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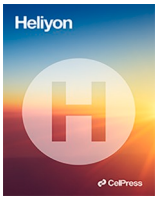

Research article

# Establishing baselines for prebiotic production in controlled environments for applications in space and vertical farming

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ABSTRACT

Prebiotics might contribute to astronauts' health in space, yet their production in Bioregenerative Life Support Systems (BLSS) remains largely unexplored. Here, chicory (*Cichorium intybus* L.) exhibited the most appropriate characteristics among seven candidate species as the ideal crop for space prebiotic production systems. Furthermore, we evaluated chicory's performance under different light intensities (250 μmol $m^{-2}$ $s^{-1}$ and 500 μmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation) and growing cycle lengths (76 and 91 days after sowing) to optimize prebiotic production. Our findings reveal chicory's remarkable adaptability to fully controlled growing conditions and its ability to accumulate high levels of inulin in the young taproot, despite the short growing cycle. The length of the growing period influenced the productivity of biomass and inulin, the latter depending more on taproot biomass than on inulin content. A growing area of 6.3 $m^2$ might be sufficient for a daily production of 12 g of inulin, the amount of "native chicory inulin" recognized by EFSA to promote health benefits in humans. These results establish important baselines for prebiotic production in BLSS or Controlled Environment Agriculture (CEA) and may be used to design fully controlled cropping systems for space and Earth based vertical farming facilities.

## 1. Introduction

Manned long-distance space travels and permanent planetary bases will require the capability to produce, recycle, and utilize resources *in situ* to limit the need for resupply from Earth. In this context, biobased systems will harness the power of photosynthesis and of the metabolism of various organisms to produce food and recycle vital resources [1]. Higher plants are the main components and primary producers of Earth's ecosystems [2] and form the basis of Earth agriculture [3]. This strongly supports the use of higher plants as pivotal elements in food-producing bioregenerative life support systems (BLSS) in space [4–6].

The integration of plants in space endeavors has been anticipated since the early days of human space programmes. Several space agencies and research groups have worked on establishing frameworks of activities, technologies, and procedures, producing data to

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support modelling and designing BLSS. Many facilities have already been constructed and used for plant-based experiments in space (as reviewed in Ref. [7]. Plants grown in space were consumed for the first time by U.S. astronauts as part of NASA's VEG-01 experiment onboard the International Space Station (ISS) [8]. More recently, cotton seeds germinated on the far side of the Moon [9]. However, we are still far from significantly contributing to the food supply for human in space through space agriculture.

In addition to space experiments, Earth-based research activities on BLSS have been conducted in research centers [7,10–12] and in harsh environmental conditions that serve as space analogs [13,14]. Nevertheless, many fundamental aspects of food production in space still require research to support advanced design. Currently, the technology readiness level of implemented systems and subsystems is too low to enable the construction of space-ready facilities for functional BLSS. The contribution of BLSS to the crew's nutritional requirements is among the many aspects still poorly defined at the research and development levels. Depending on the mission scenario, the system may be required to provide a fraction of the calories, or all the calories needed by the crew, as well as a specific subset of essential nutrients or all components of a complete diet. In this context of uncertainties, it is crucial to provide solid, research-based data to define key subsystem baselines related to food, even if focusing on a single essential nutrient or class of nutrients. This data can then inform the design of technology for specific food production functions as part of a more complex BLSS system.

Nutrition is a key need for human well-being on Earth, and this is equally true in space, as evidenced by decades of continuous presence in space and experiments in confined space analog facilities [15,16]. The approach to astronaut nutrition has evolved over time due to advances in nutritional sciences, the food technology sector, and the understanding of specific nutritional needs in space. Nutritional needs of astronauts, as defined by NASA, have gained increasing relevance in addressing health and behavioral problems affecting humans during long-term stays in space [17,18]. Among the space-related threats to human physiology and well-being, the modification of human and environmental microbiomes is particularly significant, due to the many functions influenced by the interaction between humans and microorganisms [19–22].

Nutrition is increasingly considered a countermeasure that could alleviate space-specific negative effects. One significant area of investigation concerns the interaction between nutrition and the gut microbiome [23–25]. Food affects human health directly through digestion and assimilation and indirectly by modulating the metabolism of gut microbiota. Fiber, particularly prebiotics, is among the most positive influencers of gut microbiota [26–29] and primarily derives from plant sources. Some plant species contain only residual amounts of prebiotic molecules, such as polyphenols, whose probiotic functions have been recently recognized [30]. Fortunately, other plants accumulate prebiotic molecules in considerable amounts in their edible organs [28] but only a few have been tested for acclimation and productivity in fully controlled conditions [31,32]. In addition to the lack of exhaustive data on fiber content in BLSS-derived plants produced for space consumption [33], the study of prebiotic production-specific functions and the key requirements for optimization in BLSS remain to be investigated.

In this work, a set of plant species was tested for their potential use as prebiotic producers in BLSS including *Allium schoenoprasum* L. (chives), *Brassica rapa* L. subsp. silvestris (turnip tops), *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Taraxacum officinale* L. (dandelion), *Campanula rapunculus* L. (rampion bellflower), and *Cichorium intybus* L. (chicory). These plant species were initially selected based on literature regarding previous use in space research and prebiotic content [34–36]. Chives, originating from cold areas of Europe and Asia, have shown good results when grown in fully controlled conditions in the EDEN ISS Future Exploration Greenhouse in Antarctica [14], demonstrating their potential use in BLSS. Chives can contain fructans up to 5.7 % of the leaf dry weight in commercially sourced plants [34]. *Brassica rapa* has been successfully grown in space from seed to seed [37]. The turnip tops is a widely consumed vegetable that can be grown in soilless conditions in about 40 days, from emergence to harvest [38]. Turnip tops contain significant amounts of dietary fiber in their aerial tissues, which can be consumed fresh or cooked [39]. Grasses and cereals can accumulate fructans in various organs, including seeds, depending on the growth stage and environmental conditions [40–42]. Wheat has been successfully grown in space in seed-to-seed cycles [43]. Wheat and barley exhibit notable nutritional traits, especially when consumed at early growth stages [44] and can accumulate fructans even in the caryopses [45]. Fructans can reach high concentrations in plants from the *Asteraceae* and *Campanulaceae* families [46]. Dandelion, originally from Europe, is now widely distributed across the northern hemisphere. It serves as a food source and is rich in phytochemicals, in addition to fructans [47]. Rampion bellflower is a biennial species from the *Campanulaceae* family, found in Europe, North Africa, and the Caucasus [35,48]. It is eaten fresh at the rosette stage when it offers a mix of bitter leaves and a small, white, sweet taproot [49], rich in fructans [50]. Chicory is widely cultivated for various uses [51]: the mature taproot is a commercial source of inulin, and locally chicory taproots-even at an immature stage-are traditionally consumed as vegetables [32]. Chicory can be grown under partial or full environmental control to shorten the production cycle and obtain young taproots rich in fructans [31,32,52–54].

In this work, we define the feasibility of plant prebiotic production in BLSS. More specifically, we: a) evaluated the characteristics and performance of several species known to contain prebiotics; b) tested the growth of *chicory* under fully controlled conditions; c) assessed the content of prebiotics and other fibers in the plant material; d) examined the effect of young chicory taproots on 4 microbial species known as probiotics; and e) established key system baselines for the production of prebiotic-rich vegetables to guide the design of BLSS and vertical farming facilities.

## 2. Material and methods

### 2.1. Plant material and treatments

In this work, the species: *Allium schoenoprasum* L. (chives), *Brassica rapa* L. subsp. silvestris (turnip tops), *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley) were tested as seedling producers, *Taraxacum officinale* L. (dandelion), *Campanula rapunculus* L.

