2018•2019

Faculteit Industriële ingenieurswetenschappen

master in de industriële wetenschappen: biochemie

Masterthesis

Growth and nutritional value of a selection of microgreen plants under space conditions to evaluate their use as astronauts food

PROMOTOR :

dr. ir. Kristel SNIEGOWSKI

PROMOTOR :

prof. dr. Nele HOREMANS

BEGELEIDER :

Dhr. Robin NAUTS

Sara Budé

Scriptie ingediend tot het behalen van de graad van master in de industriële wetenschappen: biochemie

Gezamenlijke opleiding UHasselt en KU Leuven

UHASSELT

KU LEUVEN

UHASSELT

KU LEUVEN

2018•2019

Faculteit Industriële ingenieurswetenschappen

master in de industriële wetenschappen: biochemie

Masterthesis

Growth and nutritional value of a selection of microgreen plants under space conditions to evaluate their use as astronauts food

PROMOTOR :

dr. ir. Kristel SNIEGOWSKI

PROMOTOR :

prof. dr. Nele HOREMANS

BEGELEIDER :

Dhr. Robin NAUTS

Sara Budé

Scriptie ingediend tot het behalen van de graad van master in de industriële wetenschappen: biochemie

UHASSELT KU LEUVEN

Preface

This master's thesis was conducted as a conclusion of my studies in biochemical engineering technology at Hasselt University and KU Leuven. It was made possible by the Belgian nuclear research centre (SCK•CEN) and more specifically the Biosphere impact studies (BIS) research unit.

First of all, I would like to thank prof. dr. Nele Horemans for allowing me to do thesis and for her guidance. Without her help, this thesis would not have been possible. I would also like to thank dr. ir. Kristel Sniegowski for her support with writing this thesis. Further I would like to thank Robin Nauts for his guidance and help in the laboratory and with processing the results. Finally, I would like to thank the people from the BIS research unit and the other interns for their company and support.

Sara Budé

Table of contents

List of tables

List of figures

Glossary of terms

BIS	Biosphere impact studies.
DNA	Deoxyribonucleic acid.
DSB	Double stranded break.
FRAP	Ferric reducing antioxidant power.
GCR	Galactic cosmic radiation.
HPS	High-pressure sodium.
HSP	Heath shock protein.
ISS	International Space Station.
LED	Light-emitting diode.
µ*g*	Microgravity.
PAR	Photosynthetic active radiation.
ROS	Reactive oxygen species.
RPM	Random positioning machine.
SCK•CEN	Studiecentrum voor Kernenergie - Centre d'Etude de l'Energie Nucléaire.
SE	Standard error.
SEP	Solar energetic particle.

Abstract

The Belgian nuclear research centre (SCK•CEN) performs research on peaceful applications of radioactivity in society. One of its goals is to determine the effect of space radiation and reduced gravity on the growth and nutritional value of microgreens. These young plants are of interest as potential food for astronauts on long-term missions because they have a higher nutrient value compared to their full grown varieties and can be grown in small, enclosed environments. This thesis aims is to determine if the adverse space conditions, microgravity and increased radiation, affect the growth and the nutritional value of a selection of microgreens.

Three microgreens, red arrow radish, red cabbage and opal basil, are selected based on their high nutrient value. The microgreens are grown for 7-14 days during which they are exposed to gamma radiation (15.1 mGy/h) and/or microgravity (~0.1 g). After harvest, proteins, antioxidative capacity, carbohydrates, lignin and pigment quantity are determined.

While radish plants that experience microgravity or radiation alone are not affected, combining these factors leads to a decrease in pigments. The red cabbage plants are more susceptible with changes to radiation and microgravity but show less significant differences when they are treated with the combination of these factors. Opal basil shows a decrease in pigments when the plants experience microgravity. Hence this pilot study showed that the nutritional value of plants is affected by adverse space conditions in a plant specific way.

Abstract in het Nederlands

Het studiecentrum voor kernenergie (SCK•CEN) onderzoekt vreedzame toepassingen van radioactiviteit. Een van hun doelstellingen is het onderzoeken van het effect van straling en verlaagde zwaartekracht in de ruimte op de groei en ontwikkeling van jonge planten genaamd microgreens. Deze planten zijn interessant om gebruikt te worden als voedingsbron voor astronauten omdat ze een hogere voedingswaarde hebben dan de volgroeide varianten en omdat ze in kleine ruimtes kunnen gekweekt worden. Het doel van deze thesis is het bepalen van het effect van de ruimtefactoren (straling en microgravitеit) op bepaalde aspecten van de voedingswaarde van een selectie microgreens.

Eerst worden er drie soorten microgreens geselecteerd op basis van hun voedingswaarde. Dit zijn red-arrow radijs, rode kool en opal basilicum. Deze microgreens worden dan voor 7-14 dagen opgekweekt terwijl ze worden blootgesteld aan gamma straling (15.1 mGy/h) en/of microgravitеit (~0.1 g). Na het oogsten wordt de hoeveelheid proteïnen, suiker en zetmeel, lignine, pigmenten en de antioxidatieve capaciteit bepaald.

