

36 OTDK Biology section

**BIOLOGICAL VALUES OF PROCESSED GREEN BIOMASS FROM
DIFFERENT VEGETABLE SPECIES**

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2022

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ABSTRACT

One of the aspects of sustainable agriculture is related to green biomass recycling. During fresh vegetable production, significant amount of green biomass waste is generated which may contain useful chemicals, health-beneficial components and could be utilized as a raw material for food production. The demand for protein ingredients has surged over the last few years due to increasing population growth. Green biomass crops have opened doors for alternative and sustainable way to meet global protein demand for consumption. The aim of present work is to increase the value of the neglected green biomass fraction of vegetable crops comparing two different processing methods. After wet fractionation green biomass of vegetable crops were processed using two different technological methods including microwave coagulation and lactic acid fermentation. As part of the technology development, microbial strains directing lacto-fermentation are compared by plant species using molecular biology tools. Also, quantitative and qualitative evaluation of the sub-products will be performed with special interest on protein content by Kjeldhal methods, amino acid composition by UPLC pre-column derivatization and protein expression pattern by SDS-PAGE. The involved plant species are broccoli, horse radish, brussels sprout and wheat sprouts. Our results showed that leaf protein concentrate (LPC) from wheat sprout had the highest protein content (up to 55 m/m%), but it was proportionally the lowest among the species. By contrast the highest LPC ratio was obtained in case of horse radish with highest water soluble solid material content. Solubilizable protein expression pattern of green biomass originated sub-products such as green juice, brown juice and leaf protein concentrate (LPC) showed clear differences in context to processing. Both microwave coagulation and lactic acid fermentation is suitable to concentrate proteins of wet-fractionated green biomass obtaining a protein enriched fractions with valuable essential amino acids. Along with it, brown juice (which represents a large proportion of the processed biomass) also contains low levels of soluble protein amino acids. From the aspects of preservation, lactic acid coagulation is more advantageous because ~pH 4.5 brown juice does not require further processing.

Key words: sustainability, biomass recycling, circular economy, leaf protein concentrate, brown juice, fermentation, lactic acid bacteria

INTRODUCTION

One of the aspects of sustainable agriculture is related to green biomass recycling. During fresh vegetable production, a significant amount of green biomass waste is generated, which may contain useful chemicals, health-beneficial components and could be utilized as a raw material for food production. Moreover, the waste generated by green biomass will present a strong damage to the environmental health.

Circular Economy (CE) is defined by a system or a model in which, consumption and production are maximized by reutilization or recycling of any material embedded in product. The practice of sustainable agriculture enable us to produce healthy foods and gives us an opportunity to teach to the succeeding generations a direction to follow, without compromising their future. Therefore, finding the right balance between the need for sustenance and the preservation of environmental ecosystems becomes the right key.

Food and Agricultural Organization (FAO) reported the issue of food waste as of high importance in the efforts to meet the sustainable development goals such as zero hunger, economic growth, responsible consumption and production (FAO, 2011).

Qualitative losses of biomass during the primary production, distribution, processing, and consumption are high in developing as well as in developed countries, even though differing in some points of handling chain (Sagar et al., 2018; Buchholz et al., 2018 and Shokri et al., 2021). Biomass waste extract has been described to be used to synthesize silver nanoparticles, which can be used for different purposes and in fields such as science and technology, and medicine (Mythili et al., 2018). Moreover, a Danish report described green biomass as strong proposal that contains high protein quality to substitute other protein sources for monogastric animals and simultaneously obtaining environmental benefits when the production of green biomass substitutes cereal production (Hermansen et al., 2017).

1. RAISING A TOPIC

Green leafy vegetables, despite their availability and relative affordability, present significant losses and waste in the fresh and processing industries, consequently becoming a serious nutritional, economic, and environmental problem (Sagar et al., 2018). This includes the potentially edible parts of the plant left on the field in normal harvest practice wastage in the fresh market due to postharvest quality deterioration and trimmings produced during consumption and processing (Shokri et al., 2021).

Numerous studies have already explored the potential impact of vegetable proteins in animal life, contributing more to the world protein in contrast to fish protein (Ghaly & Alkoaik, 2010). This essential nutrient has been trending in the minds of consumers (Hertzler et al., 2020), and the demand for protein ingredients has surged over the last few years due to increasing population growth (Ghaly & Alkoaik, 2010; Ismail et al., 2020; Małecki et al., 2021 and Henschion et al., 2017). Recent studies in green biomass crops have opened doors for an alternative and sustainable way to meet global protein demand for consumption. For example, extraction of proteins from green biomass in plants such as alfalfa and Jerusalem artichoke has now been described as a strong prospective plant species for leaf protein isolate (Bákonyi et al., 2019 and Kaszás et al., 2020).

This approach has been scientifically proven to reutilize valuable nutrients contained in the disposable green biomass, which can easily be adapted to the circular economy concepts (Bákonyi et al., 2019). As vegetable leaves are undiscussable crucial to animals, including humans, due to their multiple-use as food, medicine, energy, or shelter, studying their properties is of importance to improve food quality and security, health concerns, and sustainable agricultural practices (Oseni, 2016).

AIM OF THE STUDY

The aim of this work is to valorize the neglected green biomass of some vegetable species (including broccoli, horseradish, brussels sprout, and wheat sprout) comparing two wet processes. Some important physico-chemical parameters such as pH, crude protein content, amino acid composition and protein expression pattern is measured to evaluate the quality of obtained fractions. Moreover, we intend to examine the native lactic acid producing bacteria strains from green juice fraction of model plants which can be potentially useful during further use in the food industry.

2. LITERATURE REVIEW

2.1 Circular bioeconomy – green agrowastes

The basic principle of circular bioeconomy relies on maximizing the service provided by the material embedded in products and minimizing the loss of service with time (Sherwood, 2020 and Ubando et al., 2020). Vegetable biomass offers a great example of biodegradable material in which its conversion to energy helps in sustainable resource management, conservation, and environmental safety (Singh et al., 2012).

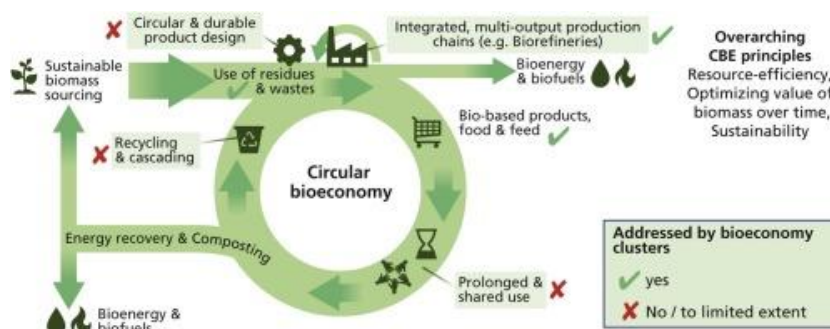


Figure 1: **Biomass cascade in the circular economy (Stegmann et al., 2020)**

Chemical ingredients found in waste biomass are primarily composed of carbon, nitrogen, and phosphorus, which play essential nutrients for the sustenance of various life forms (Mohan et al., 2016).

2.1.1. Green biorefinery methods, products

Agricultural products and processes from wastes and residues have been extensively studied (identified, quantified, extracted, and evaluated) for their potential of the bioactive compounds and biofuels (Ubando et al., 2020; Coman et al., 2020 and Sedlar et al., 2021). Biorefinery is defined as sustainable means of generating multiple bioenergy products from various biomass feedstocks comprised of four main conversion platforms: thermochemical, biochemical, and mechanical conversions (Ubando et al., 2020, Moan, 2016).

With the support of biological, physical, chemical, and technical sciences, biomass waste is being used as an important feedstock and renewable resource with a well-defined technique. For example, literatures have described traditional techniques and methods mostly used, such

as lignocellulosic feedstock biorefinery, extractive methods, gasification and liquefaction of biomass, biosynthesis, bacteria for the degradation of starch and cellulose (Moan et al., 2019; Ubando et al., 2020 and Moan et al., 2016).

Green biorefinery has been coupled with a biogas plant, as it either goes directly into the biogas plant or its ingredients such as proteins, amino acids, lactic and acetic acid, are separated (Kamm et al., 2016 and Kamn & Kamm, 2004).