(rampion bellflower), and *Cichorium intybus* L. cv Chiavari (chicory), for taproot production. Seeds of chives, turnip tops, wheat, and barley were sourced from Hortus srl (Longiano, Italy), while chicory*s* seeds were procured from L'Ortolano srl (Cesena, Italy). Mature dandelion and rampion bellflower plants were harvested from local populations around the IRET headquarters (Porano TR, Italy -Lat. 42° 41′ 3″, Long. 12° 5′ 57″, 444 m asl) in July and February 2020, respectively.

Seeds of chives, turnip tops, wheat, and barley were sown in 0.5 L plastic pots containing a mixture of commercial topsoil and sand (3:1) in a greenhouse at CNR - IRET headquarters and supplied with water as needed. Chicory was grown in 1.5 L plastic pots filled with the same soil mixture under fully controlled conditions (growth chamber Fitotron SGD170, Sanyo Gallenkamp, UK) to achieve a final plant density of 16.1 plants $m^{-2}$. Seedlings were provided with a 12 h light/12 h dark photoperiod, 150 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD, λ 400–700 nm) from cool white 5700K LED lights (model LX60; Heliospectra AB, Gothenburg, Sweden), and maintained at 400 ppm $CO_2$. Temperature and relative humidity (RH) were set at 20 ± 0.5 °C/70 % RH during the light period and 15 ± 0.5 °C/70 % RH during the dark period. Plants received a full-strength Hoagland nutrient solution once a week, adjusted to a pH of 6.5 and EC of 1.7 mS $cm^{-1}$, along with fresh water as required. Three weeks after sowing, chicory plants were divided into two groups: one was placed under high light (HL) at 500 μmol $m^{-2}$ $s^{-1}$, and the other under low light (LL) at 250 μmol $m^{-2}$ $s^{-1}$. This corresponds to a daily cumulative intensity of photosynthetically active quanta (as day light integral – DLI) of 21.6 mol $m^{-2}$ $day^{-1}$ for HL and 10.8 mol $m^{-2}$ $day^{-1}$ for LL.*2.2.*

#### *2.1.1. Harvest sampling and growth traits*

The four species intended for seedling production were harvested 20 days after sowing (DAS), while chicory was harvested at 76 and 91 DAS. For the seedling-producing species, samples of at least 15 g of fresh material were collected. For the root-producing species, a sampling unit consisted of a single plant. The harvested shoot and root materials were manually separated for shoot/root (S/R) determination. Four replicates were used for each measured variable. Dry weight (DW) and the percentage of dry matter (DM) were obtained by freeze-drying the fresh material (FW) to a constant weight. The dried material was then ground to pass through a 0.5 mm sieve for further analysis.

### *2.2. Non-structural carbohydrates (NCS) and fructans analysis*

Fructans, glucose, fructose, and sucrose from the tissues of all plant species were extracted according to Ref. [42] with some modifications: 20 mg of powdered tissue was extracted in 0.5 mL of 100 % ethanol at 80 °C until the ethanol evaporated. The dry residue was then dissolved in 2 mL of deionized water and treated at 80 °C for 1 h in a thermomixer with shaking. After cooling to room temperature, the extract was centrifuged at 6000 g for 10 min and filtered through a nylon 0.45 μm PPII syringe filter (Whatman Inc., Maidstone, UK).

Fructans concentration and average degree of polymerization (DP) were determined after mild acid hydrolysis of the extracts, conducted with 60 mM HCl (final concentration) at 70 °C for 2 h. Both non-hydrolyzed and hydrolyzed samples were diluted with water and analyzed via high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using a Dionex™ ICS-5000. An analytical CarboPac PA-100 column (4 × 250 mm) with a guard column was used (all equipment from ThermoFisher Scientific Dionex™, Waltham, MA, USA). All runs were performed at 30 °C with a flow rate of 1 mL $min^{-1}$, employing a mobile phase gradient with two aqueous solutions: (A) NaOH 1 mol $L^{-1}$ and (B) Na Acetate 1 mol $L^{-1}$ under the following conditions: 0–8 min, 100 mM NaOH; 8–18 min, 100 mM NaOH with 100 mM Na Acetate; 18–25 min, 500 mM NaOH with 500 mM Na Acetate, maintained until 35 min; 35–45 min, 100 mM NaOH, maintained until 60 min. Glucose, fructose, sucrose, 1-Kestose, and 1-Nestose were quantified against a carbohydrate standard curve prepared using HPLC-grade reagents (Sigma, Steinheim, Germany), with fucose used as an internal standard.

Starch determination in shoot and root tissues of all species was performed by extracting 10 mg of lyophilized powder in 80 % ethanol at 80 °C for 45 min under continuous shaking. The extract was centrifuged at 16.000 g for 5 min, and the pellet, containing starch, was washed four times with 50 mM Na Acetate buffer (pH 4.5). The pellet was then suspended and autoclaved at 120 °C for 45 min in 1 mL of the same buffer. After autoclaving, the sample was incubated at 50 °C for 1 h with amyloglucosidase (70 U) and α-amylase (4 U) to hydrolyze the starch to glucose. The glucose produced from starch hydrolysis was measured using a spectrophotometric coupled enzymatic assay as described by Ref. [55].

#### *2.2.1. Growth of lactic acid bacteria*

Four strains of lactic acid bacteria known for their probiotic activity: *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus rhamnosus*, were grown in De Man–Rogosa–Sharpe (MRS) growth medium (Sigma Aldrich, Milano, Italy), which was prepared according to the manufacturer's instructions and sterilized at 121 °C for 20 min. In this medium, glucose was substituted with an equal amount (20 g $L^{-1}$) of chicory taproot powder harvested at 91 DAS from both LL and HL conditions. MRS with 20 g $L^{-1}$ glucose as the energy source served as the control. Bacteria were incubated at 37 °C for 24 h, without shaking, and growth was assessed by measuring the OD600nm (insert make of spectrophotometer). The analysis was conducted in triplicate.

### *2.3. Statistical analysis*

For the experimental trial with chicory, statistical analysis was performed using one-way ANOVA with the STATISTICA software package (StatSoft 8 for Windows, 1998), considering HL and LL as factors. Differences between averages were assessed using Fisher's

post hoc test, with a significance level set at $p = 0.05$, and results were indicated with different letters. The results of the tests on the prebiotic potential of chicory were analyzed using one-way ANOVA followed by Tukey's multiple range test in SPSS (IBM).

## 3. Results and discussion

### 3.1. Fructans in leafy vegetables

A set of species was tested for their possible use as prebiotic producers in BLSS. In our conditions, the fructans content in the aerial parts of chives and turnip tops was negligible (Fig. 1S). This discrepancy with the literature may stem from our protocol, where leaves were harvested at a very young stage. Further research on the cultivation of these two species could promote their use in controlled environment cropping systems, both on Earth and in space. However, they were excluded from further tests due to their low fructans content.

When grown in our greenhouse, the fructans content in barley (Fig. 2S) and wheat (Fig. 3S) increased from seeds to the aerial parts of 20-day-old plants, reaching a maximum estimated content of 13 % of dry weight (data not shown). It is important to note that this value, measured after hydrolysis, may be partially overestimated due to the presence of β-glucans [40], because acidic hydrolysis can hydrolyze the β-glucans that are present in barley and wheat producing glucose molecules that can interfere with the quantification of fructans. Remarkably, wheat and barley accumulate substantial storage carbohydrates in the form of fructans at such early stages. Nevertheless, the fructans content is not expected to increase significantly in older plants [41] and remains low compared to other candidates.