De hoeveelheid pigmenten van de radijsplanten verschilt meer van de controlestalen wanneer ze behandeld worden met de combinatie van straling en microgravitеit terwijl de rode kool planten dan juist minder verschillen in antioxidanten en pigmenten vertonen. De basilicum die microgravitеit heeft ervaren, heeft een lagere hoeveelheid pigmenten. Deze pilootstudie toont dus aan dat er een soort-afhankelijke reactie is op de verschillende behandelingen.

1 Introduction

Exploring space has always been something triggering people's imagination. Just looking at the night sky can make you wonder how many things you cannot see from tiny planet earth. This curiosity is one of the drivers for the desire of humans for space exploration.

Since the first manned space flight in 1961 [1], humans have travelled to space, but only a few, like the ones from NASA's Apollo missions, have been outside the atmosphere and the magnetic field of the earth [2]. At the moment, institutes like NASA are researching the possibilities of travelling to other planets and even living in space [3]. But it is currently not yet possible to send astronauts on interplanetary missions because of technical limitations in bringing sufficient recourses along like water and food on the one hand, and adverse space conditions that might be life threatening on the other hand [4]. Table 1.1 shows the different gravitational accelerations and radiation levels on the earth, the International Space Station (ISS), the moon, in deep space and on Mars.

As stated above, one problem that comes with long space missions is that it is impossible to take enough food and water along to provide for the whole crew [5]. A crew of four astronauts who would travel to Mars and back would take more than three years to complete this mission which would be equal to more than ten tons of food. Additionally, an astronaut in the ISS uses approximately 11 liters of water a day. Assuming that they could not recycle the water, they should bring more than 12 000 liters of water per astronaut for a three year trip to Mars and back [6]. Therefore, research facilities examine the possibilities for recycling water and the production of food and oxygen in space stations (an advanced regenerative life support system). One of these research facilities is the Belgian nuclear research facility SCK•CEN [5].

SCK•CEN (Studiecentrum voor Kernenergie - Centre d'Etude de l'Energie Nucléaire) is a Belgian research facility located in Mol that explores the peaceful applications of radioactivity and develops nuclear technologies that are of use in society. SCK•CEN consists of three scientific institutes: the institute for nuclear materials science, the institute for advanced nuclear systems, and the institute for environment, health and safety [7]. This master thesis is covered by the latter institute, and more specifically by the research unit Biosphere Impact Studies (BIS) which falls under the Interdisciplinary Biosciences expert group [8]. One of the major objectives of the BIS unit consists out of studying the "biological effects induced in plants by radiation, radionuclide uptake and mixed contaminant conditions at different levels of biological organization"[9]. The subject of this master thesis falls under this objective and aims to examine the effect of space radiation and microgravity on the growth and nutritional value of young plant seedlings called microgreens.

Microgreens are young, immature plants that differ from sprouts and baby greens because they are smaller than baby greens and harvested later than sprouts (see Figure 1.1) [10]. These plants have been the focus of recent research because they have a higher nutrient value [11], [12] and lower amounts of nitrates [13] than their mature counterparts and because they provide good

sources for many minerals [13], [14]. Due of these traits, microgreens are investigated to be cultivated inside a space station during long-term space missions [15].

Figure 1.1: Example of microgreens: 1.5 week old red cabbage (Brassica oleracea L. var. capitate) plants

On its voyage to, for example, Mars, a spaceship and its crew will be mostly outside the magnetic field of the earth [16]. This field, along with the atmosphere, protects the earth and its inhabitants from among others space radiation [17]. This is a type of ionizing radiation, coming from several different sources, that can do significant harm to living organisms [17]. Another factor that objects in space experience is microgravity. This is the weightlessness that is observed when an object is in free-fall in a vacuum [18], [19]. Microgravity is also used to indicate environments with reduced gravity, which means, where lower gravitational forces than the ones experienced on earth exist (1 g, see Table 1.1) [20].