The mechanical technique presents the one commonly used by researchers. In recent years biomass plants have been used to further convert and yield products such as green juice (GJ), fiber, leaf protein concentrate (LPC), and brown juice (BJ) (Bákonyi et al., 2019; Kaszás et al., 2020; Andersen & Kiel, 2000 Kerfai et al., 2011; Nynäs et al., 2021; Kisvarga et al., 2020 and Martinez et al., 2018).

The green liquid bio-based product contains non-denatured proteins free amino acids, which are suitable feed additives for mono-gastric animals (Bákonyi et al., 2019 and Martinez et al., 2018). The process is followed by the residual liquid fraction obtained during heat treatment (coagulation) or lactic acid (LA) fermentation termed differently by researchers as deproteinized leaf juice, whey, brown liquid, or just as BJ (Bákonyi et al., 2019; Martinez et al., 2018; Feng et al., 2021; Barna et al., 2021 and Kisvarga et al., 2020).

These by-products are highly appreciated due to their richness in protein and phenols as well as water-soluble carbohydrates and minerals (Bákonyi et al., 2019 and Martinez et al., 2018) besides other applications in agricultural practices and medicine (Bákonyi et al., 2019).

2.2. Fermentation with lactic acid bacteria

2.2.1. Significance of applications of LAB

Lactic acid bacteria (LAB) microorganisms are considered gram-positive and facultative anaerobic bacteria that reside in substrates rich in carbohydrates which have been extensively used for many years in different cultures and in agricultural systems to improve and preserve perishable foods, soils, control disease, promote plant growth and beverages (Caplice & Fitzgerald, 1999 and Lamont et al., 2017). These microorganism can be classified as homo-fermenters and hetero-fermenters, where through biosynthetic pathways obtain their energy by catabolizing sugars (Lübeck, & Lübeck, 2019).

LAB serves as a stable and significant source of proteins, vitamins, minerals, and other nutrients (Lamont et al., 2017; Tamang et al., 2016; Caplice & Fitzgerald, 1999 and Sathe et al., 2007). Despite the substantial geographical and cultural differences, cow's milk is the most dairy fermented product around the world, especially in western culture (Tamang et al., 2020 and Ashaolu & Reale, 2020).

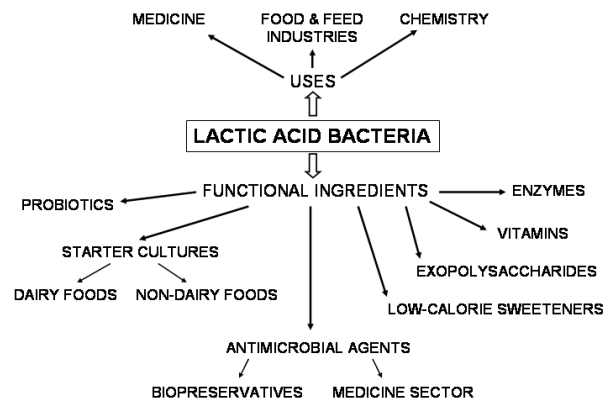


Figure 2: LAB significance and functionality (Florou-Paneri et al., 2013)

In some African countries for example, due to a large impact on nutrition, fermented foods represent a significant component of main meals which serve as a low-cost source of protein in the diet (Odunfa & Oyewole, 1998 and Steinkraus, 1994). Common fermented known foods, beverages, and condiments are named as *gari*, obtained from cassava, *ogi* and *mahewu* are obtained from maize, *kaffir a* drink obtained from sorghum, cowpea leaves or pumpkin leaves, and condiments usually made of protein-rich seeds and tubers, but also involves meat, and dairy products (Odunfa & Oyewole, 1998; Misci et al., 2021; Steinkraus, 1994 and Franz et al., 2014). In Asia, fermentation technology is used as a traditional technique, and many of the products are prepared at home, which includes mainly soybean, rice, black gram, bengal gram, mung beans, fish, and seafood (Tamang et al., 2020; Reddy et al., 1982 and Steinkraus, 1994).

2.2.2. Endophytic microbiota as a tool for fermentation

All above mentioned fermented foods, several genera of microorganisms have been used. The most common dairy LAB includes species from four main genera: *Alkalibacterium*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*

Pediococcus, *Tetragenococcus*, *Oenococcus*, *Vagococcus*, and *Weissella* (Tamang et al., 2020 and Ashaolu & Reale, 2020).

Species of *Bacillus* in green leafy vegetables widely used for fermentation, includes, *Bacillus amyloliquefaciens*, *Bacillus alimentarius*, *Carnobacterium piscicola*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Bacillus plantarum*, *Bacillus fermentum*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus subtilis variety natto*, and *Bacillus thuringiensis* (Leroy & De Vuyst, 2004; Tamang et al., 2016; Steinkraus, 1994 and Reddy et al., 1982).

2.3 Vegetable species as a green biomass source

Vegetable biomass is a particular group of versatile renewable energy that needs to be characterized to clearly understand its nature for application as raw material and to propose the best methodology for its proper utilization (Singh et al., 2012). The four vegetables (Broccoli, horseradish, brussels sprout, and wheat sprout) are going to be described in detail in the present work.

2.3.1 Description of Broccoli

2.3.1.1. Taxonomy of Broccoli

Brassica genus, belonging to the Brassicaceae family (also known as Cruciferae), includes several crops with high economic interest, cultivated for their edible roots, leaves, stems, buds, flowers, mustard, and oilseeds (Velasco et al., 2021 and Poveda et al., 2020).

Broccoli (*Brassica oleracea* var. *Italica*) is a vegetable crop, fast-growing annual plant, perishable, and attractive with high nutritive value (Padaga et al., 2000; Li et al., 2019 and Das et al., 2021). Broccoli belongs to the Brassicaceae family, a large family comprised of approximately 3,000 described species apportioned among 350-380 genera (Buck, 1956 and Gray, 1982). The genus *Brassica oleracea*, in which broccoli belongs, includes a ramified important number of vegetables, e.g., cabbage, cauliflower, Brussels sprouts, kale, Kohlrabi, Turnip, Rutabaga and Mustard.

2.3.1.2 History of Broccoli

According to the history, the plants were taken to Persia after the Aryan invasion of Europe, from where they diffused throughout the Mediterranean region and later developed in varieties

of sprout broccoli by advanced horticultural art mainly by Italians and Greeks (Buck, 1956; Gray, 1982 and Branca, 2008).

Literatures argue about a considerable confusion in both scientific and lay nomenclature of this plant. The term “broccoli” is an Italian word from the Latin brachium, meaning an arm or branch, which is also used for the young edible floral shoots on brassica plants, including cabbages and turnips, and was originally applied to sprouting forms but now includes heading forms, which develop a large, single, terminal inflorescence (Buck, 1956; Gray, 1982; Branca et al., 2018; Li et al., 2019 and Das et al., 2021). However, the same term was described as certain types of cauliflower, notably in Britain, where the term heading, or winter broccoli, is traditionally reserved for biennial types (Buck, 1956; Gray, 1982). And finally, the term “broccoli” without qualification is applied in America to the annual green-sprouting form known in Britain and Italy as calabrese (Buck, 1956; Gray, 1982 and Das et al., 2021).

2.3.1.3 Morphology of Broccoli

Although we are focused on the study of broccoli, several studies on the relationship between cauliflower and broccoli have been performed over recent years based on bio-morphological, anatomical, biochemical, and molecular traits (Branca, 2008 and Li et al., 2019).

Cauliflower and broccoli are differentiated from other crops of *Brassica oleracea* by the morphological modification of the reproductive organs which represent the produce.

Broccoli heads are harvested in correspondence with a more developed flowering stage characterized by the presence of fertile flower buds (Branca, 2008). The produce represents about 20-30% of the whole cauliflower plant, 30- 40% of the broccoli one.

The flower of broccoli conforms to the symmetry typical of the *Cruciferae*. The floral shoot consists of primary tissues from apical meristems of the faceted stem, whereas anatomically, it presents trilacunar nodes, also found in other members of *Brassica* (Wiebe, 1975).