The four leafy crops considered here (chives, turnip tops, wheat, and barley seedlings) can generate substantial biomass in a relatively short time, can be grown in a controlled environment, and possess significant nutritional value, indicating great potential for space and ground vertical farming systems. However, as leafy crops, they all have a low percentage of dry matter and low fructans content in the fresh produce. Even considering the content of other prebiotics (such as β-glucans, pectin, or raffinose oligosaccharides) and fiber, the amount of produce required to provide the daily recommended intake of these compounds would be impractical for human consumption and space applications.

### 3.2. Fructans in taproots crops

We tested the concentration of fructans in the tissues of dandelion, rampion bellflower, and chicory. For dandelion, the total fructans content in the taproot averaged 44.3 % of the dry weight (Table 1S), while it was relatively negligible in the leaves. Both the leaves and roots contained measurable amounts of 1-kestose and nestose—two oligofructans that consist of one and two galactose molecules linked to sucrose, respectively. These compounds serve as precursors for the synthesis of longer fructans molecules. In the leaves, the content of these fructans was lower than that of soluble sugars (glucose, fructose, sucrose, GFS), suggesting they do not significantly contribute to fructans storage in dandelion leaves (data not shown). In contrast, the combined content of 1-kestose and nestose in the taproot exceeded the total amount of GFS (data not shown).

The total fructans content we found in dandelion taproots (Table 1S) and the role of the taproot as a fructans accumulator corroborate previously published data [35]. While our data indicate that dandelion has great potential as a source of prebiotics for human nutrition, the plant size and that of its taproot in particular are much smaller than those of other fructans-accumulating plants (data not shown). The dandelion plants were grown from seeds harvested from wild populations with minimal environmental control.

**Table 1**
Influence of growing period duration and light intensity on fresh and dry weight of chicory leaves and taproots, dry matter percentage, specific leaf dry weight, and shoot/root ratio. Data are reported as average ± the standard error (S.E.).

| DAS | Growth light | Chicory leaves | | | | Chicory taproots | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | FW (g plant$^{-1}$) | DW (g plant$^{-1}$) | Dry matter (%) | SLDW (mg m$^{-2}$) | FW (g plant$^{-1}$) | DW (g plant$^{-1}$) | Dry matter (%) | Shoot/ Root |
| **76** | **LL** | 84.0 ± 6.29 | 6.96 ± 0.43 | 8.50 ± 0.45 | 2.37 b ± 0.14 | 27.0 ± 5.72 | 5.45 ± 1.27 | 20.0 ± 0.47 | 1.42 ± 0.32 |
| | **HL** | 101.9 ± 8.91 | 9.57 ± 0.89 | 9.4 ± 0.14 | 2.92 a ±0.08 | 37.4 ± 5.36 | 7.72 ± 1.06 | 20.7 ± 0.14 | 1.28 ± 0.13 |
| **Statisticalsignificance** | | n.s. | n.s. | n.s. | * | n.s. | n.s. | n.s. | n.s. |
| **91** | **LL** | 158.3 ± 8.94 | 16.67 ± 0.83 | 10.5 ± 0.12 | 3.05 ± 0.24 | 78.2 ± 12.13 | 18.1 ± 2.86 | 23.1 ± 0.29 | 1.03 ± 0.19 |
| | **HL** | 184.7 ± 25.77 | 17.47 ± 1.58 | 9.88 ± 0.95 | 3.78 ± 0.31 | 112.5 ± 16.53 | 26.6 ± 4.22 | 23.5 ± 0.54 | 0.69 ± 0.06 |
| **Statistical significance** | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

Note: The 76 days after sowing (DAS) corresponds to the harvest date of September 2$^{nd}$, while 91 DAS corresponds to September 17$^{th}$ Low light intensity (LL) = 250 PPFD; High light intensity (HL) = 500 PPFD. For each harvest date, n.s. and * stand for non-significant or significant at $p \leq 0.05$ respectively. Different letters correspond to significant differences according to F test.

It may be possible to enhance dandelion biomass and increase fructans accumulation through plant breeding and the modulation of growth conditions.

It should be noted that dandelion can also accumulate oxalic acid [56] that can chelate bivalent calcium ions in the digestive tract. This may not pose a problem for occasional consumption on Earth but should be avoided in space, where microgravity already contributes to bones mass loss. Since the accumulation of oxalate is influenced by environmental and agronomical factors [55], the effects of controlled growth conditions must be carefully evaluated before including this species in space programs. For these reasons, dandelion was not further studied under controlled conditions in this research.

Both leaves and taproots of rampion bellflower accumulated fructans, but with different degrees of polymerization (DP) and accumulation capacities (Table 2S). While the dry leaves contained only about 1 % fructans, the taproot accumulated approximately 15 % of its dry weight in fructans. The root fructans had a much lower DP than those in the leaves, indicating possible depolymerization due to acclimation to low temperatures or preparation for bolting. Fructans are known to be present in the *Campanulaceae* family and have been previously identified in rampion bellflower [50]. Type-I inulin fructans were found in its close relative, *C. rapunculoides* ([57], this is the first report measuring fructans content and DP in both the leaves and taproot of wild rampion bellflower during the winter period. The amounts of fructans and their DP are important for both the rampion bellflower environmental adaptation and its nutritional value. While rampion bellflower is enjoyed in salads, its relatively modest fructans content and the lack of studies on its cultivation under fully controlled conditions led us to conclude that this species is unsuitable for space-oriented controlled environment agriculture (CEA) activities.

The cultivation cycle for chicory taproots intended for industrial fructans extraction typically lasts at least six months, though shorter cycles can be employed for fresh consumption. Previous research has explored the potential for growing root chicory in controlled environments [52–54], and more recent studies have focused on productivity and quality in relation to space-oriented activities [31,32]. In our study, chicory plants were grown for either 76 or 91 days after sowing (DAS) under fully controlled conditions, producing taproots with average dry weights of 6.6 g and 22.4 g (Table 1), fructans contents of 57 % and 59 % (Table 2), respectively (averaged across LL and HL growth conditions for each harvest time) and a DP similar to that found in field grown plants [54]. The fructans concentrations found in taproots produced under our protocol were lower than those typically observed in field-grown plants intended for industrial production [58,59], but similar to those found in young roots grown under comparable conditions [31,32]. The productivity and density of fructans in chicory taproots are promising characteristics that set it apart from other tested species, positioning chicory as the most viable candidate for fructans production in space and vertical farming systems. However, further clarification of the plant's responses to controlled environments is needed before establishing baseline systems and subsystems for fructans production in BLSS.

### 3.3. Manipulation of fructans content by the variation of growth condition parameters

Controlled environment variables can significantly influence the efficiency of fructans production, impacting various yield components such as taproot weight per plant and per cultivated area, the length of the cropping period, fructans concentration in the tissues, and the resources required for fructans production. These data are crucial for designing the fructans production subsystem within BLSS.