Table 1.1: Gravitation and radiation levels on earth, the International Space Station (ISS), the moon, deep space and Mars

	Earth	**ISS**	**Moon**	**Deep space**	**Mars**
Gravitation (g)	1	0.89 [19]	0.17 [20]	≈ 0	0.38 [20]
Radiation (mSv/y)	2.4 [21]	200-400 [22]	110-380 [23]	620 [24]	220 [24]

It is known that the environmental factors in a space station (radiation and microgravity) have an influence on humans and other organisms including plants. For example, Hampp et al. [25] demonstrated that microgravity causes a decrease in necessary metabolic energy to regenerate a damaged membrane in Tobacco protoplasts. It is also demonstrated in some plants that microgravity has an influence on the amounts of calcium in the cells, on the expression level of a number of genes, and on the quantity of poly- and monosaccharides [26]. The expression of genes can also be up or down-regulated by gene alterations caused by chronic low-dose radiation. Functions of commonly affected genes are: cell wall synthesis, and secondary metabolite synthesis [27]. These genes are typically affected in plants under stress and can lead

to a changed metabolism and growth and development. These changes in expression of genes can affect the nutrient value of the plants in a positive or a negative way. Not every plant reacts the same to radiation and microgravity, which makes it hard to predict a specific plant's response [28]. The effect of the radiation and microgravity on the nutritional value of the selected microgreens has not been studied thoroughly [15]. Because of this, it is currently not possible to determine if these microgreen plants are appropriate to use as astronauts food.

If the effect of these environmental space factors on the nutritional value of microgreens is not known while they are being used as a food source for astronauts, the wellbeing of the astronauts might be at risk for deficiency disorders and their health can be compromised [29], [30]. Additionally, if enhanced radiation or microgravity would cause the plants to grow slow or not at all, the astronauts could have a shortage of food. Because of this, it is important to know the effects of a space environment on the growth and the nutritional value of plants that are used as a nutrient source for some essential amino acids, vitamins, antioxidants, and other nutrients during a space mission [31].

This concludes to the main question of this research: What is the effect of simulated space radiation and microgravity on the nutritional value of a selection of microgreen plants?

The aim of this master thesis is to determine if radiation and/or microgravity has a significant effect on the nutritional value of a selection of microgreen plants. To accomplish this goal, the following research objectives need to be achieved.

First, a literature study is conducted to gather information about space conditions, the simulation of them and how they influence the growth of plants. Further, the parameters that influence the nutritional value of plants which are analysed during this research are clarified. This literature study was necessary as this research topic is a pilot project of the BIS research unit. After this, three species of microgreen plants that have a high nutritional value (red arrow radish (*Raphanus sativus* L.), red cabbage (*Brassica oleracea* L. var. *capitate*), and opal basil (*Ocimum basilicum* L.)) are cultivated during which they are irradiated and/or experience microgravity.

After the harvest, analyses are conducted to determine a number of parameters important in the nutritional value of the microgreens. The parameters that are examined are: total protein content, the total antioxidative capacity, the photosynthetic pigment content, the amount of soluble sugars and starch, and the lignin quantity. Additionally, the photosynthetic activity of the radish plants is measured. These parameters can give a view on the effects of radiation and microgravity on the nutrient value of a plant. The photosynthetic capacity as one of the first to respond to stress will be used as well as a parameter for the plant's health and for the capacity to produce oxygen. Higher antioxidant levels are beneficial for humans but are in plants also a defence mechanism to protect cells to enhanced oxidative stress and therefore indicate the plant's stress level.

The results of these experiments are then statistically compared to determine if there are significant differences between the irradiated plants, the plants that were irradiated while

experiencing microgravity, the plants that only experienced microgravity, and the control plants.

Obtaining these results should lead to a better view on the usefulness of the selected microgreens as food for astronauts on long space missions.

2 Literature study

To fully understand the topic of this research and to determine which tests are necessary for the determination of the nutritional value of the microgreens, a literature study is required. First, the difference between microgreens and normal plants is clarified. After this, the factors that generally influence the growth and development of plants are listed. Then, the effect of factors specific to a space environment on the development of plants is investigated, along with the different methods to simulate these factors. Finally, the aspects influencing the nutritional value that are researched in this thesis and the methods used to determine these factors are explained.

2.1 Microgreens

Microgreens are young immature plants that differ from sprouts and baby greens because they are smaller than baby greens and harvested at approximately 7-21 days after germination depending on the species, which is later than sprouts [10], [32]. These microgreens have been the focus of recent research because they have a higher nutritional value than their mature counterparts [11], [12], provide good sources for many minerals [13], [14] and have lower quantities of nitrates [13]. The lower amount of nitrate in the plant is beneficial because nitrate can be converted into nitrite in humans. This nitrite can then bind to haemoglobin and prevent oxygen from being distributed through the body [33]. Some microgreens even have significant concentrations of glucosinolates [34], these are the precursors of isothiocyanates, molecules that are known to induce specific pathways in mammals that block the growth of tumours [35]. Table 2.1 shows the most common families of microgreens with at least one example for each. This thesis focusses on red arrow radish (Raphanus sativus L.), red cabbage (*Brassica oleracea* L. var. *capitate*), and opal basil (*Ocimum basilicum* L.) because these plants contained the highest amount of antioxidants of a selection of 25 microgreens [11].