Broccoli contains copious quantities of many nutrients and phytonutrients, including flavanols which are a class of naturally occurring secondary metabolites synthesized in plants through the phenylpropanoid pathway (Duan et al., 2021 and Liu et al., 2018).

2.3.2 Description of Horseradish

2.3.2.1 Taxonomy and history of Horseradish

Horseradish (*Armoracia rusticana*) is a hardy perennial plant belonging to the Cruciferae family, to which cabbage, turnip, radish, kale, Brussel sprouts, cauliflower, and broccoli also belong and contains nearly 340 genera and more than 3350 species (Kadow and Aderson, 1940; Egamberdieva et al., 2020).

Horseradish is known for over 2,000 years for its pungent and fleshy root, which was brought to the Nordic countries by monks used as a condiment and medicinal plant and thought to be native to Eastern Europe (Courter et al., 1969; Sarli. et al., 2012 and Wedelsbäck Bladh, 2014). The most primitive name for horseradish is “chren”, a common Slavic language of eastern Europe (Courter et al., 1969). Several denotations emerged in different western European languages, hence less primitive than the name “cren” (Courter et al., 1969).

Many early botanists reported that horseradish rarely produced viable seeds and, until the early part of the 20th century, the plant was considered to be sterile (Courter et al., 1969).

2.3.2.2 Morphology of Horseradish

The morphology of horseradish varies throughout the growing season, where the plant produces entire laminate leaves in the summer, turning to more pinnate leaves in the autumn. The plants start to produce the pinnate leaves when they prepare for winter dormancy, probably induced by the colder temperature in the autumn (Shehata et al., 2008). The leaves are also most likely to regenerate adventitious shoots from veins (Shehata et al., 2008).

The plant flowers in early summer with white flowers having long and white to light yellow-fleshed, strong-tasting roots (Wedelsbäck Bladh, 2014). The basal leaves are lancet to heart-shaped with toothed margins and long stalks.

Horseradish, a strong spice that was successfully applied in phytotherapy for its valuable effects on health, presents high content in vitamins and especially vitamin C (Agneta et al., 2013 and Cirimbei et al., 2013). Egamberdieva et al., (2020) reported the antimicrobial activity of horseradish secondary metabolites against pathogenic microbes such as *Candida albicans* and *Fusobacterium nucleatum*.

Horseradish peroxidase is one of the most used peroxidases due to its wide application in various fields such as analytical chemistry, environmental chemistry, or clinical trials (Cirimbei et al., 2013).

2.3.3 Description of Brussels sprout

2.3.3.1 Taxonomy and history of Brussels sprout

Brussels sprouts (*Brassica oleracea* var. *gemmifera*) are part of a large group of plants known as Cole crops. Cole crops are a group in the Brassicaceae or mustard family (previously Cruciferae or crucifers), originated from the word *caulis*, meaning stem or stalk of a plant, a biennial, but generally grown as annuals (Respondek and Zvalo, 2008).

According to Ockendon and Smith (1993), the origin of this plant remains obscure since the earliest known reference is to *spruyten*, mentioned in the market regulations of Belgian towns. The crop only became widely known in Europe in the late eighteenth and early nineteenth centuries, and the first reference to the breeding of brussels sprouts was probably by Van Mons (Ockendon and Smith, 1993).

2.3.3.2 Morphology of Brussels sprout

Early maturing cultivars are considered shorter than later ones as sprouts do not develop until the stem has almost reached its full height, but the taller the plant, the greater the potential number of sprouts (Ockendon and Smith, 1993). The lateral buds develop as tiny round vegetative buds, representing an important seasonal vegetable in temperate regions (Naylor, 2003). The inflorescence bears a large number of flowers that ripen over a long period of time. (Naylor, 2003).

The constant increase in brussels sprout cultivation is due not only to nutritional benefits, such as high protein content; high level of antioxidative activity; unsaturated fatty acids and mineral compounds; and low content of nitrates and heavy metals but also to the attractive sensory traits of this vegetable (Lisiewska et al., 2008). The leguminous plant used worldwide both as a food and as a medicinal plant is attracting attention to researchers in charge of biological studies for its richness in vitamin A and ascorbic acid and contain appreciable amounts of riboflavin, niacin, calcium, and iron (Kim et al., 2001., Gupta and Rana, 2003; Yamanouchi et al., 2018 and Renner et al., 2020). Furthermore, the plant contains rutin, which is a flavonol glycoside compound used against edema, hemorrhagic diseases, and stabilizing high blood pressure (Kim et al., 2001 and Yamanouchi et al., 2018).

Brussels sprouts produce a class of cancer-preventing compounds known as glucosinolates, which are nitrogen and sulfur-containing secondary compounds produced by plants of the order

Brassicales, and they are particularly abundant in the economically important Brassicaceae family (Renner et al., 2020).

2.3.4 Description of Wheat sprout

2.3.4.1 Taxonomy and history of Wheat sprout

According to Harlan et al. (1973), cereals are genetically classified by gene pool, where wheat belongs to secondary gene pools such as *Triticum*, *Secale*, and *Aegilops*.

Seed sprouts such as barley, buckwheat, corn, millet, oats, rice, rye, triticale, and wheat have been used as a food since early human history, and it is considered to be among the oldest and most extensively grown of all crops (Lorenz, 1980; Finnie et al., 2019; González-Pérez & Arellano, 2009 and Benincasa et al., 2019). Historical evidence points to the cultivation of wheat in the Nile valley around 5,000 BC, the Indus and Euphrates valleys by 4,000 BC, China by 2,500 BC and England by 2,000 BC (González-Pérez & Arellano, 2009), and it became popular in the western world (Lorenz, 1980; González-Pérez & Arellano, 2009; Amici et al., 2008 and Benincasa et al., 2019). Wheat is highly rich source of antioxidants (Calzuola, Marsili & Gianfranceschi 2004; Amici et al., 2008; Sytar et al., 2018; Benincasa et al., 2019 and Miyahira et al., 2021).

2.3.4.2. Morphology of Wheat sprout

The sprouting of grains is short. It does not require sunlight or even soil which can be easily done at home by the consumer without sophisticated equipment, and the yields are fairly high (Lorenz, 1980; Miyahira, Lopes & Antunes, 2021 and Benincasa et al., 2019).

Germination of seeds and their development in water or another medium produce a green shoot with very young leaves; the caryopsis is the edible part of the grain of cereal (Benincasa et al., 2019)

Products made with sprouted grain are growing rapidly in the food industry, and projections suggest that research and consumption will continue to increase due to health benefits perceived by consumers and pharmaceutical industries (Pagand et al., 2017; Calzuola, Marsili & Gianfranceschi 2004 and Sytar et al., 2018)

3. MATERIAL AND METHODS

3.1 Location of research

The location of the present work was in the Department of Applied Plant Biology at the Faculty of Agriculture, Food Science, and Environmental Management, University of Debrecen. Experiments and measurements were carried out in the laboratories and the Biodrome (Biological Plant Growing and Experimental Greenhouse) of the Department.

3.1.1 Sample collection

Four crops species were involved in this experimental work. The green biomass as agro-wastes were collected from Broccoli leaves, Brussels sprouts leaves, and Horseradish leaves, which were planted in the demonstration garden of the Faculty of Agriculture, Food Science and Environmental Management, University of Debrecen, and home garden, too. The wheat sprout was grown in the hydroponic culture of the Biodrome experimental greenhouse.

3.2 Processing of green biomass

We pressed the vegetable biomass and pulped it mechanically using Angel Juicer (5500, Angel Ltd., Praha, Czech Republic) into fiber and green juice (GJ) fractions. From GJ, we obtained leaf protein concentrate and a brown-colored liquid fraction (phytoserum) known as brown juice (BJ), product candidates using two different coagulation methods. These methods include heat coagulation used by microwave radiation and lactic acid fermentation as detailing in below.

Figure 3, shows the schematic drawing of processed green biomass described above.

The leaf protein concentrate (LPC) and green juice (GJ) were freeze-dried by lyophilization technic (Alpha 1-4 LSC plus -Martin Christ) keeping them for long time.