Chicory root growth and fructans accumulation are described as following a three-phase pattern [54]. Fructans accumulation in the taproot begins during the first growth phase, just before the formation of the secondary cambial meristem, which drives rapid radial growth of the taproot [53]. Before fructans accumulation starts, the taproots contain relatively high levels of glucose and fructose. However, as fructans synthesis commences, the levels of glucose and fructose decrease both in absolute terms and in relation to sucrose. During the second growth phase, characterized by a rapid increase in taproot size, the fructans concentration remains relatively stable, with a DP higher than 10 which might decrease in mature root exposed to low temperatures [54]. This suggests that new tissue

**Table 2**
Effects of growing period length and light intensity on carbohydrate composition in chicory: hexoses and sucrose (glucose, fructose, sucrose), starch, total GFS plus starch in leaves and taproots, and fructans with average degree of polymerization (DP) in taproots. Carbohydrate contents are expressed as a percentage on a dry matter basis (% DM). Data presented as average ± the standard error (S.E.).

| DAS | Growth light | Chicory leaves | | | Chicory taproots | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GFS | Starch | GFS + Starch | GFS | Starch | GFS + Starch | Fructans | DP |
| **76** | **LL** | 4.62 ± 0.96 | 0.29 ± 0.03 | 4.91 ± 0.97 | 4.60 b ± 0.20 | 0.04 ± 0.01 | 4.64 b ± 0.16 | 58.0 ± 3.18 | 11.3 ± 0.69 |
| | **HL** | 7.03 ± 0.97 | 0.37 ± 0.08 | 7.40 ± 1.01 | 5.86 a ±0.26 | 0.05 ± 0.005 | 5.91 a ±0.26 | 56.4 ± 2.97 | 14.2 ± 0.91 |
| **Statistical significance** | | n.s. | n.s. | n.s. | * | n.s. | n.s. | n.s. | n.s. |
| **91** | **LL** | 4.68 ± 0.32 | 0.58 a ±0.15 | 5.26 ± 0.59 | 1.48 ± 0.21 | 0.08 a ±0.01 | 1.55 ± 0.22 | 59.1 ± 2.58 | 11.1 ± 0.51 |
| | **HL** | 4.18 ± 0.55 | 0.27 b ± 0.04 | 4.45 ± 0.35 | 1.55 ± 0.20 | 0.03 b ± 0.01 | 1.59 ± 0.20 | 59.6 ± 3.51 | 10.5 ± 0.46 |
| **Statistical significance** | | n.s. | * | n.s. | n.s. | * | n.s. | n.s. | n.s. |

Note: The 76 days after sowing (DAS) corresponds to the harvest date of September 2nd, while 91 DAS corresponds to September 17th Low light intensity (LL) = 250 PPFD; High light intensity (HL) = 500 PPFD. For each harvest date, n.s. and * stand for non-significant or significant at $p \leq 0.05$ respectively. Different letters correspond to significant differences according to F test.

formation is coordinated with carbohydrate allocation to fructans.

Consequently, the timing of young chicory taproot harvest can greatly influence fructans productivity by affecting both taproot size, fructans content and the fructans DP. In this study, plants were harvested at 76 and 91 DAS, by which time field-grown roots typically enter the second growth phase [53]. The average total fresh weight of plants was 141.1 g at 76 DAS and 300.7 g at 91 DAS, with total dry weights of 14.4 g and 41.1 g, respectively (averaged across LL conditions) (Table 4S). The substantial difference in weight between the two harvest times indicates rapid biomass accumulation, with total biomass roughly doubling in just two weeks. Fructans with different DP might promote differential growth of gut microbes and have different prebiotic effects. This could be relevant in space related activities and deserves more specific research where DP class distribution is evaluated in relation to growth conditions and environmental control.

The percentage increase in dry weight from the first to the second harvest was higher in the root (+239 %) compared to the shoot (+107 %) (Table 1), leading to a decreased shoot/root (S/R) ratio at 91 DAS relative to 76 DAS (Table 1). Dry biomass yield per unit area averaged 3.3 g $m^{-2}$ $day^{-1}$ and 7.3 g $m^{-2}$ $day^{-1}$ for the first and second harvest dates, respectively (Table 3). The high fructans content in taproots at 76 DAS indicates that fructans accumulation had already begun. The decrease in glucose and fructose content between the first and second harvest (Table 3S), as well as the decline in the ratio of hexoses to sucrose (data not shown), supports this observation.

All these findings suggest that by 76 DAS, young chicory plants have entered the second growth phase, marked by rapid taproot weight increase and high fructans content. During this phase, fructans yield becomes more reliant on the taproot yield per unit area rather than on variations in fructans content, which increases slowly only at later growth stages.

Planting density is likely key to enhancing young taproot biomass productivity in controlled environments. In conventional fields, chicory is planted at a density of 15 plants $m^{-2}$. Increasing density may reduce the weight per root; however, total productivity remains relatively unaffected by high plant density [60]. For shorter growth cycles, like those employed in our experiments, increasing plant density could enhance biomass (and fructans) production by boosting total biomass yield without negatively impacting fructans content per unit of biomass.

The light regime is a crucial environmental variable affecting plant performance in natural ecosystems, conventional farming, and controlled environment agriculture. In fully controlled plant growth systems, the light regime significantly influences overall productivity, product quality, and energy conversion efficiency into biomass and edible products [61]. This aspect is particularly important for designing space-oriented fully controlled plant growth systems and vertical farming.

In this study, we examined the role of light intensity on young chicory taproot growth and fructans accumulation by investigating two light regimes: 250 and 500 μmol $m^{-2}$ $s^{-1}$, corresponding to DLI of 10.8 and 21.6 mol $m^{-2}$ $day^{-1}$, respectively. Data on biomass accumulation (Tables 1 and 3) consistently indicates an increase in biomass at higher light levels, though the differences were not statistically significant. This lack of significance may be attributed to the limited number of replicates in the experiment, constrained by space limitations, as well as variability in the genetic material used.

In field-grown chicory, total dry matter accumulation has been shown to correlate linearly with the canopy's absorbed photosynthetically active radiation [58]. Additionally, data in Ref. [62] demonstrated that young chicory plants grown in a partially controlled environment at an average DLI of 19 mol $m^{-2}$ $day^{-1}$ exhibited high photosynthetic rates (exceeding 20 μmol $m^{-2}$ $s^{-1}$) when tested at 800 μmol $m^{-2}$ $s^{-1}$. This suggests that the HL intensity of 500 μmol $m^{-2}$ $s^{-1}$, corresponding to a DLI of 21.6 mol $m^{-2}$ $day^{-1}$, does not induce stress on the photosynthetic function.

Furthermore, analysis of leaf carbohydrates (Tables 2 and 3S) revealed no significant differences in non-structural carbohydrates between the two light regimes. In particular, there was no accumulation of sucrose or starch—the expected end products of photosynthesis—suggesting that the plants were not experiencing sink limitations in allocating photosynthates for growth and storage [63]. This evidence indicates that plants grown under higher light intensity were able to effectively utilize photosynthetic products without

**Table 3**
Chicory yield components, efficiency, and system baselines for fructans production under fully controlled conditions. For the September 2nd harvest, data for the low light treatment are based on 3 replicates, while data for the high light treatment are based on 4 replicates. For the September 17th harvest, data are derived from 5 replicates.

| Variables | DAS | Light Intensity | | Statistical significance |
|---|---|---|---|---|
| | | LL | HL | |
| **Total plant biomass yield. (g DM $m^{-2}$ $day^{-1}$)** | 76 | 2.79 ± 0.39 | 3.87 ± 0.39 | n.s. |
| | 91 | 6.35 ± 0.61 | 8.15 ± 1.02 | n.s. |
| **Total plant biomass production per LUE. (g dm $mol^{-1}$ $PAR^{-1}$)** | 76 | 0.26 ± 0.03 | 0.18 ± 0.02 | n.s. |
| | 91 | 0.59 **a** ± 0.06 | 0.38 **b** ± 0.05 | * |
| **Fructans yield. (g $m^{-2}$ $day^{-1}$)** | 76 | 0.67 ± 0.19 | 0.96 ± 0.18 | n.s. |
| | 91 | 1.91 ± 0.31 | 2.84 ± 0.55 | n.s. |
| **Fructans production per LUE. (g $mol^{-1}$ $PAR^{-1}$)** | 76 | 0.06 ± 0.02 | 0.04 ± 0.01 | n.s. |
| | 91 | 0.16 ± 0.04 | 0.13 ± 0.03 | n.s. |
| **Cultivation area to produce 12 g of fructans. ($m^2$)** | 76 | 21.83 ± 7.32 | 14.00 ± 2.63 | n.s. |
| | 91 | 7.59 ± 2.07 | 4.90 ± 0.88 | n.s. |

Note: The 76 days after sowing (DAS) corresponds to the harvest date of September 2nd, while 91 DAS corresponds to September 17th. Low light intensity (LL) = 250 PPFD; High light intensity (HL) = 500 PPFD. LUE, light use efficiency. For each harvest date, n.s. and * stand for non-significant or significant at $p \leq 0.05$ respectively. Different letters correspond to significant differences according to F test.

