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RESEARCH ON THE ACHIEVEMENT OF A FUNCTIONAL INGREDIENT FROM CAULIFLOWER LEAVES BY LYOPHILIZATION

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Abstract: The European Commission has established food waste as one of the priority areas of the Action Plan on the European Strategy for the Circular Economy. Of all the biomass generated by cauliflower and broccoli crops, only 30% is used for food (the inflorescence); the rest of the parts, including leaves, stems, are considered as by–products (waste) which are mainly used for composting or incorporated into the soil. This paper presents the results of the research undertaken to obtain a functional ingredient (powder) by lyophilization of cauliflower leaves, resulting from the culture of this vegetable species. The functional ingredient was analyzed from a sensory, biochemical and microbiological point of view. This functional ingredient comes in the form of a green powder with a characteristic taste and smell. The biochemical analysis revealed that the powder obtained from the processing of cauliflower leaves is notable for its content in glucosinolates, total polyphenols, vitamin C, α–tocopherol, total carotenoids, β–Carotene, lutein, chlorophyll a, chlorophyll b. Due to the high content in bioactive compounds, the functional ingredient obtained from cauliflower leaves, has antioxidant capacity (21.94 µmol TE/g; 24.87 µmol TE/g).
Keywords: cauliflower, leaves, functional ingredient, lyophilization, bioactive compounds

INTRODUCTION

The consumption of green leafy vegetables represents only 37% of the recommended daily dose. Agricultural waste not only impede food security but also pollutes the environment (*Gebrechristos & Chen, 2018*).

The vegetable processing industry produces over one million tons of vegetable by–products every year, which are waste, but which constitute a potential source of bioactive ingredients (*Galali et al., 2020; Rafiuddin et al., 2019, 2021*). Studies undertaken internationally have shown that there is a huge potential for the use of by–products from plant sources for the development of food products (*Amoah et al., 2023; San José et al., 2018; Tamasi et al., 2019*). Cauliflower by–products have a high potential for valorization due to their complex biochemical composition. It is worth noting that cauliflower waste (leaves, stems) represents about 60% of the total weight of this vegetable (*Ribeiro et al., 2015*).

The European Commission has established food waste as one of the priority areas of the Action Plan on the European Strategy for the Circular Economy (*European Commission, Directorate–General for Research and Innovation, 2018*). This Plan includes a "zero waste" strategy (targeting agro–food waste) to reduce environmental pollution. This strategy is based on the superior recovery of food waste, through:

- obtaining functional ingredients with nutritional value and antioxidant potential, which can be used in the fortification of food products;
- extraction and concentration of bioactive compounds from food waste with a complex biochemical composition and their use in innovative and economically profitable applications in the food, cosmetic and pharmaceutical industries (*Saini et al., 2019; Jiménez–Moreno et al., 2019*).

International studies have shown that cauliflower by–products are an exceptional source of protein, vitamins, minerals, ascorbic acid, carotenoids, antioxidants and dietary fiber (*Montone et al., 2018; Munir et al., 2018; Stojceska et al., 2008*).

Cauliflower leaves have bioactive peptides found within native proteins that require to be released by enzymatic hydrolysis and bacterial fermentation during digestion. These bioactive peptides can improve the viability of human vascular endothelial cells by triggering the inhibition of intracellular xanthine oxidase activity and modulation of superoxide dismutase. Thus, cauliflower by–products can be additional sources of protein (*Caliceti et al., 2019*).

Cauliflower leaves which are a rich source of dietary fiber, crude protein, calcium, iron, carotene, lysine, tryptophan and natural antioxidants including phenols, and therefore can be used in different value added products (*Revathi et al., 2019*). These authors used the powder obtained from the dehydration of cauliflower leaves at a temperature of 60–70°C, for the fortification of cookies (fortification levels of 5% and 10%). The sensory evaluation (appearance, taste, texture and aroma) of the fortified cookies, on a 9–point hedonic evaluation scale, revealed that the optimal level of fortification is 5%.