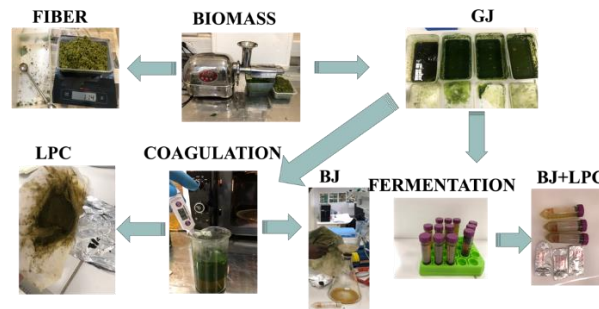


Figure 3: **Schematic of green biomass process**

3.2.1 Microwave coagulation process

First, the green juice was thermally treated at 80 ± 2 °C using microwave coagulation (MW) to coagulate mainly the chloroplastic and cytoplasmic proteins (Patent of Fári – Domokos-Szabolcsy, 2022). Hereinafter referred to as MW. The coagulated green cottage was left at room temperature for approximately 10 min and then separated via vacuum pump with filter paper to obtain leaf protein concentrate (LPC MW) and brown juice (BJ MW) fractions.

3.2.2 Fermentation Process

Lacto-fermentation of GJ was the other coagulation method by using 5% 1 M lactic acid added to fresh GJ, and incubated for four days at 36 °C in anaerobic conditions. During the incubation period (4 days), no measurements have been carried out to avoid infection due to experience in our previous experiment. The fermented green cottage was also separated via vacuum pump with filter paper to obtain leaf protein concentrate (LPC+F) and brown juice (BJ+F) fractions. Finally, all fractionated products (i.e., GJ, BJ, and LPC) were measured quantitatively and qualitatively.

3.3 Sample management and determination of physicochemical parameters of processed samples

3.3.1 Determination of pH

We measured the pH of green juice and brown juice fractions by pH-meter (Mettler Toledo S20 Seven Easy, Switzerland).

3.3.2 Determination of Brix%

Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix represents 1 gram of sucrose in 100 grams of solution and expresses the strength of the solution in % by weight. Degree Brix was recorded manually by a refractometer (RBR32-ATC, Germany).

3.3.4 Determination of Crude protein content

The total protein content of the vegetable species were measured as total N content using the Kjeldahl method (Salo-väänänen & Koivistoinen, 1996). In detail, 1 g lyophilized and grinded sample was weighed in digestion tube; after 15 mL concentrated sulfuric acid and catalyst tablets were added. The digestion was performed by the Tecator Digester unit (VELT, VWR Ltd.) at 420°C for 1.5 h. Then the total N content was measured by titration from the digested sample. The total protein content of the sample was calculated using the following equation: Total protein % = total N content × 6.25 (Kaszás et al., 2020).

3.3.4.1 Protein expression pattern by SDS PAGE

Sample preparation was made by weighting 10 mg of the lyophilised (or crashed in liquid nitrogen) plant samples into an Eppendorf tube. Then it was added 800 µL 2x Laemmli buffer, vigorous vortexing, and incubated for 5 min at 95 °C, followed by 15 min centrifugation at max. speed at 4°C. Finally the supernatant was used for the observation.

Separation of the protein patterns of the different fractions by mass was performed by SDS PAGE in a vertical system on a discontinuous polyacrylamide gel. For this purposes two different gels were prepared:

The preparing Separating/Resolving gel: 12,5% gel (6 mL), contained 2,57 mL MilliQ water, 1,8 mL 40% acrilamide solution, 1,5 mL, 1,5M TRIS pH 8,8, buffer 60 µL 10% fresh SDS 60 µL 10% ammonium persulfate APS (fresh preparation) and 6 µL TEMED.

In case of Stacking gel (3,5 mL), it was used the following, 2,2 mL MilliQ water 350 µL 40% acrilamide solution, 875 µL 0,5M TRIS pH 6,5 buffer, 35 10%-os µL SDS, 35 µL 10%-os APS and 3,5 µL TEMED.

The running parameters were as follows : 90V - 15 min until it reaches the separating gel, then we raised the voltage to 180V for 40-50 min.

After gel run, 2x washing in distilled water (DW), staining in Coomassie G250 staining solution for ~12 hours, then washing off excess stain in DW. Finally, the gel was analyzed using the BioRad ChemiDoc MP Imaging System.

3.3.5 Determination of amino acid composition

3.3.5.1 Sample hydrolysis

The lyophilized and ground samples or liquid phytoserum fraction were weighted in digestion tubes (0,02g) and hydrolyzed with 6 M HCl at 150 °C at 1 hour in the Microwave Digestion Unit of CEM MARS One. The pH of the hydrolyzed acidic sample was adjusted with 6M NaOH. The sample was filtered by a 3kDa PTFE membrane filter.

3.3.5.2 Aminoacid separation by UHPLC

For the analysis of the amino acid composition, an Ultra High-Pressure chromatography (UHPLC) Waters Acquity UPLC System H class plus (Waters, Milford, MA, USA) was used. The separation was based on AccQTag pre-column derivatization chemistry using PDA detector.

Derivatization was made by preheating the vial in the heat block to 55°C for 10 minutes. The mixture containing 70 µl AccQ Tag ultra Borate buffer, 10 µl 500 pmol/µl standard (10 µl Waters aminoacid standard (2500 pmol/µl) +40 µl MQ water and 20µl AccQ Tag ultra reagent) stood for 1 minute at room temperature. Sample derivatization process was the same as used in case of standards.

The separation of the derivatized amino acids was carried out on an AccQ-tag Ultra C18 column (1.7 µm; 2.1 × 100 mm, Waters, Milford, MA, USA) guarded by an Acquity in-line filter (0.2 µm; 2.1 mm, Waters, Milford, MA, USA). Flow rate was 0,100ml/min and 54 °C column temperature in gradient elution (11 min long gradient). A Quaternary gradient pump mixed the Solvent A which was 100% AccQ-tag Ultra eluent A, solvent B was 10% AccQ-tag Ultra eluent B in LC–MS grade water, solvent C was LC water and solvent D was 100% AccQ-tag Ultra eluent B.

The results were evaluated with Waters Empower 3 software (Waters, Milford, MA, USA).

3.4 Identification of microorganisms

Lactobacilli-specific PIL solid media (pH: 6,5) (composition shown in table 5.) was prepared by protocol provided by Dr. Endre Gabriella and Dr. Jenei Sándor at Biological Research Centre, Szeged (BRC) and poured in an 8-cm plastic Petri Dishes.

The green juices from all the examined plant species, obtained by fractionation were diluted by G bioscience molecular grade sterile water by 10^{-1} and 10^{-2} , and spread (500ul / Petri dish) in PIL solid media in 3 repeats under sterile conditions. Petri dishes were placed in the thermostat at 24 °C, and colonies were counted daily to determine Colony Forming Unit (CFU).

Table 1: Composition of PIL solid selective growth medium

Component	g/L
Glucose	3
Sodium Acetate x 3 H ₂ O	2,27
KH ₂ PO ₄	1,36
MgSO ₄ x 7H ₂ O	0,23
MnSO ₄ x 2H ₂ O	0,05
FeSO ₄ x 7H ₂ O	0,02
(NH ₄) ₂ SO ₄ x 6H ₂ O	0,02
Trypton	7,5
Yeast Extract	2,5
Agar Agar	20

3.4.1 Collecting bacterial cells from solid plates

At the point no more colonies have appeared on the solid PIL medium, colonies with different morphologies were selected and isolated to make pure cultures. Colonies of the bacterial strains found in the largest number in the sample were selected. Solitary colonies were isolated on freshly prepared PIL medium under sterile conditions, and after their development in large numbers, sufficient biological material could be collected for DNA isolation. Bacteria colonies were collected from the solid medium by adding sterile water and using inoculating loop by rubbing on agar plates of the selective growth medium. Pure cultures were established in triplicate for all four plant species studied, for two of the most typical lactic acid-producing bacterial strains.

3.5 Statistical analysis

The experimental results were analyzed using Microsoft Excel, and the One-way ANOVA, followed by shapiro test for normality $p > 0.05$, by *R statistical software* package.