**Table 4**
Validate probiotic activity of chicory taproot on the growth of four strains of lactic acid bacteria precisely *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus rhamnosus* expressed as $OD_{600}$ nm.

| Treatment | Lactic acid bacteria strains | | | |
|---|---|---|---|---|
| | *L. acidophilus* | *L. casei* | *L. plantarum* | *L. rhamnosus* |
| **MRS Control** | 2.42 a ±0.10 | 2.49 a ±0.13 | 2.45 a ±0.12 | 2.80 a ±0.13 |
| **Chicory root LL** | 1.48 b ± 0.11 | 1.47 b ± 0.09 | 1.48 b ± 0.11 | 1.50 b ± 0.15 |
| **Chicory root HL** | 1.52 b ± 0.14 | 1.50 b ± 0.14 | 1.50 b ± 0.13 | 1.53 b ± 0.12 |
| **Statistical significance** | * | * | * | * |

Data are the average of three independent experiments ± SD for MRS medium with 20 g $L^{-1}$ glucose (MRS Control) MRS medium with taproot homogenate from low light 91 DAS treatment sample (Chicory root LL) and from high light 91 DAS treatment sample (Chicory root HL). N.s and * stand for non-significant or significant at $p \leq 0.05$ respectively. Different letters correspond to significant differences according to F- test.

stress.

The efficiency of light conversion into plant biomass was significantly influenced by light intensity, showing lower efficiency at high light compared to low light (Table 3). The rapid increase in biomass accumulation between the first and second harvests markedly enhanced light conversion efficiency, which remained significantly higher at the lower light intensity, reaching 0.59 g of dry matter accumulated per mole of PAR. This efficiency is higher than that observed for wheat and soybean, but comparable to the best-performing potato crops [64]. More recently, similar light use efficiency values have been reported for various salad and vegetable crops, indicating that chicory has promising potential [14]. This potential could be further realized through optimization of growth conditions. Importantly, the entire chicory plant is edible, resulting in a harvest index close to one, meaning minimal waste, a positive attribute for use in BLSS. In contrast, studies have shown that the ratio of edible to total biomass production is less than 0.30 for wheat, under 0.40 for soybean, below 0.50 for tomato, below 0.70 for potato, and above 0.90 only in lettuce [64]. While light intensity did not significantly impact the average daily productivity of root mass and inulin, further research is needed to confirm this observation. Fructans production increased from the first (76 DAS) to the second harvest (91 DAS), reaching an average of 2.4 g of inulin produced daily per square meter (average of the LL and HL at 91 DAS in Table 3). This value represents more than half of that obtained in open-field conditions as in Refs. [58,66] underscoring the significant potential of chicory taproots for fructans production in fully controlled environments.

The European Union recommends a daily intake of 12 g of inulin for adults [65]. Under the HL conditions employed in our experiments, a cultivation area of 6.2 $m^2$ of chicory can produce this amount daily (Table 3, average for HL conditions). Such a growing area is challenging for space facilities, even for those eventually built in the future on the Moon or Mars surface. However, the area needed to provide the daily production of 12 g of inulin could be strongly reduced by i) further optimization of chicory cultivation ii) considering that a relevant fraction of prebiotic could be provided by other food sources. These results establish, for the first time, important baselines for prebiotic production in BLSS and are a relevant starting point for system design for BLSS and Earth based vertical farming facilities.

### *3.4. Chicory prebiotics can sustain microbial growth*

An increasing body of evidence supports the idea that prebiotics positively influence various human functions and health, showcasing significant potential for space exploration [67]. While several recognized prebiotics, such as inulin, are classified as dietary fibers by different authorities, not all dietary fibers qualify as prebiotics; cellulose and lignin, for example, do not. Consequently, analyzing fiber production does not equate to evaluating a crop's prebiotic potential.

We investigated the potential of extracts from chicory taproots to support the growth *in vitro* of four commercially available strains of lactic acid bacteria known for their probiotic activity: *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus rhamnosus*. Given that fructans accumulation in taproot samples harvested at 76 and 91 DAS was similar, we focused our prebiotic activity assessment on the homogenates from samples harvested at 91 DAS. The microbial test results (Table 4) indicate that chicory taproots from both LL and HL conditions supported the growth of all four probiotic strains. Although the plant extracts are not as metabolically accessible as glucose, the bacteria exhibited growth, with absorbance values exceeding 1.4 (OD600 nm) in all cases. Thus, all samples stimulated the growth of the probiotic microorganisms used in the experiments.

While the prebiotic role of isolated fructans is well established in both *in vitro* and *in vivo* studies [68], the potential of whole young chicory taproot tissue in this context has not been previously explored. Although we could not identify any antagonistic or synergistic effects of other phytochemical components present in the root on bacterial growth through this experiment, our findings highlight the *in vitro* ability of chicory root to stimulate the growth of known probiotics and thus its potential as part of symbiotic formulations.

## 4. Conclusions

Our work identifies chicory as the most promising species for efficient prebiotic production under fully controlled growth conditions for BLSS and vertical farming facilities. Recently, our group has begun defining the system requirements for prebiotic production in space, an area previously lacking even in Earth-based analogue systems [31,32]. The results presented here provide the first quantification of the cultivation area needed to meet the daily recommended dose of inulin and assess the efficiency of light used for

this production in fully controlled environments (Table 4). Such data are crucial for designing both space and terrestrial plant-based systems.

Further research is needed to understand how environmental variables and agronomic practices can enhance chicory performance without adversely affecting the nutritional content. Increasing planting density and accelerating growth cycles through environmental modulation are promising strategies.

Our findings also indicate that various chicory tissues positively influence different known probiotic microorganisms, and this effect may be influenced by growth conditions. Although fructans are not directly digestible by the human gut, they are fully fermented by gut microbiota, producing metabolites (primarily short-chain fatty acids) that benefit the host. We have demonstrated that chicory taproot homogenates can sustain the growth of four commercially available lactic acid bacteria with proven probiotic activity. Additionally, a portion of dietary fiber, such and fructans, is fermented in the colon producing metabolites that might enter the host metabolism, hence fiber contributes approximately 2.0 calories per gram ingested [69]. Consequently, a significant portion of the chicory plant could enhance human health and nutrition on multiple levels.

While confirming that chicory can thrive in a fully closed environment, our data show that the resource requirements to provide the recommended daily dose of fructans for adults can vary significantly based on crop management. Future studies aimed at optimizing environmental and agronomic techniques could further boost biomass and fructans production in fully controlled conditions. We believe our work lays the groundwork for more research into prebiotic production for space applications, aligning with the growing interest within the space research community regarding the role of food in enhancing astronaut well-being and mitigating space-related health risks, including alterations to the gut microbiome.