Furthermore, cauliflower and its by–products contain glucosinolates in very high concentrations up to 75,000 µg/g (*Sanlier & Guler, 2018*).

This paper presents the results of the research undertaken to obtain a functional ingredient (powder) by lyophilization of cauliflower leaves. The functional ingredient was analyzed from a sensory, biochemical and microbiological point of view.

MATERIALS AND METHODS

Waste (leaves) resulting from the conventional cauliflower culture, from farmers in Ialomița county, were used in our experiments. Pretreated cauliflower leaves (sorting, washing, boiling in water at 98–100°C for 2–3 minutes, cutting) were subjected to lyophilization process in a freeze dryer (Heto Power Dry PL 3000, from Thermo Electron Corporation) at – 55°C to a moisture which allows their milling and conversion into flours and, in the same time, gives their stability in terms of quality.

Figure 1 – Pretreated and freeze–dried cauliflower leaves

Milling of dried semi–finished products was performed by using Retsch mill (Knife Mill GRINDOMIX GM 200). Functional ingredient (powder) obtained from cauliflower leaves was packed in hermetically sealed glass containers, protected with aluminum foil and kept in dry and cool spaces (at a maximum temperature of 20°C), until the sensory, biochemical and microbiological analyzes were performed.

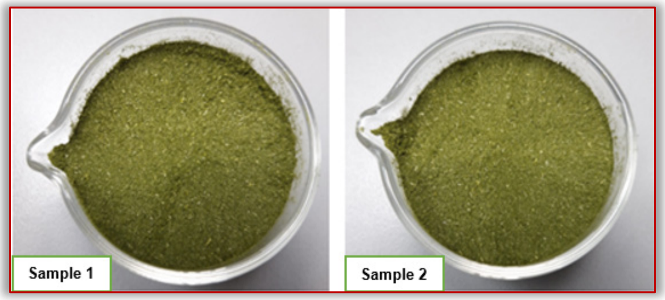

Figure 2 – "Functional ingredient from cauliflower leaves (powder)"

For the qualitative characterisation of the Functional ingredient from cauliflower leaves (powder) were used standardized methods and developed and validated methods in IBA Bucharest.

Measurement of the color parameters of samples was performed at room temperature, using a CM–5 colorimeter (*Konica Minolta, Japan*), equipped with SpectraMagic NX software, to register CIELab parameters (L*, a*, b*). The moisture content was determined according to the AACC 44–15A method. Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005).

Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005). Total dietary fibre (TDF) was determined by enzymatic method using the assay kits: KTDFR ''Total dietary fibre'' (AOAC Method 991.43).

Determination of iron, copper, manganese and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP–MS, model NexION300Q, Perkin Elmer) after dry digestion of the samples. The determination of sodium, potassium, calcium and magnesium was carried out by High–Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 – High–Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

Determination of the vitamin C was performed by high performance liquid chromatography coupled with mass spectrometry (*Catană et al., 2017*). Determination of vitamin E (a–tocopherol) content was performed by high–performance liquid chromatography (HPLC–DAD) (*Popović et al., 2015*). Determination of β–carotene and all–trans lutein content was performed by high–performance liquid chromatography (HPLC–DAD) (*Catană et al., 2020*). Total phenolic content was determined by Folin–Ciocalteu procedure, and antioxidant capacity by DPPH method (Horszwald and Andlauer, 2011 with some modifications (concerning extraction media, time and mode of extraction, extract volumes of the used sample and reagents, using UV–VIS Jasco V 550 spectrophotometer). Determination of glycosinolate content was carried out using a rapid spectrophotometric method (*Mawlong et. al., 2017*). Determination of total carotenoids and chlorophyll a and b was carried out spectrophotometrically (*Chinnadurai et al., 2017*).

The water activity (Aw) was determined by the method ISO 21807:2004. Yeasts and molds were determined by the method SR ISO 21527–1:2009 and SR ISO 21527–2:2009. *Enterobacteriaceae* were determined according to the SR EN ISO 21528–2:2017 method and *Escherichia coli* by SR ISO 16649–2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579–1:2017.