4. RESULTS

4.1 Characteristics and quantities of obtained fractions from different vegetables

The yield of the processed vegetable biomass in different methods is presented in Figure 13. The results have shown all significant differences in ratio after biomass fractionation with up to 50%. The brown liquid juice was the main fraction, leaf protein concentrate was in lower ratio. Regarding the two different methods used, lacto-fermentation process showed to yield the higher amount of BJ in contrast to the LPC. As for different species, wheat sprout showed significant differences with the highest in yield of BJ, and lowest in LPC. By contrast the highest amount of LPC-s could be obtained from Horseradish regardless of coagulation methods.

Processed biomass were measured qualitative and quantitatively in GJ product, MW and Lacto-fermentation (F) methods in g/100 ml as standard.

The results displayed in the table 6 shows the average and the standard deviation measurements of the four species, based on the following variables, pH, degree brix, fresh and dry weight as shown in table 6. The significant level was set by 0.05 where we obtained a p-value = 0.000712.

Table 2: Features and amounts of different fractions processed by MW and Lacto-fermentation (F) Sample size (n = 3, $\pm$ S.D). Different letters in the same columns show significant differences at the level of $p < 0.05$.

	GJ		MW LPC (from g/100ml GJ)		FLPC (from g/100ML GJ)		MW BJ				FBJ			
SPECIES	pH	BRIX %	FW (g)	DW (g)	FW (g)	DW (g)	ml/100 ml GJ	FW (g/100 ml GJ)	pH	BRIX	ml/100 ml GJ	FW (g/100 ml GJ)	pH	BRIX
BROCCOLI	6,27 $\pm$ 0,47 bc	8,03 $\pm$ 0,12 bc	28,96 $\pm$ 4,93 bc	5,48 $\pm$ 1,28 bc	6,78 $\pm$ 1,44 bc	1,7 $\pm$ 0,27 bc	53,33 $\pm$ 0,00 bc	56,29 $\pm$ 3,69 bc	5,94 $\pm$ 0,02 bc	7,53 $\pm$ 0,34 bc	82 $\pm$ 2,00 bc	81,12 $\pm$ 1,51 bc	4,74 $\pm$ 0,18 bc	5,03 $\pm$ 0,12 bc
HORSERA DISH	6,27 $\pm$ 0,02c	11,43 $\pm$ 0,33 c	37,31 $\pm$ 4,64 c	7,0986 $\pm$ 0,50 c	28,89 $\pm$ 4,21 c	5,746 $\pm$ 0,33 c	54,44 $\pm$ 3,85c	54,91 $\pm$ 4,93 c	6,35 $\pm$ 0,04 c	11,13 $\pm$ 0,25 c	52,53 $\pm$ 2,84 c	56,76 $\pm$ 3,65 c	4,57 $\pm$ 0,03 c	10,97 $\pm$ 0,12 c
BRUSSELS SPROUT	6,18 $\pm$ 0,00 ab	7,7 $\pm$ 0,14 ab	19,96 $\pm$ 7,06 ab	3,49 $\pm$ 0,88 ab	8,81 $\pm$ 2,40 ab	1,65 $\pm$ 0,27 ab	71,11 $\pm$ 7,70 ab	71,73 $\pm$ 6,62 ab	6,143 $\pm$ 0,02 ab	7,03 $\pm$ 0,05 ab	76,33 $\pm$ 1,53 ab	76,27 $\pm$ 2,84 ab	4,4 $\pm$ 0,02 ab	7,2 $\pm$ 0,00 ab
WHEAT SPROUT	6,52 $\pm$ 0,01 a	6 $\pm$ 0,00 a	9,045 $\pm$ 2,30 a	2,12 $\pm$ 0,20 a	5,43 $\pm$ 0,73 a	0,97 $\pm$ 0,03 a	86,67 $\pm$ 5,77 a	84,87 $\pm$ 3,99 a	6,60 $\pm$ 0,01 a	5,23 $\pm$ 0,12 a	76 $\pm$ 5,29 a	75,28 $\pm$ 5,98 a	4,73 $\pm$ 0,03 a	5 $\pm$ 0,00 a

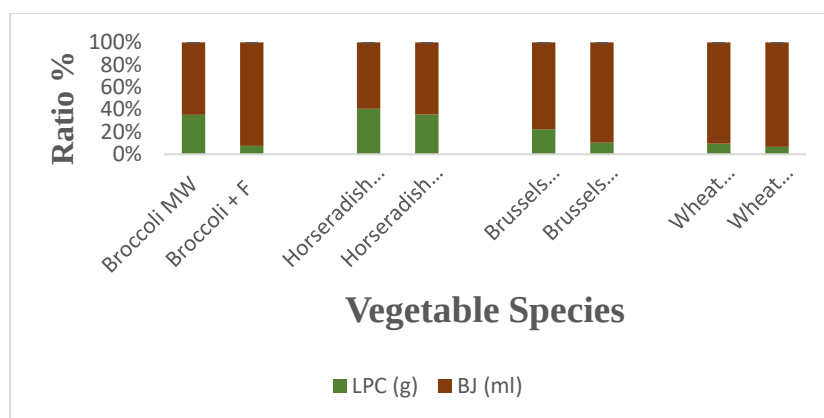


Figure 4 : The LPC and BJ yield of processed biomass of different vegetable species, isolated from green juice by MW (microwave coagulation) and F (Lacto-fermentation). Sample size (n = 3).

4.2. Physical and positional appearance of different fractions by MW and lacto-fermentation

During lacto-fermentation all the species displayed different physical position and appearance between BJ colour (yellowish-green and redish-brown), the position of BJ, and LPC position (bottom, middle and top). The results in three vegetable species (broccoli, brussels sprout and wheat sprout), showed that BJ colour, position and LPC position were uniform. However, in one species (horseradish) it differed significantly in LPC position located on the top and bottom and the position of BJ was located in the middle as shown in table 7. Furthermore, MW coagulation resulted a compact structure of the LPC fraction, located above the BJ fraction (top) which facilitated the filtration. While the lacto-fermentation resulted in a mostly sludgy structure of the LPC fraction, located below the BJ fraction (button), and thus in the different structure and opposite location of the LPC fraction in comparison to the MW.

4.3. pH and degree BRIX degree during the lacto-fermentation

The pH of green juice were monitored during the lacto-fermentation. As shown in the figure 16, it was observed that the freshly pressed GJ presented an average pH of 6, after biomass fractionation. However, the lacto-fermentation process was stabilized on the fourth day of incubation with an average pH of 4. The pH of broccoli and wheat sprouts had a significant changed as the results of fermentation and stabilized at pH 4.7. In contrast to horseradish and brussels sprout, the final pH was reached at 4.5, as shown in the figure. Simultaneously the

degree Brix refer for the waters-soluble sugar content was also measured, in which significant difference was observed for all vegetable species. The only interesting result was in case of broccoli species, in which the water soluble sugar content dropped significantly to the end of the incubation process (9.90-5.0), followed by brussels sprout (9.90-7.20) respectively.