## CRediT authorship contribution statement

**Alberto Battistelli:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Simona Proietti:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriele Paglialunga:** Writing – review & editing, Visualization, Validation, Investigation, Data curation. **Michele Mattioni:** Methodology. **Filomena Nazzaro:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Florinda Fratianni:** Writing – review & editing, Validation, Data curation. **Giuseppe Colla:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Mariateresa Cardarelli:** Writing – review & editing, Visualization, Validation, Supervision, Data curation. **Marta Del Bianco:** Writing – review & editing. **Stefano Moscatello:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Data availability statement

Data will be made available on request. For requesting data, please write to the corresponding author.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42112.

## References

[1] V. De Micco, C. Amitrano, F. Mastroleo, G. Aronne, A. Battistelli, E. Carnero-Diaz, S. De Pascale, G. Detrell, C.-G. Dussap, R. Ganigué, Ø.M. Jakobsen, L. Poulet, R. Van Houdt, C. Verseux, S.E. Vlaeminck, R. Willaert, N. Leys, Plant and microbial science and technology as cornerstones to Bioregenerative Life Support Systems in space, Npj Microgravity 9 (2023) 69, https://doi.org/10.1038/s41526-023-00317-9.

[2] Y.M. Bar-On, R. Phillips, R. Milo, The biomass distribution on Earth, Proc. Natl. Acad. Sci. U.S.A. 115 (2018) 6506–6511, https://doi.org/10.1073/pnas.1711842115.

[3] B.S. Halpern, M. Frazier, J. Verstaen, P.-E. Rayner, G. Clawson, J.L. Blanchard, R.S. Cottrell, H.E. Froehlich, J.A. Gephart, N.S. Jacobsen, C.D. Kuempel, P. B. McIntyre, M. Metian, D. Moran, K.L. Nash, J. Többen, D.R. Williams, The environmental footprint of global food production, Nat. Sustain. 5 (2022) 1027–1039, https://doi.org/10.1038/s41893-022-00965-x.