RESULTS

Following the sensory analysis, it was found that the functional ingredient from cauliflower leaves is in the form of a powder with a characteristic taste and smell and dark green colour (Figure 2). Following the instrumental color value, it was found that these powders recorded negative values of the color parameter a* and positive values of the luminance L* and the color parameter b* (Figure 3).

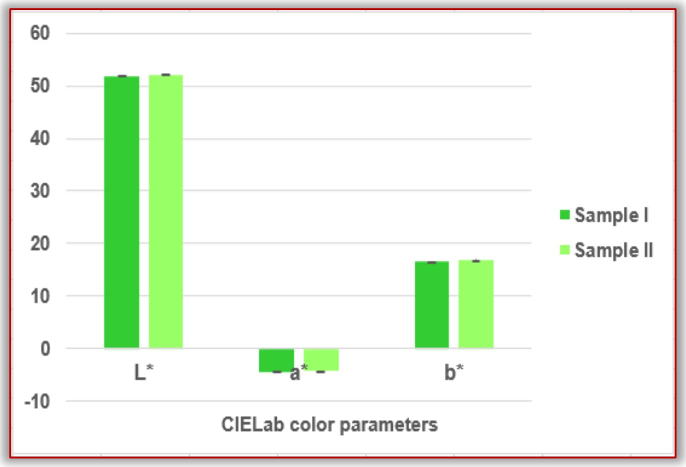

Figure 3 – Colour parameters of the "Functional ingredient from cauliflower leaves (powder)"

Physico–chemical analysis revealed that the functional ingredient of cauliflower leaves (powder) stands out for its content in protein, ash, total sugar and total fiber (Table 1). It is worth noting that Sample 2 recorded higher values of the determined physico–chemical indicators compared to Sample 1.

Table 1. The physico–chemical indicators of the"Functional ingredient from cauliflower leaves (powder)"

Physico–chemical indicators	"Functional ingredient from cauliflower leaves (powder)"	
	Sample 1	Sample 2
Moisture (%)	5.23±0.13	5.11±0.13
Ash (%)	10.64±0.16	10.95±0.16
Protein (%)	29.40±0.44	29.86±0.45
Fat (%)	4.38±0.06	4.70±0.06
Carbohydrates (%)	50.35±0.33	49.38±0.32
Total sugar (%)	18.30±0.12	19.16±0.12
Total fibre (%)	31.20±0.58	29.83±0.55
Energy value (kcal/100g)	296	300
Energy value (kJ/100g)	1237	1253

The "Functional ingredient from cauliflower leaves (powder)" has a higher ash, protein, fat content compared to that reported by Tukassar et al. (2023) in the case of cauliflower by–products powder (Protein = 22.80%; Ash = 5.81%; Fat = 2.95%).

The "Functional ingredient from cauliflower leaves (powder)" stands out for its content in mineral elements (Figure 4 and 5). Among the determined mineral elements, in the case of the "Functional ingredient from cauliflower leaves (powder)", potassium it recorded the highest content (Figure 4). Potassium is the most abundant cation in the human body, approximately 2% being located in the extracellular fluid (3.5–5.0 mEq/L) and 98% being in the intracellular area (140 mEq/L) (*Kovesdy et al., 2017; Kovesdy et al., 2017*). Potassium plays a crucial role in normal cell membrane electrophysiology in neurons, muscle, and cardiac cells (*Kovesdy et al., 2017*).

At the same time, according to our results, the "Functional ingredient from cauliflower leaves (powder)", can be considered an important source of calcium and magnesium. Calcium and magnesium are two mineral elements with an important role in the human body. Calcium and magnesium affect muscle mass and function and are important for optimal vitamin D status (*Hibler et al. (2020*). Insufficient dietary intake of nutrients such as calcium and magnesium can be associated with increased risk of cancer, cardiovascular diseases, metabolic diseases and mortality (*Schwingshack et al, 2015; Asemi et al., 2015*).