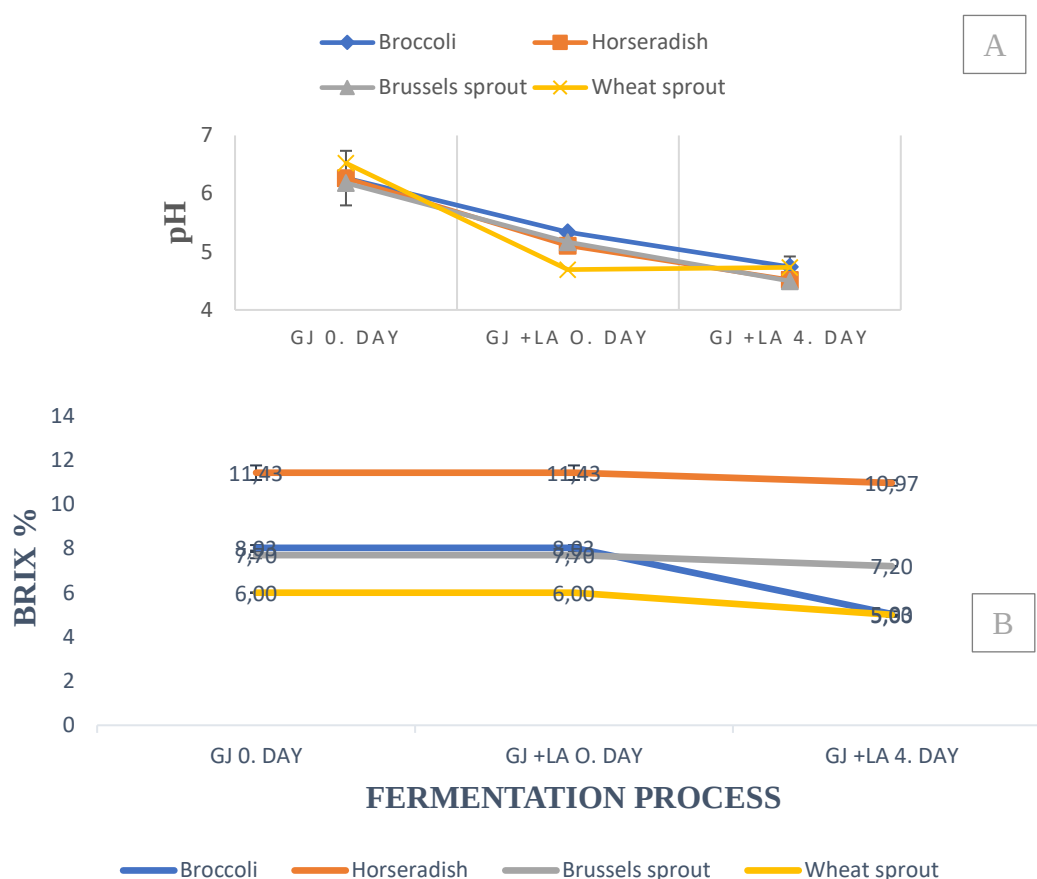


Figure 5: **pH (A) and degree BRIX% (B).** Sample size (n = 3, ± S.D).

4.4. Protein content by Kjedahl

The total protein content (m/m%) of different vegetable species generated from fresh green biomass are displayed in figure 17. It shows that, out of four selected vegetables species, wheat sprout presented significantly the highest in protein concentration (55%), followed by brussels sprout (37%), and horseradish (25%), broccoli (21%) showed the lowest respectively. Comparing the method, microwave technique showed to yield the highest average in protein concentration.

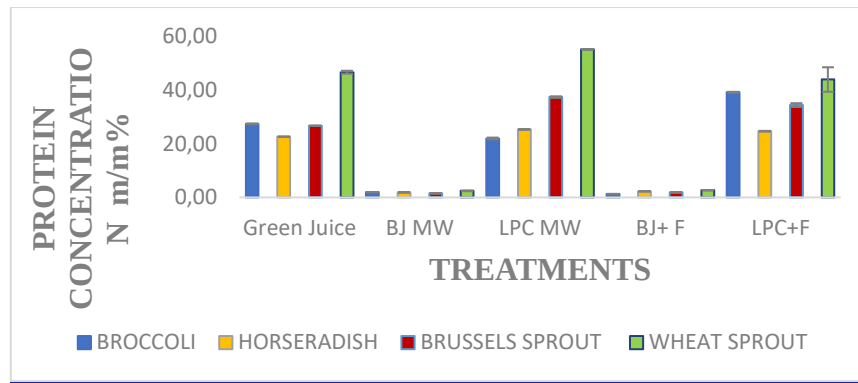


Figure 6: **Protein concentration between species with different treatments.** Sample size ($n = 2, \pm S.D.$).

The protein expression pattern displayed interesting visibility in four vegetables species. Green juice showed the highest number of bands in all four species, indicating that this fraction contains the highest amount of soluble protein. Microwave-derived LPC contained fewer bands when compared to the Fermented-derived LPC. This can be explained by the fact that microwave heating (above 80 °C) coagulates and aggregates most of the proteins. These can be only slightly re-solubilized. In comparison, fermentation resulted more soluble proteins as the higher number of LPC bands shows. Stronger bands can be seen in almost all sample near to 55 kDa, which is the size of large subunit of Rubisco protein as most abundant protein in green leaves. When comparing the species, broccoli showed different expression pattern and visibility in contrast to horseradish or other species. For example, green juice was seen to be expressed stronger bands in Broccoli compared to horseradish.

Brown juices showed very few protein bands in all species. This is understandable, since coagulated proteins are typically retained in the LPC fraction during filtration.

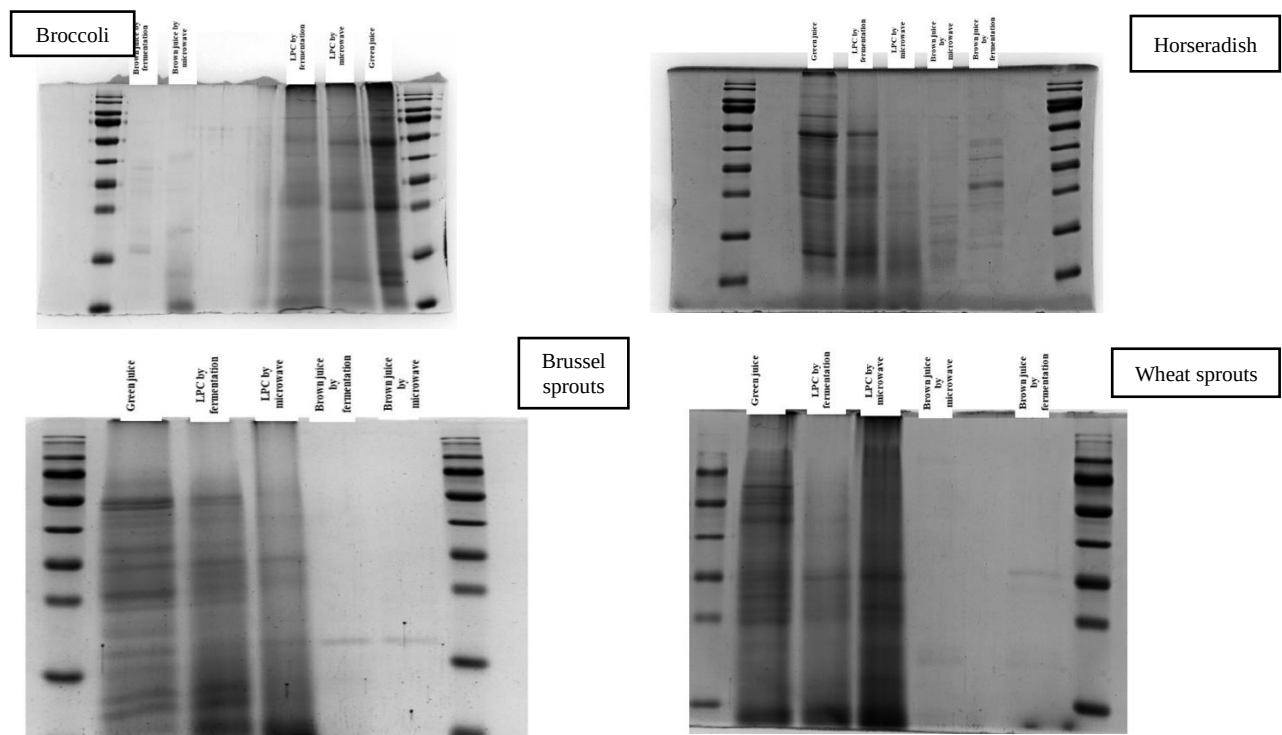


Figure 7: **Protein expression pattern of processed broccoli, horseradish, Brussels sprouts and Wheat sprouts green biomass by 1D SDS PAGE**

4.5. Amino acid composition

The results in amino acid content is shown in table 3. We quantified the proteinogenic amino acid content from the green juice, microwave coagulated LPC and lacto-fermented LPC fractions of all vegetables species.