[4] S. De Pascale, C. Arena, G. Aronne, V. De Micco, A. Pannico, R. Paradiso, Y. Rouphael, Biology and crop production in Space environments: challenges and opportunities, Life Sci. Space Res. 29 (2021) 30–37, https://doi.org/10.1016/j.lssr.2021.02.005.
[5] T.W. Halstead, F.R. Dutcher, Plants in space, Annu. Rev. Plant Physiol. 38 (1987) 317–345, https://doi.org/10.1146/annurev.pp.38.060187.001533.
[6] C.M. Johnson, H.O. Boles, L.E. Spencer, L. Poulet, M. Romeyn, J.M. Bunchek, R. Fritsche, G.D. Massa, A. O'Rourke, R.M. Wheeler, Supplemental food production with plants: a review of NASA research, Front. Astron. Space Sci. 8 (2021), https://doi.org/10.3389/fspas.2021.734343.
[7] R.M. Wheeler, Agriculture for space: people and places paving the way, Open Agric 2 (2017) 14–32, https://doi.org/10.1515/opag-2017-0002.
[8] P. Zabel, M. Bamsey, D. Schubert, M. Tajmar, Review and analysis of over 40 years of space plant growth systems, Life Sci. Space Res. 10 (2016) 1–16, https://doi.org/10.1016/j.lssr.2016.06.004.
[9] G. Xie, J. Ding, Y. Zhang, M. Ren, D. Qiu, X. Wang, The first biological experiment on lunar surface for Humankind: device and results, Acta Astronaut. 214 (2024) 216–223, https://doi.org/10.1016/j.actaastro.2023.10.023.
[10] C. Dong, L. Shao, Y. Fu, M. Wang, B. Xie, J. Yu, H. Liu, Evaluation of wheat growth, morphological characteristics, biomass yield and quality in Lunar Palace-1, plant factory, greenhouse and field systems, Acta Astronaut. 111 (2015) 102–109, https://doi.org/10.1016/j.actaastro.2015.02.021.
[11] F. Gòdia, J. Albiol, J. Pérez, N. Creus, F. Cabello, A. Montràs, A. Masot, Ch Lasseur, The MELISSA pilot plant facility as an integration test-bed for advanced life support systems, Adv. Space Res. 34 (2004) 1483–1493, https://doi.org/10.1016/j.asr.2003.08.038.
[12] J. Li, N. Zhang, J. Luo, Q. Yu, W. Ai, L. Zhang, Y. Tang, Growth and biomass yield of 25 crops in the 4-subject 180-day integrated experiment, Acta Astronaut. 162 (2019) 336–343, https://doi.org/10.1016/j.actaastro.2019.06.028.
[13] M.T.P. Nguyen, M. Knowling, N.N. Tran, A. Burgess, I. Fisk, M. Watt, M. Escribà-Gelonch, H. This, J. Culton, V. Hessel, Space farming: horticulture systems on spacecraft and outlook to planetary space exploration, Plant Physiol. Biochem. 194 (2023) 708–721, https://doi.org/10.1016/j.plaphy.2022.12.017.
[14] P. Zabel, C. Zeidler, V. Vrakking, M. Dorn, D. Schubert, Biomass production of the EDEN ISS space greenhouse in Antarctica during the 2018 experiment phase, Front. Plant Sci. 11 (2020), https://doi.org/10.3389/fpls.2020.00656.
[15] G.L. Douglas, M. Cooper, D. Bermudez-Aguirre, M.E. Leidos, T. Sirmons, M. Perchonok, Evidence report: risk of performance decrement and crew illness due to an inadequate food system [online]. Available at: https://humanresearchroadmap.nasa.gov/Risks/risk.aspx?i=87, 2016.
[16] G.L. Douglas, D. DeKerlegand, H. Dlouhy, N. Dumont-Leblond, E. Fields, M. Heer, S. Krieger, S. Mehta, B.V. Rooney, M.G. Torralba, S.E. Whiting, B. Crucian, H. Lorenzi, S.M. Smith, M. Young, S.R. Zwart, Impact of diet on human nutrition, immune response, gut microbiome, and cognition in an isolated and confined mission environment, Sci. Rep. 12 (1) (2022) 20847, https://doi.org/10.1038/s41598-022-21927-5.
[17] S.M. Smith, S.R. Zwart, M. Heer, Evidence Report: Risk Factor of Inadequate Nutrition, NASA Technical Report, 2015.
[18] H. Tang, H.H. Rising, M. Majji, R.D. Brown, Long-term space nutrition: a scoping review, Nutrients 14 (1) (2021) 194, https://doi.org/10.3390/nu14010194.
[19] K.F. Al, J.A. Chmiel, G.A. Stuivenberg, G. Reid, J.P. Burton, Long-duration space travel support must consider wider influences to conserve microbiota composition and function, Life 12 (8) (2022) 1163, https://doi.org/10.3390/life12081163.
[20] A.P. Koehle, S.L. Brumwell, E.P. Seto, A.M. Lynch, C. Urbaniak, Microbial applications for sustainable space exploration beyond low Earth orbit, npj Microgravity 9 (1) (2023) 47, https://doi.org/10.1038/s41526-023-00285-0.
[21] A.A. Voorhies, C.M. Ott, S. Mehta, D.L. Pierson, B.E. Crucian, A. Feiveson, C.M. Oubre, M. Torralba, K. Moncera, Y. Zhang, E. Zurek, H.A. Lorenzi, Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome, Sci. Rep. 9 (1) (2019) 9911, https://doi.org/10.1038/s41598-019-46303-8.
[22] E. Gonzalez, M.D. Lee, B.T. Tierney, N. Lipieta, P. Flores, M. Mishra, L. Beckett, et al., Spaceflight alters host-gut microbiota interactions, bioRxiv (2024), https://doi.org/10.1101/2024.01.
[23] F. Asnicar, S.E. Berry, A.M. Valdes, L.H. Nguyen, G. Piccinno, D.A. Drew, E. Leeming, et al., Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals, Nat. Med. 27 (2) (2021) 321–332, https://doi.org/10.1038/s41591-020-01183-8.
[24] A.L. Kau, P.P. Ahern, N.W. Griffin, A.L. Goodman, J.I. Gordon, Human nutrition, the gut microbiome and the immune system, Nature 474 (7351) (2011) 327–336, https://doi.org/10.1038/nature10213.
[25] M. Valles-Colomer, C. Menni, S.E. Berry, A.M. Valdes, T.D. Spector, N. Segata, Cardiometabolic health, diet and the gut microbiome: a meta-omics perspective, Nat. Med. 29 (3) (2023) 551–561, https://doi.org/10.1038/s41591-023-02260-4.
[26] G.R. Gibson, R. Hutkins, M.E. Sanders, S.L. Prescott, R.A. Reimer, S.J. Salminen, K. Scott, et al., Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics, Nat. Rev. Gastroenterol. Hepatol. 14 (8) (2017) 491–502, https://doi.org/10.1038/nrgastro.2017.75.
[27] J.I. Ramírez-Pérez, P.E. Álvarez-Gutiérrez, M.C. Luján-Hidalgo, S.L. Ovando-Chacón, R.E. Soria-Guerra, M.Á. Ruiz-Cabrera, A. Grajales-Lagunes, M. Abud-Archila, Effect of linear and branched fructans on growth and probiotic characteristics of seven Lactobacillus spp. isolated from an autochthonous beverage from Chiapas, Mexico, Arch. Microbiol. 204 (7) (2022) 364, https://doi.org/10.1007/s00203-022-02984-w.
[28] K.P. Scott, R. Grimaldi, M. Cunningham, S.R. Sarbini, A. Wijeyesekera, M.L.K. Tang, J.C.-Y. Lee, et al., Developments in understanding and applying prebiotics in research and practice—an ISAPP conference paper, J. Appl. Microbiol. 128 (4) (2020) 934–949, https://doi.org/10.1111/jam.14424.
[29] K.S. Swanson, W.M. de Vos, E.C. Martens, J.A. Gilbert, R.S. Menon, A. Soto-Vaca, J. Hautvast, et al., Effect of fructans, prebiotics and fibres on the human gut microbiome assessed by 16S rRNA-based approaches: a review, Benef. Microbes 11 (2) (2020) 101–129, https://doi.org/10.3920/BM2019.0082.
[30] M.C. Rodríguez-Daza, E.C. Pulido-Mateos, J. Lupien-Meilleur, D. Guyonnet, Y. Desjardins, D. Roy, Polyphenol-mediated gut microbiota modulation: toward prebiotics and further, Front. Nutr. 8 (2021), https://doi.org/10.3389/fnut.2021.689456.
[31] S. Moscatello, S. Proietti, A. Severoni, A. Battistelli, Simple procedure to enhance pulsed amperometric detector (PAD) response stability for inulin-type fructans analysis. Application to a case study with chicory taproot, Food Chem. 430 (2024) 136923, https://doi.org/10.1016/j.foodchem.2023.136923.
[32] G. Paglialunga, S. Proietti, M. Cardarelli, S. Moscatello, G. Colla, A. Battistelli, Chicory taproot production: effects of biostimulants under partial or full controlled environmental conditions, Agronomy 12 (11) (2022) 2816, https://doi.org/10.3390/agronomy12112816.
[33] R.M. Wheeler, C.L. Mackowiak, J.C. Sager, W.M. Knott, W.L. Berry, Proximate composition of CELSS crops grown in NASA's biomass production chamber, Adv. Space Res. 18 (4–5) (1996) 43–47, https://doi.org/10.1016/0273-1177(95)00860-H.
[34] N.Tr Petkova, I.G. Ivanov, M. Raeva, M.G. Topuzova, M.M. Todorova, P.P. Denev, Fructans and antioxidants in leaves of culinary herbs from Asteraceae and Amaryllidaceae families, Food Res. 3 (5) (2019) 407–415, https://doi.org/10.26656/fr.2017.3(5).030.
[35] K. Schütz, R. Carle, A. Schieber, *Taraxacum*—a review on its phytochemical and pharmacological profile, J. Ethnopharmacol. 107 (3) (2006) 313–323, https://doi.org/10.1016/j.jep.2006.07.021.
[36] V. Vinelli, P. Biscotti, D. Martini, C. Del Bo, M. Marino, T. Meroño, O. Nikoloudaki, et al., Effects of dietary fibers on short-chain fatty acids and gut microbiota composition in healthy adults: a systematic review, Nutrients 14 (13) (2022) 2559, https://doi.org/10.3390/nu14132559.
[37] M.E. Musgrave, A. Kuang, Y. Xiao, S.C. Stout, G.E. Bingham, L.G. Briarty, M.A. Levinskikh, V.N. Sychev, I.G. Podolski, Gravity independence of seed-to-seed cycling in *Brassica rapa*, Planta 210 (3) (2000) 400–406, https://doi.org/10.1007/PL00008148.
[38] S. De Pascale, F. Orsini, S. Vallone, G. Barbieri, Crop season effects on yield and quality of hydroponically grown *Brassica rapa* var. *sylvestris*, Acta Hortic. 807 (2009) 427–432, https://doi.org/10.17660/ActaHortic.2009.807.60.
[39] D. Giannino, G. Testone, C. Nicolodi, L. Giorgetti, L. Bellani, M. Gonnella, M. Ciardi, P. Cappuccio, S. Moscatello, A. Battistelli, V. Longo, Nutritive parameters and antioxidant quality of minimally processed "Cime di Rapa" (*Brassica rapa* subsp. *sylvestris*) vary as influenced by genotype and storage time, Pol. J. Food Nutr. Sci. (2020) 337–346, https://doi.org/10.31883/pjfns/126617.
[40] E. Botticella, F. Sestili, F. Sparla, S. Moscatello, L. Marri, J.A. Cuesta-Seijo, G. Falini, A. Battistelli, P. Trost, D. Lafiandra, Combining mutations at genes encoding key enzymes involved in starch synthesis affects the amylose content, carbohydrate allocation and hardness in the wheat grain, Plant Biotechnol. J. 16 (10) (2018) 1723–1734, https://doi.org/10.1111/pbi.12908.
[41] C.J. Pollock, A.J. Cairns, Fructan metabolism in grasses and cereals, Annu. Rev. Plant Biol. 42 (1) (1991) 77–101, https://doi.org/10.1146/annurev.pp.42.060191.000453.