Table 2.1: Common families of microgreens with examples [11], [36], [37]

Botanic family	**Example(s)**
Brassicaceae	Broccoli, radish and cabbages
Lamiaceae	Basil
Asteraceae	Lettuce
Apiaceae	Carrot, dill and celery
Amarillydaceae	Garlic, onion and leek
Amaranthaceae	Amaranth, beet and spinach
Cucurbitaceae	Cucumber and melon

Recently, researchers began examining the cultivation of microgreens under space conditions [15], [38], [39]. The reason for this is because microgreens could be used as a food source, to produce oxygen and to recycle water [15]. Another advantage that comes with the usage of plants in space stations besides the production of oxygen and fresh food, is that they are beneficial for the metal health of the astronauts [40], [41]. However, a problem with these microgreens for their potential use in space is that they generate little oxygen which could be solved by growing them in multi-layered farming-systems or combining them with larger edible crops [15].

2.2 Plant growth influencing factors

The most important factors that can influence the growth of plants in growth chambers are light, temperature, carbon dioxide concentration in the air, and the production of ethylene by the plants [42]. Table 2.2 shows the optimal growth conditions the three plants that are used in this thesis.

Table 2.2: Optimal growth conditions of plants used in this thesis (No value found: /)

	Radish (*Raphanus sativus* L.) [11]	**Basil (*Ocimum basilicum* L.)** [11]	**Red cabbage (*Brassica oleracea* L. var. *capitate*)** [11]
Light intensity (µmol/m^{2}s)	240-300 [43]	500 [44]	480 [45]
Growth temperature (°C)	24 [46]	30 [47]	15.5 – 18 [48]
CO_2 (ppm)	5000 [49]	/	1000 [45]

2.2.1 Light

One of the most important environmental factors that can influence the growth and development of a plant is the illumination. It has to meet several demands in terms of spectral quality, quantity and the duration of the lighting period (photoperiod) [50], [51]. The spectral quality of light refers to its ability to deliver the wavelengths that the plants need in order to develop and function properly [52]. Not every wavelength is equally absorbed by the plants, chlorophyll, which absorbs the light energy used for photosynthesis, absorbs far red (700 - 800 nm), red (600 - 700 nm) and blue light (400 - 500 nm) [50]. Research has shown that blue light is necessary for the normal development of plants, but the optimal amount of blue light depends on the plant species [53]. There are however other wavelengths that can affect the growth and development of a plant. Green and far red light can function as an indicator for growing conditions of plants and alter its development [54], [55]. As indicated above, the light intensity also has an influence on the growth and development of plants. An increased light intensity will generally lead to a higher photosynthetic activity. There is however a maximal light intensity after which the photosynthetic activity of the plant stays constant, this is the light saturation

point [56]. Finally, the photoperiod refers to the number of hours the plants are illuminated a day. Based on the photoperiod, there are three types of plants: the short-day plants, the long-day plants and the day-neutral plants. The short-day plants flower when the lighting period is shorter than the dark period while the long-day plants need longer lighting periods than dark periods. The flowering of day-neutral plants does not depend on the length of the lighting period [56].

The most commonly used lamps in greenhouses are high-pressure sodium (HPS) lamps. This is because they are more efficient in converting electrical energy to photosynthetic active radiation (PAR) than other high intensity discharge lamps like metal halide and fluorescent lamps [57]. Photosynthetic active radiation is the electromagnetic radiation that can be used by green plants to produce energy via photosynthesis [58]. However, 75% of the electrical energy used by these lamps is lost in thermal radiation, which causes the lamp to heat up to 450°C at the surface. Because of this, there has to be enough distance between the lamp and the plant to avoid scorching [59]. Since several years, light emitting diodes (LEDs) are getting more common as light source in greenhouses [57]. Because of the lower temperature of the LEDs, the lamps can be placed closer to the plants without harming them. This makes it possible to grow the plants in multiple layers and save space which is already limited in a space station.

2.2.2 Temperature

Every plant has its own temperature range in which it can grow with a minimum, optimal and maximum temperature. The yield is the highest when the plants are grown at their optimal growth temperature [60].

The response of a plant to a shift in the temperature is also dependent on the plant species. These responses can additionally vary throughout the plant's lifetime depending on the developmental stage [61]. In a growth chamber, the temperature should be controlled and adjusted if necessary. Besides the heath that is produced directly by the lamps, high-density discharge type lamps, like HPS lamps, can also heat up the plant using far infrared radiation (1500 – 30 000 nm) which is mostly absorbed by the plant and converted into heat [62]. Because of the high energy demand of a cooling system, these high-density discharge lamps cannot be used in space applications [42].

2.2.3 Carbon dioxide

Several researches have been conducted to determine the effect of higher than normal carbon dioxide concentrations on plants. Mainly, the results state that a high CO_2 concentration leads to a reduction in nitrogen inside the plant [63]–[65]. Little is known about the response of plants on low CO_2 concentrations [66]. Just like the temperature, the response on variations in the carbon dioxide concentration depends on the species of the plant [66]. In space applications, the plants are grown in a closed system. This asks for a gas exchange system to remove or add carbon dioxide that is produced or used by the plants [42].