Differences in the composition of amino acids can be observed in relation to the methods. Consistent with the crude protein content, each amino acid was also detected in highest amounts in wheat sprout originated LPC. For example the concentration of all essential amino acids were highly expressed in wheat sprout amongst all four species. It is noteworthy that lysine (4.09 g/ 100 g DW), a limiting amino acid, was detected in high amounts in MW-LPC. Horseradish presented with the lowest concentration respectively. Comparing to the concentration of essential amino acids, leucine, appeared with the highest concentration content. Tryptophan, methionine and cysteine showed lower values than would be expected from the literature, which can be explained by the fact that these amino acids are particularly sensitive to the acid hydrolysis used in sample preparation.

g/100 g sample	GJ				MW-LPC (coagulation by microwave)			
Aminoacids	Brussels sprout	Wheat sprout	Horseradish	Broccoli	Broccoli	Horseradish	Wheat sprout	Brussels sprout
His	0,00 ± 0,00	0,59 ± 0,05	0,54 ± 0,53	0,32 ± 0,18	0,23 ± 0,01	0,62 ± 0,07	1,53 ± 0,09	1,25 ± 0,14
Asn	0,00 ± 0,00	0,19 ± 0,07	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,23 ± 0,15	0,19 ± 0,03	0,41 ± 0,30
Ser	1,61 ± 0,30	2,17 ± 0,14	0,82 ± 1,01	2,11 ± 0,69	1,73 ± 0,04	2,58 ± 0,07	5,15 ± 0,39	3,15 ± 1,75
Gln	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
Arg	1,75 ± 0,28	1,98 ± 0,18	1,07 ± 0,24	1,88 ± 0,74	1,44 ± 0,01	2,21 ± 0,08	5,08 ± 0,34	3,89 ± 0,46
Gly	1,12 ± 0,16	1,15 ± 0,05	0,89 ± 0,19	1,60 ± 0,71	1,19 ± 0,02	2,07 ± 0,07	4,01 ± 0,28	2,91 ± 0,92
Asp	2,08 ± 0,59	8,31 ± 0,52	1,63 ± 0,39	2,92 ± 1,05	2,13 ± 0,11	3,16 ± 0,62	9,22 ± 0,74	5,57 ± 0,65
Glu	2,55 ± 0,64	1,87 ± 0,09	1,68 ± 0,40	3,47 ± 1,22	2,03 ± 0,06	3,11 ± 0,48	6,23 ± 0,42	5,67 ± 0,36
Thr	0,93 ± 0,18	1,08 ± 0,08	0,76 ± 0,17	1,20 ± 0,43	0,94 ± 0,01	1,62 ± 0,04	3,28 ± 0,20	3,48 ± 0,66
Ala	1,02 ± 0,25	1,19 ± 0,04	0,78 ± 0,19	1,48 ± 0,56	1,14 ± 0,04	1,84 ± 0,14	4,42 ± 0,31	2,73 ± 1,21
Pro	1,91 ± 0,45	0,90 ± 0,02	1,38 ± 0,31	1,26 ± 0,45	1,08 ± 0,02	2,49 ± 0,12	3,43 ± 0,26	3,68 ± 0,19
Cys	0,00 ± 0,00	0,05 ± 0,08	0,03 ± 0,02	0,00 ± 0,00	0,04 ± 0,00	0,10 ± 0,03	0,35 ± 0,01	1,70 ± 1,91
Lys	0,99 ± 0,37	1,21 ± 0,05	0,85 ± 0,24	1,51 ± 0,52	1,10 ± 0,03	1,66 ± 0,48	4,09 ± 0,26	1,92 ± 2,41
Tyr	0,77 ± 0,05	0,90 ± 0,05	0,58 ± 0,11	0,99 ± 0,37	0,91 ± 0,01	1,47 ± 0,07	2,14 ± 1,12	3,27 ± 0,53
Met	0,00 ± 0,00	0,22 ± 0,02	0,12 ± 0,02	0,41 ± 0,18	0,28 ± 0,02	0,48 ± 0,02	1,04 ± 0,10	1,53 ± 0,74
Val	0,94 ± 0,15	1,32 ± 0,06	0,70 ± 0,12	1,31 ± 0,56	0,98 ± 0,01	1,69 ± 0,16	3,70 ± 0,16	2,03 ± 1,93
Ile	0,64 ± 0,10	0,84 ± 0,03	0,49 ± 0,09	0,91 ± 0,41	0,70 ± 0,00	1,27 ± 0,16	2,71 ± 0,10	2,92 ± 0,48
Leu	1,58 ± 0,29	1,61 ± 0,06	1,19 ± 0,25	2,24 ± 0,88	1,87 ± 0,04	2,89 ± 0,22	6,56 ± 0,41	4,16 ± 2,70
Phe	1,12 ± 0,06	1,17 ± 0,06	0,78 ± 0,15	1,51 ± 0,65	1,27 ± 0,02	1,97 ± 0,10	4,26 ± 0,26	5,23 ± 1,64
Trp	0,00 ± 0,00	2,54 ± 0,02	0,03 ± 0,05	0,03 ± 0,05	0,02 ± 0,04	0,17 ± 0,01	0,30 ± 0,02	2,30 ± 2,91
FLPC (coagulation by lactic acid)								
g/100 g sample	Broccoli	Horseradish	Wheat sprout	Brussels sprout				
Aminoacids	0,25 ± 0,01	0,63 ± 0,02	1,20 ± 0,06	0,89 ± 0,00				
His	0,74 ± 1,05	0,07 ± 0,01	0,49 ± 0,23	0,11 ± 0,00				
Asn	1,67 ± 0,49	2,22 ± 0,08	4,17 ± 0,00	3,06 ± 0,00				
Ser	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00				
Gln	2,37 ± 0,007	1,55 ± 0,04	3,70 ± 0,13	2,55 ± 0,00				
Arg	1,24 ± 0,02	1,95 ± 0,04	3,17 ± 0,05	2,55 ± 0,00				
Gly	1,87 ± 0,40	3,20 ± 0,10	7,76 ± 0,45	4,31 ± 0,00				
Asp	2,06 ± 0,19	3,24 ± 0,08	4,63 ± 0,13	4,45 ± 0,00				
Glu	0,75 ± 0,27	1,56 ± 0,06	2,56 ± 0,05	2,03 ± 0,00				
Thr	1,18 ± 0,07	1,85 ± 0,04	3,26 ± 0,16	2,66 ± 0,00				
Ala	1,19 ± 0,50	2,27 ± 0,06	2,48 ± 0,08	2,62 ± 0,00				
Pro	0,07 ± 0,05	0,10 ± 0,00	0,37 ± 0,08	0,17 ± 0,00				
Cys	1,09 ± 0,08	1,92 ± 0,08	2,78 ± 0,18	2,48 ± 0,00				
Lys	0,67 ± 0,02	1,40 ± 0,03	2,51 ± 0,11	1,93 ± 0,00				
Tyr	0,32 ± 0,02	0,50 ± 0,01	1,06 ± 0,02	0,93 ± 0,00				
Met	1,05 ± 0,04	1,75 ± 0,03	2,89 ± 0,01	2,32 ± 0,00				
Val	0,78 ± 0,03	1,34 ± 0,03	2,19 ± 0,03	1,86 ± 0,00				
Ile	1,81 ± 0,06	2,87 ± 0,06	4,92 ± 0,07	4,23 ± 0,00				
Leu	1,27 ± 0,01	1,96 ± 0,04	3,55 ± 0,15	2,99 ± 0,00				
Phe	0,00 ± 0,00	0,16 ± 0,00	0,25 ± 0,01	0,21 ± 0,00				
Trp								

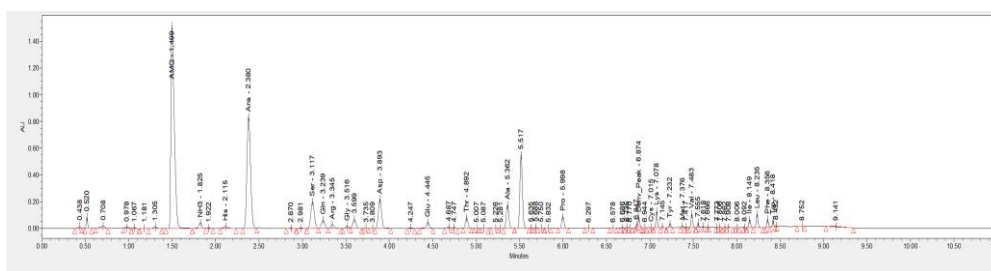


Figure 8: **Representative chromatogram of derivatized amino acid composition by UHPLC separation**

4.6. LAB Microorganisms from different origin of GJ

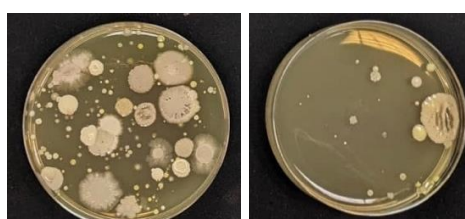
The number of colonies were counted daily and the colony forming unit (CFU) was calculated, by using the standard formula. Table 9 illustrates the averages with standard deviation per plant species.