[42] J. Verspreet, S. Cimini, R. Vergauwen, E. Dornez, V. Locato, K. Le Roy, L. De Gara, W. Van den Ende, J.A. Delcour, C.M. Courtin, Fructan metabolism in developing wheat (*Triticum aestivum* L.) kernels, Plant Cell Physiol. 54 (12) (2013) 2047–2057, https://doi.org/10.1093/pcp/pct144.
[43] E.N. Baranova, M.A. Levinskikh, A.A. Gulevich, Wheat space odyssey: "From seed to seed". Kernel morphology, Life 9 (4) (2019) 81, https://doi.org/10.3390/life9040081.
[44] A. Niroula, S. Khatri, R. Timilsina, D. Khadka, A. Khadka, P. Ojha, Profile of chlorophylls and carotenoids of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) microgreens, J. Food Sci. Technol. 56 (5) (2019) 2758–2763, https://doi.org/10.1007/s13197-019-03768-9.
[45] R.J. Henry, A comparison of the non-starch carbohydrates in cereal grains, J. Sci. Food Agric. 36 (12) (1985) 1243–1253, https://doi.org/10.1002/jsfa.274036120.
[46] L.D. Incoll, G.D. Bonnett, The occurrence of fructan in food plants (1993) 309–322, https://doi.org/10.1016/B978-0-444-89369-7.50046-4.
[47] M. González-Castejón, F. Visioli, A. Rodriguez-Casado, Diverse biological activities of dandelion, Nutr. Rev. 70 (9) (2012) 534–547, https://doi.org/10.1111/j.1753-4887.2012.00509.x.
[48] C. Schlindwein, D. Wittmann, C.F. Martins, A. Hamm, J.A. Siqueira, D. Schiffler, I.C. Machado, Pollination of *Campanula rapunculus* L. (Campanulaceae): how much pollen flows into pollination and reproduction of oligolectic pollinators? Plant Syst. Evol. 250 (3–4) (2005) 147–156, https://doi.org/10.1007/s00606-004-0246-8.
[49] M. Bencivenga, B. Romano, Aspetti della biologia di *Campanula rapunculus* L., Riv. Ortofrutt. Ital, 1984, pp. 235–247.
[50] J.S.D. Bacon, Carbohydrates of the rampion, *Campanula rapunculus* L, Nature 184 (4703) (1959) 1957, https://doi.org/10.1038/1841957a0, 1957.
[51] R.A. Street, J. Sidana, G. Prinsloo, *Cichorium intybus*: traditional uses, phytochemistry, pharmacology, and toxicology, Evid.-Based Complement, Altern. Med. 2013 (2013) 1–13, https://doi.org/10.1155/2013/579319.
[52] J. De Roover, A. Van Laere, W. Van den Ende, Effect of defoliation on fructan pattern and fructan metabolizing enzymes in young chicory plants (*Cichorium intybus*), Physiol. Plantarum 106 (2) (1999) 158–163, https://doi.org/10.1034/j.1399-3054.1999.106202.x.
[53] N. Druart, J. De Roover, W. Van den Ende, P. Goupil, A. Van Laere, S. Rambour, Sucrose assimilation during early developmental stages of chicory (*Cichorium intybus* L.) plants, Planta 212 (3) (2001) 436–443, https://doi.org/10.1007/s004250000414.
[54] J. van Arkel, R. Vergauwen, R. Sévenier, J.C. Hakkert, A. Van Laere, H.J. Bouwmeester, A.J. Koops, I.M. van der Meer, Sink filling, inulin metabolizing enzymes and carbohydrate status in field-grown chicory (*Cichorium intybus* L.), J. Plant Physiol. 169 (15) (2012) 1520–1529, https://doi.org/10.1016/j.jplph.2012.06.005.
[55] S. Proietti, S. Moscatello, G.A. Giacomelli, A. Battistelli, Influence of the interaction between light intensity and CO2 concentration on productivity and quality of spinach (*Spinacia oleracea* L.) grown in fully controlled environment, Adv. Space Res. 52 (6) (2013) 1193–1200, https://doi.org/10.1016/j.asr.2013.06.005.
[56] S.A. Petropoulos, Â. Fernandes, N. Tzortzakis, M. Sokovic, A. Ciric, L. Barros, I.C.F.R. Ferreira, Bioactive compounds content and antimicrobial activities of wild edible *Asteraceae* species of the Mediterranean flora under commercial cultivation conditions, Food Res. Int. 119 (2019) 859–868, https://doi.org/10.1016/j.foodres.2018.10.069.
[57] R. Vergauwen, W. Van den Ende, A. Van Laere, The role of fructan in flowering of *Campanula rapunculoides*, J. Exp. Bot. 51 (348) (2000) 1261–1266, https://doi.org/10.1093/jexbot/51.348.1261.
[58] A. Monti, M.T. Amaducci, G. Pritoni, G. Venturi, Growth, fructan yield, and quality of chicory (*Cichorium intybus* L.) as related to photosynthetic capacity, harvest time, and water regime, J. Exp. Bot. 56 (415) (2005) 1389–1395, https://doi.org/10.1093/jxb/eri140.
[59] R.G. Wilson, J.A. Smith, C.D. Yonts, Chicory root yield and carbohydrate composition is influenced by cultivar selection, planting, and harvest date, Crop Sci. 44 (3) (2004) 748–752, https://doi.org/10.2135/cropsci2004.7480.
[60] J. Panahandeh, S. Abdollahi, H.D. Kazemnia, N. Mahna, Effects of plant density on root yield and leaf area in chicory (*Cichorium intybus* L.), Acta Hortic. 946 (2012) 245–249, https://doi.org/10.17660/ActaHortic.2012.946.27.
[61] S. Proietti, S. Moscatello, F. Riccio, P. Downey, A. Battistelli, Continuous lighting promotes plant growth, light conversion efficiency, and nutritional quality of *Eruca vesicaria* (L.) cav. In controlled environment with minor effects due to light quality, Front. Plant Sci. 12 (2021), https://doi.org/10.3389/fpls.2021.730119.
[62] M. D'Imperio, F.F. Montesano, M. Renna, B. Leoni, D. Buttaro, A. Parente, F. Serio, NaCl stress enhances silicon tissue enrichment of hydroponic "baby leaf" chicory under biofortification process, Sci. Hortic. 235 (2018) 258–263, https://doi.org/10.1016/j.scienta.2018.03.001.
[63] S. Moscatello, S. Proietti, A. Augusti, A. Scartazza, R.P. Walker, F. Famiani, A. Battistelli, Late summer photosynthesis and storage carbohydrates in walnut (*Juglans regia* L.): feed-back and feed-forward effects, Plant Physiol. Biochem. 118 (2017) 618–626, https://doi.org/10.1016/j.plaphy.2017.07.025.
[64] R.M. Wheeler, Carbon balance in bioregenerative life support systems: some effects of system closure, waste management, and crop harvest index, Adv. Space Res. 31 (1) (2003) 169–175, https://doi.org/10.1016/S0273-1177(02)00742-1.
[65] EFSA, Scientific Opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006, EFSA J. 13 (1) (2015) 3951, https://doi.org/10.2903/j.efsa.2015.3951.
[66] J.R.A. Baert, E.J. Van Bockstaele, Cultivation and breeding of root chicory for inulin production, Ind. Crops Prod. 1 (2–4) (1992) 229–234, https://doi.org/10.1016/0926-6690(92)90023-O.
[67] S. Turroni, M. Magnani, P. Kc, P. Lesnik, H. Vidal, M. Heer, Gut microbiome and space travelers' health: state of the art and possible pro/prebiotic strategies for long-term space missions, Front. Physiol. 11 (2020), https://doi.org/10.3389/fphys.2020.553929.
[68] S. Kolida, K. Tuohy, G.R. Gibson, Prebiotic effects of inulin and oligofructose, Br. J. Nutr. 87 (S2) (2002) S193–S197, https://doi.org/10.1079/BJN/2002537.
[69] EFSA, Scientific opinion on dietary reference values for energy, EFSA J. 11 (1) (2013) 3005, https://doi.org/10.2903/j.efsa.2013.3005.