2.2.4 Ethylene

Ethylene is a gaseous plant hormone produced by most tissues of the plant. It influences various mechanisms inside the plant like seed germination, growth and stress responses. Ethylene also causes growth reduction in most plant cells by inhibiting the expansion of the cell and leads to

early senescence [67], [68]. Because of this growth inhibition, ethylene cannot be allowed to accumulate in the growth chamber of the plants. The maximal allowable concentration of ethylene in the air is 50 nmol ethylene/mol air. Like carbon dioxide, ethylene needs to be removed in closed growth chambers before the levels get to high [42].

2.3 Plant growth influencing factors in space environment

The last part described the common parameters that influence the growth and development of plants. In a space station, there are however more factors of which the variation can affect the growth of plants. These additional factors include microgravity and radiation.

2.3.1 Microgravity

Microgravity is the weightlessness that is observed when an object is in free-fall in a vacuum. Here, the object will experience no drag force, which will cause all objects to fall at the same rate [18], [19]. A free-falling object will accelerate with 10^{-4} - 10^{-6} g [39]. However, microgravity is also used for reduced gravitational forces. This means that the gravity is lower than on earth [20]. Table 2.3 shows the different gravitational accelerations on the earth, the International Space Station, the moon, and on Mars.

Table 2.3: Gravitational acceleration on earth, ISS, the moon and Mars

	Earth	**ISS**	**Moon**	**Mars**
Gravitational acceleration	1 g = 9.81 m/s²	0.89 g = 8.73 m/s² [19]	0.17 g = 1.624 m/s² [20]	0.38 g = 3.71 m/s² [20], [69]

2.3.1.1 Effect on plants

The growth of plants is also guided by gravity, this ability is called gravitropism and it causes a better nutrient uptake in the roots and a better solar energy capture in the leaves [70]. Gravitropism is a complex process that uses many different signalling and regulatory components [71], [72]. Plants and more specifically, their roots, can sense gravity using amyloplasts containing starch grains that sediment in the direction of the gravitational vector. These amyloplasts are called statoliths [39], [71], [73]. When there is a displacement of these statoliths due to a change in direction of the gravitational vector, a biochemical signal is produced with affects the concentration of auxin in the root cap cells. This signal includes a change in the Ca^{2+} concentration in the cells, which affects the auxin transport [71], [72]. Auxin is a plant growth hormone that stimulates the differential growth of plants by inhibiting the cell elongation in the cells of the lower region of the roots. This will cause the roots to curve in the directional of the gravitational vector [72], [74].

Research has shown that plants adapt to the oxidative stress caused by microgravity during long exposure to microgravity. “Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences, which may lead to tissue injury” [75, p. 3]. The plants that were exposed to microgravity had

an altered expression of heat shock proteins (HSPs) [70]. These are proteins that protect other proteins against aggregation when the cell experiences stress [76]. Plants also undergo metabolic adaptations when they experience microgravity. These changes range from a thinner cell wall to a more effective energy state [70].

Differences in phototropism were noticed when plants were exposed to reduced gravity (0.3 *g* and 0.1 *g*) [20]. Phototropism is the ability to reorient the growth of a plant's organ in the direction of a light source or away from it [77]. When the tested plants experienced 0.3 *g*, the phototropism in their hypocotyls was reduced. However, when the plants were exposed to 0.1 *g*, the phototropism in their roots was reduced [20].

2.3.1.2 Simulation

To investigate the influence of microgravity on processes, the experiment has to be conducted in simulated microgravity conditions or in real free-fall conditions. The problem with the methods that simulate microgravity is that the sample experiences specific sensations that are not linked to microgravity but to the machine used for the simulation. For example, when a sample is placed into a clinostat or a random positioning machine, the sample will experience centrifugal accelerations and vibrations [78]. Because of these sensations, the results obtained using these simulations are hard to compare with the results of experiments conducted in real free-fall conditions like in drop towers or during a parabolic flight [78].

During this thesis, a random positioning machine (RPM) (Figure 2.1) was used to simulate microgravity. This machine consists out of two frames that rotate randomly and independently from each other. When a plant is place in the middle of the frames, it experiences low gravitational forces of which the average over time is equal to zero [79]. A limitation of this technique is that the object that can be placed between the frames is limited in size. An advantage of the RPM is that it is mobile and can thus be moved to the chamber where the plants are irradiated.

The use of an RPM does not give a completely accurate view of the response of plants to microgravity because the placement of the light source with reference to the plants constantly changes while plants grown in a space station can still grow towards the fixed light.