The content in Cu, Zn and Mn of the "Functional ingredient from cauliflower leaves (powder)", recorded lower values, compared to other determined mineral elements, being in the range of 1.42–6.20 mg/100g.

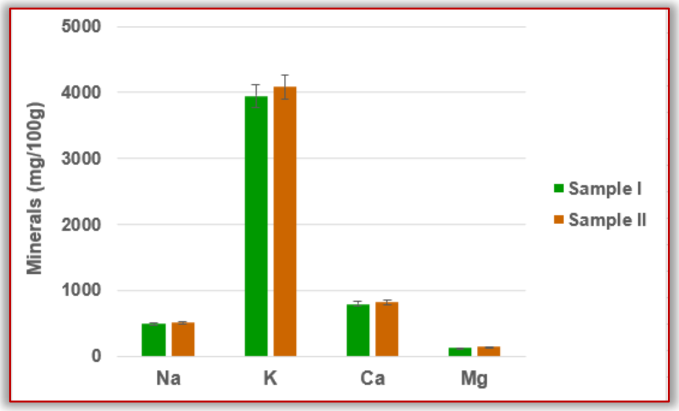

Figure 4 – Mineral content (Na, K, Ca, and Mg) of the "Functional ingredient from cauliflower leaves (powder)"

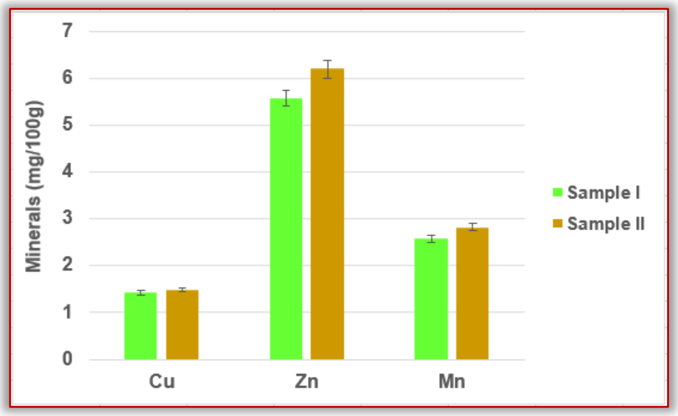

Figure 5 – Mineral content (Cu, Zn and Mn) of the "Functional ingredient from cauliflower leaves (powder)"

The "Functional ingredient from cauliflower leaves (powder)" stands out for its Fe content: Sample 1 = 41.65 mg/100g; Sample 2 = 48.52 mg/100g. Iron is an important bioelement and an essential dietary mineral for most life forms. In humans, iron is present in the body in various tissues and cells and plays an essential role in oxygen transport. Iron also plays a role in cell signaling, gene expression, and regulation of cell growth and differentiation.

Normally, total body iron remains within a relatively narrow range of normal values, about 5 g in adults, with about 65%–70% present in red blood cells as hemoglobin. Iron homeostasis is highly regulated through tightly controlled iron absorption. Iron deficiency is the most common micronutrient deficiency worldwide, accounting for about one–half of all cases with nutrient deficiency, and has a high incidence in developing countries driven by poor nutrition and parasitic infections (*Serai et al., 2020*).

The "Functional ingredient from cauliflower leaves (powder)" has the content in Mg, Fe, Zn and Cu, higher compared to that reported by Tukassar et al. (2023) in the case of cauliflower by–products powder (Mg = 36.3 mg/100g; Fe = 20.03 mg/100g; Zn = 1.46 mg/100g; Cu = 0.83 mg/100g).

It is worth noting that The "Functional ingredient from cauliflower leaves (powder)" is an important source of bioactive compounds: total polyphenols, glucosinolates, vitamin C, β–Carotene, lutein, chlorophyll a, chlorophyll b (Table 2).

