. Ellis proclaimed it as the most abundant protein in the world (Ellis, R. J. 1979).

Leaf biomass has a high content of insoluble carbohydrates, which play a crucial role in maintaining cell wall integrity (Tamayo Tenorio et al., 2018). Therefore, protein extraction from leafy biomass starts with tissue disruption in order to release proteins in plant juice. Usually, this is achieved using mechanical pressing, where two fractions are obtained, protein-rich green juice and fibrous pulp. The efficiency of this process directly affects the final LPC yields, as it determines the protein content in the juice (Bals et al., 2012). During disruption of leaves with low-fiber content percentage of intact cells can reach 10%. On the other hand, carbohydrate fraction of the leaf has high water holding capacity and can hamper extraction by retaining proteins in the pulp. In order to avoid these protein losses juicing process should be quick (Tamayo Tenorio et al., 2016). Beside mechanical pressing, other technologies for membrane disruption are gaining more interest in odred to achieve better protein yields, including ultrasound-assisted extraction, pulse electric field-assisted extraction and enzyme-assisted extraction (Balfany et al., 2023; Vernès et al., 2019).

Further processing includes precipitation of unwanted components, namely green proteins along with cell debris, parts of chloroplast and other, while retaining RuBisCo and other soluble proteins in the solute. This is often done using thermal coagulation, since green proteins aggregate at lower temperatures than the white fraction (Tamayo Tenorio et al., 2016). RuBisCo can be further purified by pH precipitation.

In order to make this process more lucrative, better exploitation of side streams, which are still rich in proteins, is necessary. Application of subsequent enzyme assisted-extraction of proteins from fibrous pulp can affect overall protein yield, and fibrous pulp can be further used for biofuel production or as supplement on the fields from where the leaves were collected (Tamayo Tenorio et al., 2017b).

4. NUTRITIONAL VALUE OF LEAF PROTEINS

Depending on the type of proteins extracted from the leaf, LPC can contain only white or green protein fractions or both. Usually, if intended for human consumption LPC containing white protein fraction is the target and the extraction process is optimised towards a high yield of these proteins. The reason is LPC green colour and unpleasant taste when green proteins are extracted along with white fraction (Tamayo Tenorio et al., 2016). From the nutritional viewpoint, LPC has higher amounts of threonine and lower content of isoleucine, methionine and lysine compared to animal proteins. Still, some plants such as spinach, broccoli and duckweed have lysine and methionine ratios similar to those of animal-based proteins and in accordance with FAO standards (Anoop et al., 2023). Essential amino acids account for 57% of RuBisCo of its total amino acids, while this number is a bit lower for LPC (Anoop et al., 2023). When comparing white and green fractions, the superiority of white proteins is obvious in all amino acid concentrations except isoleucine, however, both fractions have concentrations above recommended. White proteins exhibit a better amino acid profile when compared to other common plant proteins like soybean seed protein, particularly in terms of sulphur amino acids (methionine and cysteine), which are typically lacking in plant-based proteins (Chiesa et al., 2011).

In terms of digestibility, expressed as protein digestibility corrected amino acid score (PDCAAS), leaf proteins rank lower than animal-based proteins. The reason is a low concentration of at least one essential amino acid along with the presence of antinutritional factors. However, processing of leaf proteins increases the PDCAAS score, with the white fraction having the highest one, even higher than soy proteins. In addition, in terms of digestibility, white proteins are superior to green ones with the note that extraction technique could seriously affect this feature (Balfany et al., 2023; Chiesa et al., 2011). From the viewpoint of nutritional value, white proteins are superior to green ones and therefore their selective extraction is justified. However, recent attempts were made to further exploit side streams of LPC production, trying to utilise green fractions using different extraction methods, which could alter their nutritive and functional performance (Tamayo Tenorio et al., 2017a).

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