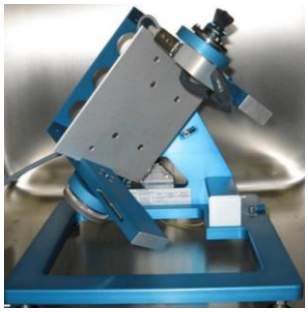

Figure 2.1: Random positioning machine (RPM) [80]

2.3.2 Radiation

Radiation can be described as energy in the form of electromagnetic waves or high speed particles. In general, radiation can be divided into two types of radiation: ionizing and non-ionizing radiation [2].

Non-ionizing radiation is low energy electromagnetic radiation that cannot remove electrons from their orbit around the nucleus of an atom [2], [81]. This type of radiation is used everywhere in the daily life on earth, for example as light, in microwaves and as radio signals [81].

Ionizing radiation includes high energy electromagnetic waves and particles. This radiation can create very unstable and reactive ions by removing electrons other than the ones on the outer orbit [2].

The activity of a radioactive source is indicated using the SI unit Becquerel (Bq). This measures the amount of radionuclides that decay per second [82]. The amount of radiation a tissue receives is indicated as the absorbed dose and has the unit Gray (Gy) [83]. The amount of radiation that is absorbed depends on the intensity of the radiation, the energy of one particle, and the material that is irradiated. An older unit to express the absorbed dose is the rad, this equals to 0.01 Gy [84]. The effective dose is expressed in Sievert (Sv) and indicates the biological risk of the radiation dose that a tissue received [21], [83]. This can be calculated by multiplying the absorbed dose (in Gy) with the quality factor. This is the amount of gamma or X-rays (in rad) the material needs to be irradiated with to cause the same amount of biological damage as 1 rad of the radiation concerned [84].

$$\text{Effective dose (Sv)} = \text{Absorbed dose (Gy)} \cdot \text{Quality factor} \quad (1)$$

The quality factor of gamma radiation, which is used in this research to irradiate the selected plants, is 1. This means that the effective dose for gamma radiation equals the absorbed dose. The effective dose is however only used for human tissues, the dose to non-human biota like plants is expressed in Gy or as dose rate in Gy/h [85].

2.3.3 Space radiation

While radiation on earth is composed of alfa, beta and gamma radiation, and X-rays, space radiation consists of protons and ions [86]. Most of these particles cannot reach the earth's surface because it is shielded by the atmosphere and the magnetic field [87]. This is shown in Table 2.4.

Table 2.4: Radiation levels on earth, the International Space Station (ISS), the moon, deep space and Mars

	Earth	**ISS**	**Moon**	**Deep space**	**Mars**
Radiation (mSv/y)	2.4 [21]	200-400 [83]	110-380 [23]	620 [24]	220 [24]

The radiation belts shown in Figure 2.2 are magnetic rings that surround the earth. The inner radiation belt consists of high energetic protons that are captured inside the magnetic field of the earth. The outer radiation belt mostly holds high energetic electrons [2]. In Figure 2.2 can be seen that the International Space Station (ISS), which orbits at an altitude of around 400 km, is still partly protected from space radiation by the magnetic field of the earth [88].

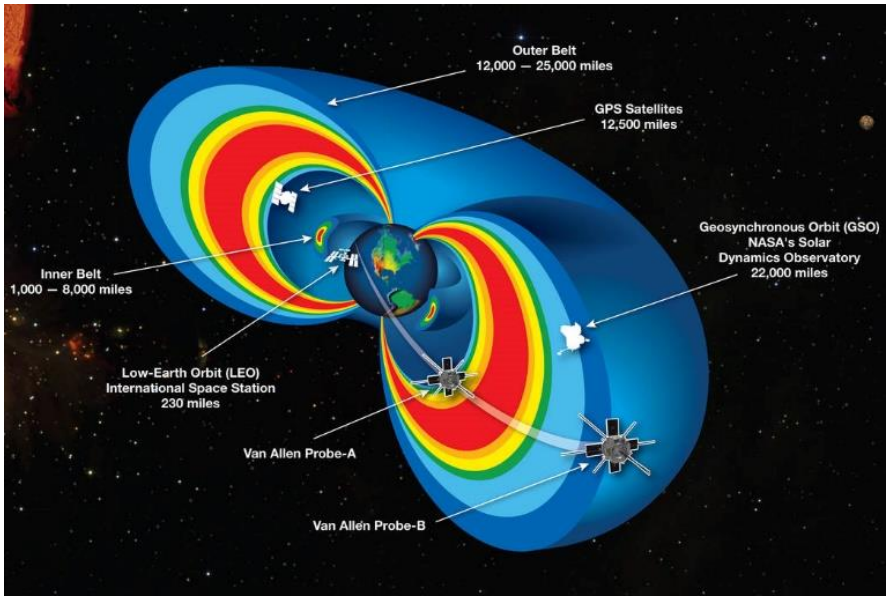

Figure 2.2: Schematic overview of magnetic field of earth as it is subdivided in different belts. The position of the International Space Station (ISS) in low-earth orbit is also indicated [89]

Space radiation can be divided in three different types: trapped radiation, solar energetic particles and galactic cosmic radiation. These three types are shortly explained in the next paragraphs [2], [17], [90].