Table 2. Bioactive compounds content of the"Functional ingredient from cauliflower leaves (powder)"

Bioactive compounds	"Functional ingredient from cauliflower leaves (powder)"	
	Sample 1	Sample 2
Total polyphenols (mg GAE/g d.m.)	8.97±0.22	9.95±0.25
Glucosinolates (mmol/g d.m.)	338.72±8.13	383.22±9.20
Vitamin C (mg/100g)	164.48±4.93	195.76±5.87
α–Tocopherol (mg/100g d.m.)	4.36±0.12	5.11±0.14
β– Carotene (mg/100g)	47.73±1.10	53.54±1.23
Lutein (mg/100g)	17.82±0.41	21.18±0.49
Chlorophyll a (mg/g)	1.98±0.02	2.19±0.02
Chlorophyll b (mg/g)	3.68±0.04	3.82±0.04

The "Functional ingredient from cauliflower leaves (powder)" obtained by lyophilization at –55°C has a higher content of bioactive compounds, compared to that obtained by dehydration with hot air, at a temperature of 50°C, as follows (Catană et al, 2023):
- 2.15 times, respectively, 2.44 times higher, in the case of vitamin C
- 1.88 times, respectively, 2.01 times higher, in the case of vitamin E
- 1.21 times, respectively, 1.26 times higher in the case of β–carotene
- 1.53 times, respectively, 1.60 times higher in the case of lutein
- 1.56 times, respectively, 1.68 times higher in the case of total polyphenols
- 1.41 times, respectively, 1.52 times higher in the case of glucosinolates.

At the same time, the total polyphenol content of this functional ingredient is 1.67–1.85 times higher, compared to that reported by Tukassar et al. (2023) in the case of cauliflower by–products powder (537.40 mg GAE/100g)

Due to the high content of antioxidants (β–carotene, vitamin C, vitamin E, total polyphenols, etc.) the "Functional ingredient from cauliflower leaves (powder)", shows antioxidant capacity: Sample 1 = 21.94 μmol TE/g; Sample 2 = 24.87 μmol TE/g. This functional ingredient obtained by lyophilization has an antioxidant capacity of 1,64–1,74 times higher compared to that of the ingredient obtained by dehydration with hot air, at 50°C (*Catană et al, 2023*).

Following the microbiological analysis, it was found that the "Functional ingredient from cauliflower leaves (powder)" falls within the provisions of the legislation in force and presents low water activity values (Table 3).

Table 3. Microbiological analysis of the"Functional ingredient from cauliflower leaves (powder)"

Microbiological indicators	"Functional ingredient from cauliflower leaves (powder)"	
	Sample 1	Sample 2
Yeast and mold (CFU/g)	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10
Salmonella (in 25 g)	Absent	Absent
Water activity	0.157	0.146

CONCLUSIONS

The "Functional ingredient from cauliflower leaves (powder)" obtained by lyophilization has high nutritional value and antioxidant potential. Thus, this functional ingredient stands out for its protein content, mineral elements (Na, K, Ca, Mg, Zn and Fe) and bioactive compounds (total polyphenols, glucosinolates, vitamin C, α–Tocopherol, β–Carotene, lutein, chlorophyll a, chlorophyll b).

The "Functional ingredient from cauliflower leaves (powder)" is a source of protein (min. 29%), total fiber (min. 28%), K (3500 mg/100g), Fe (min. 41 mg/100g), Ca (min. 750 mg/100g), Mg (min. 110 mg/100g). At the same time, this functional ingredient stands out for its total content of polyphenols (min. 8.5 mg GAE/g d.m.), glucosinolates (min. 300 mmol/g d.m.), vitamin C (min. 150 mg/100g), α–Tocopherol (min 4 mg/100g), β–Carotene (min. 40 mg/100g), lutein (min. 16.5 mg/100g), chlorophyll a (min. 1.4 mg/100g) and chlorophyll b (min. 3 mg/g) .

Due to the high antioxidant content, the "Functional ingredient from cauliflower leaves (powder)" has antioxidant capacity (min. 21.2 µmol TE/g).

Due to nutritional qualities and antioxidant capacity, the "Functional ingredient from cauliflower leaves (powder)" can be used for fortification of conventional and gluten–free bakery and pastry products.

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