Figure 21, shows the result of colony growth in Petri dishes made in two dilution 10^{-1} and 10^{-2} with three replicates each respectively. Out of four vegetables species, the highest number of LAB colonies were recorded in horseradish ($8,6 \cdot 10^3$), followed by broccoli ($6,03 \cdot 10^3$). Brussels sprout were Too Few to Count (TFC). Not only was there a difference in the total number of colonies, but also in their shape, suggesting that different strains of bacteria are present in the green biomass depending on the plant species. The most representative colonies per species were selected for DNA-based identification.

Table 3: Number of LAB colony forming units. Sample size (n = 3, $\pm$ S.D).

SPECIES	FINAL NUMBER OF LAB COLONIES		
	10^{-1}	10^{-2}	CFU/1ml GJ
BROCCOLI	$60,33 \pm 2,52$	$15,00 \pm 5,00$	$6,03 \cdot 10^3$
BRUSSELS SPROUT	$0,67 \pm 0,58$	$2,67 \pm 2,31$	TFC*
WHEAT SPROUT	$34,67 \pm 3,06$	$5,67 \pm 2,08$	$3,46 \cdot 10^3$
HORSERADISH	$86,00 \pm 21,28$	$16,00 \pm 6,08$	$8,6 \cdot 10^3$

*TFC: Too Few to Count



**Figure 9: Growth of colonies originated from Horseradish GJ
(10^{-1} dilution on the left, 10^{-2} dilution on the right)**

5. CONCLUSION AND RECOMMENDATION

Nowadays green biomass recycling get more attention as it contribute to sustainable development and production. During fresh vegetable production, significant amount of green biomass waste is generated which may contain useful chemicals, health-beneficial components and could be utilized as a raw material for food production.

The present work highlighted the increased value of the neglected green biomass fraction of vegetable crops using two different technological methods including microwave coagulation (MW) and lactic acid fermentation (F) with four vegetable crops (broccoli, horseradish, brussels sprout, and wheat sprout). Physico-chemical parameters of fractions from wet fractionation of these plant biomass were measured, such as pH, Brix degree, extraction ratio, crude protein content, amino acid composition and protein expression pattern, also native lactic acid producing bacteria were isolated for further potentially use in the food industry, as well as the physical and positional appearance of different fractions by MW and lacto-fermentation were evaluated.

Conventional methods described in the literatures such as alkaline extraction, hammer mill have described to extract proteins from the biomass, but offering limitations in the yield and qualities of the products, due to factors such as economical, crop plant, lands and the methods used (Hermansen et al., 2017 and Bals & Dale, 2017). In contrast, microwave and lacto-fermentation techniques used in the present study to process vegetable biomass showed to be affordable with less limitation and considerable yield of by products such leaf protein concentration (LPC) and brown juice (BJ). The yields obtained in the fractionation of the green juice contained considerable amounts of biological elements including high quality of protein concentration up to 50%, essential aminoacids which could be further used for different industrial purposes direct and/or indirect way. Because of the high sugar content of the product and to prevent spoilage, the lacto-fermentation process proved to be effective, with the pH stabilized at around 4 for all species. The results clearly showed that broccoli and brussels sprout, are more prone for lactofermentation, as the pH decreased, sugar content also dropped significantly. Regarding LAB, previous studies had highlighted that due to the considerable number of LAB microorganism in the vegetable's green juice, lacto-fermentation could result in more nutritious food with alternative to store for longer periods (Breidt et al., 2012)

As the world faces an increasing demand for protein, only soybean presents the main source of protein to date (Solati et al., 2018). Moreover, there is a trade-off between meeting the protein demand and the practices of sustainable agriculture, as the world governments look urgently for alternatives with sustainable development goals. The protein content of the leaf protein concentrate fractions could be potential candidates as alternative protein sources, thus valorising the waste plant parts into a valuable value-added food feedstock. Hermansen et al., 2017 reported that animals present different amino acid requirements specially for lysine, methionine and cysteine, which are also found in the samples we examined. It can be concluded that this processing and valorisation of waste plant parts contribute to the objectives of circular farming to the implementation of a circular approach.

Additionally, in this research LAB colonies were isolated from green juice of all plant species to find native, potentially effective bacteria that can be used in fermentation processes. LAB has great potential in several ways. For example fermentation process have been used to increase limiting amino acid lysine (Lübeck & Lübeck, 2019), showing that amino acids are one of the most important components for food and feed, specially the essential and limiting amino acids. In this study, almost all amino acids including essential and limiting amino acids were identified in our four vegetable species using different methods.

Preserving these biological valuable contents from the vegetables biomass, presents the priority to meet sustainability and resources rationalization, for further use.

Hence, our work faced with challenges mainly time factor which limited to show further conclusions specifically in the identification of LAB microorganism. As mentioned, above-earlier this is an ongoing project with the aim to publish and unveils the biological significance of fractionated vegetable's biomass in form of scientific article.

Biorefinery with the use of right technology is giving us a chance to produce beneficial products with enormous health and environmental benefits. Our recommendations lie on further research of the related field to create global awareness to the relevant stakeholders such as food and chemical industries, policy makers, environmentalists and individuals regarding sustainable practices to reuse and reduce food waste in order to meet the sustainable development goals specifically the zero hunger and responsible consumption and production.

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ACKNOWLEDGEMENT

I truly believe that, there is nothing more attractive in education than having a professor or a mentor with the ability to see potential in a student or a mentee, and pushes you, challenges you, to discover it and makes you grow.

I would like to thank God for giving me the opportunity to meet **Dr. Nóra Bákonyi and Dr. Éva Domokos-Szabolcsy** my supervisors, for their dedication and keen interest to make me grow, to challenge me and for their unmeasurable support since day one of this thesis, I am eternally grateful. This work became a reality thanks to you dear Professors.

I also would like to express my appreciation to professor Miklós Fári, Zoltán Kovács, Döme Barna, László Kaszás, Judit Koroknai and Ibolya Oláhné Tóth, for their technical support and their input in this work.

Last but not least, I also thank my Parents and friends for their social and emotional support that were crucial to fulfil this work.

Geraldo Jorge Domingos

A 36. OTDK
BIOLÓGIA SZEKCIÓ FELHÍVÁSÁNAK
2. SZÁMÚ MELLÉKLETE

TÉTELES SZERZŐI HOZZÁJÁRULÁS

Alulírott (**Geraldo Jorge Domingos**) nyilatkozom, hogy a 36. OTDK-ra benyújtott (**Biological values of processed green biomass from different vegetable species**) című pályamunkámban leírtak esetében az alábbi munkafolyamatok elvégzésében vettem részt:

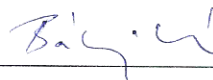
Részfeladat ^{1*}	A részvétel mértéke (ötfokozatú skálán [#] jellemezve)
Növényi zöld biomassza begyűjtése, feldolgozása figyelembe véve a különböző módszereket (nedves frakcionálás, mikrohullámú koagulálás, tejsavas fermentáció)	5
A feldolgozási módszerekhez tartozó alapvető mérések elvégzése (pH, Brix mérés)	5
Nyers fehérje tartalom meghatározás Kjeldahl módszerrel	2
Fehérje mintázat vizsgálata SDS PAGE módszerrel	3
Aminosav összetétel meghatározás UPLC módszerrel	2
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Az adatok statisztikai feldolgozása	5

Kelt: 2022. 05. 27.

(Kérjük a dőlttel jelölt részeket értelemszerűen adják meg. A táblázat szükség szerint értelemszerűen módosítható).


Pályamunkát benyújtó hallgató
(**Geraldo Jorge Domingos**)

A nyilatkozatban foglaltak helyességét megerősítem:

 
Témavezető (**Dr. Bákonyné Nóra és Dr. Domokos-
Szabolcsy Éva Debreceni Egyetem, Alkalmazott
Növénybiológia Tanszék**)

^{1*} Az alábbi lista mintául szolgál, szabadon módosítható / bővíthető.



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