A solar wind consists mostly of protons and electrons and to a lesser extent, of ions of most chemical elements. When a solar wind reaches the earth's magnetic field, the particles will not easily penetrate the field but form a shockwave around it. However, some particles can penetrate the magnetic field and will form belts inside the field, these are the radiation belts that are mentioned before [2].

Solar energetic particles (SEPs) are constantly emitted by the sun in low quantities but they can also be released in higher quantities during coronal mass ejections and solar flares [90], [91]. This type of radiation consists mostly out of medium energy protons. Their energy is lower than the energy of the protons in galactic cosmic radiation, which is a type of space radiation that is discussed in the next section. Because their low energy, these particles cannot penetrate the shielding of space stations. Besides protons, this type of radiation contains helium nuclei and ions of heavier elements [90], [92].

The third but probably most dangerous type of space radiation is the Galactic Cosmic radiation (GCR). This radiation originates from outside the solar system [93], [94], moves at nearly the speed of light [16], and is formed in cataclysmic events like explosions of supernovae [93],

[94]. GCR is a threat for space travel because the current shielding on space stations cannot deflect these particles because of their high energy (10^{10}-10^{15} eV) [90], [95]. It consists mostly of high-energy protons, helium nuclei and heavier high-energy nuclei [93], [94], [96], [97]. These last ones are also known as high charge and energy particles. They collide with nuclei and cause them to shatter, which will produce neutrons, charged particles and gamma radiation [96].

2.3.3.1 Effect on plants

Ionizing radiation can induce DNA damage directly by damaging the nucleotides or DNA structure, or indirectly by the oxidative stress caused by the radiation. This damage includes double stranded breaks (DSBs) in the DNA [98]. If these DSBs are not repaired, they can lead to genomic rearrangements which can cause cell death [99]. Because ionizing radiation can lead to the production of free radicals, it will also induce oxidative stress in the cells which can lead to DNA injury [98].

Plants have the ability to quickly repair DNA that has been damaged by ionizing radiation. When a plant is irradiated using high acute doses, differences in the expression of specific genes occur. Genes coding for DNA repair and antioxidative defence are upregulated and genes of the metabolism of sugar and starch are also affected. For chronic low doses, there is less difference in expression of genes that code for DNA repair and antioxidative defence. However, genes coding for defence and cell wall synthesis are generally upregulated under chronic low dose whereas genes for storage functions and non-specific metabolic pathways are downregulated [27].

Most plant cells have a higher radio-resistance than mammalian cells. Some mechanisms that contribute to plant radio resistance are the ability to adapt thickness of the cell wall, to increase the amount of radical scavengers that are produced, and to increase the phenolic content of some cells which can act as a radiation shield [28]. The lethal dose for acute high doses for plants ranges from 10 to 1000 Gy, while a lethal dose of 2 Gy was observed for a mammal [27], [100].

2.3.3.2 Simulation

Most simulations of space radiation focus on mimicking GCR. The dose of this type of radiation is mostly constant regarding to radiation coming from SEPs, which depends on the solar cycle [92]. Because space radiation consists of protons and ions, particle accelerators are used to simulate this type of radiation on earth. These particle accelerators generate high-energy ion beams with only one type of ions (single ion beams) or with multiple different ions [101].

It is currently possible to produce beams with different ions and varying energies using particle accelerators. These beams are more similar to real GCR than single ion beams, which contain only one type of ions at fixed energies. However, single ion beams can still be used to investigate the effect of one type of ions on a target, which cannot be done when beams with multiple ions are used [101].

Because it is not possible to use a particle accelerator for this research at SCK•CEN, the plants are treated with high dose (15.1 mGy/h) gamma radiation by placing them at a distance of

2.5 m from a caesium-137 source (662 keV) [102]. The energy of these particles is much lower than the energy of GCRs (10^{10}-10^{15} eV [95]) but this should be compensated because the absorbed dose of the gamma rays used in this study (15.1 mGy/h) is a 1000-fold higher than the absorbed dose of GCR in space (0.017 mGy/h [103]).

2.4 Nutritional value and other factors

The nutritional value of food is

> an indication of the contribution of a food to the nutrient content of the diet. This value depends on the quantity of a food which is digested and absorbed and the amounts of the essential nutrients (protein, fat, carbohydrate, minerals, and vitamins) which it contains. This value can be affected by soil and growing conditions, handling and storage, and processing [104].

In the next paragraphs, the different components that are measured are clarified and the principle of the tests that are used is explained.

2.4.1 Carbohydrates

Carbohydrates are molecules built out of monosaccharides that can form a high variation of structures [105]. The most common mono- and disaccharides are glucose, fructose, galactose, sucrose, lactose and maltose. These soluble saccharides are hydrolysed and ingested by the human body after which they can be used for energy production. There are however saccharides like the ones form the raffinose series, that cannot be digested by the human body [105], [106]. When a plant experiences stress due to environmental factors like light, water, temperature or radiation, it affects the efficiency of the photosynthesis and thereby affects the amount of soluble sugars in the plant [107].

Another type of carbohydrate that can be found in plants is starch. This structure is built from amylose and amylopectin and functions as a nutritional reservoir for saccharides [105]. Amylose is a mostly linear polymer of glucose molecules bonded via α-1,4-linkages (Figure 2.3(a)). Glucose molecules bonded via α-1,4-linkages and α-1,6-linkages form a much more branched molecule, named amylopectin (Figure 2.3(b)) [105], [108].

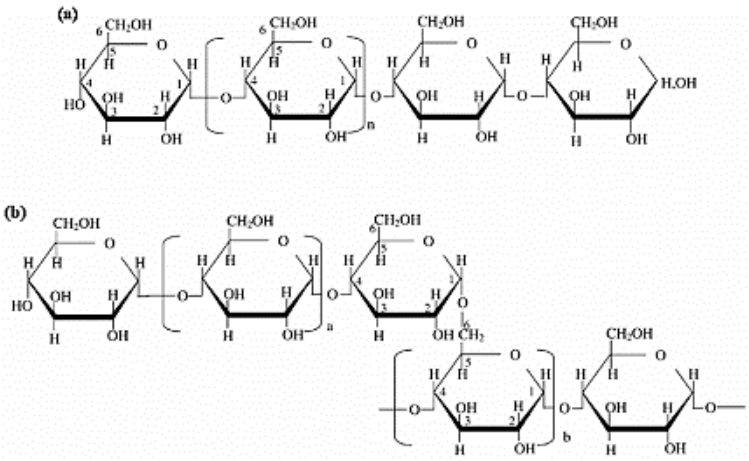

Figure 2.3: Structure of the components of starch (a) amylose and (b) amylopectin [108]

Different researches showed the increase of soluble sugars in plants that were irradiated with doses of 5-20 Gy and 50 Gy [109], [110]. This increase can be caused when the gamma radiation damages the photosynthetic apparatus, which can lead to an increase in sugar production [110]. Several researches show varying results for different plants that experienced microgravity. An increase in sugar and starch was noticed in sweet potato while no significant difference in starch was found in dwarf wheat plants. Further, a decrease in sugar and starch was observed in rocket seedlings that were grown while experiencing microgravity [111]–[113]. So it can be said that the variation in carbohydrates in plants that experienced microgravity depends on the species.

The anthrone reagent can be used to determine the total amount of soluble sugars and starch. This leads to the formation of a green colour that can be measured photospectrometrically [114], [115].

2.4.2 Proteins

Besides the carbohydrates, plants can also function as a source for proteins. These can provide the human body with amino acids and non-specific nitrogen [116].

Amino acids can be divided into three groups, the essential amino acids (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine), the conditionally essential amino acids (Cysteine, Tyrosine, Glycine, Arginine, Glutamine and Proline) and the non-essential amino acids (Aspartic acid, Asparagine, Glutamic acid, Alanine and Serine) [116]. Humans cannot synthesize essential amino acids and therefore have to ingest them. Conditionally essential amino acids can be produced by the human body but during illness or stress, a shortage can occur and the amino acid has to be provided via food [116], [117].

Besides functioning as a source of amino acids, plants can also provide non-specific nitrogen. These molecules are digested and used as a source to build non-essential amino acids or other important nitrogen molecules like nucleic acids [116], [117].

An important group of proteins are the heat shock proteins (HSP). These proteins are expressed by all living organisms to cope with stress. HSPs function as chaperones for other proteins when

the cell experiences heat stress to avoid unfolding of the proteins. When the cell however experiences other types of stresses which do not cause the unfolding of proteins, the function of HSPs is not known and could be different [118].

Rosemary that is irradiated with chronic gamma radiation (10-20 Gy) shows a gradual increase in total soluble proteins with an increasing dose. This variation is due to changes in the expression of genes that have various functions like signal transduction and stress defence [109]. When plants experience microgravity, the expression of a wide range of genes changes. These genes function in many different cellular processes like the total metabolism, calcium signalling and lipid signalling. It is also shown that the expression of HSPs increases in *Arabidopsis* that is grown during orbital flight [26].

To determine the protein quantity in the samples, a variety of Lowry's method is used. The protein sample is first treated with a copper tartrate solution in alkaline medium after which the Folin-phenol reagent is added [119]. This reagent contains sodium tungstate and sodium molybdate. The Folin-phenol reagent is reduced which leads to the formation of a blue colour that can be measured using a spectrophotometer [120], [121].

2.4.3 Antioxidants

The function of antioxidants is to neutralise free radicals. These are molecules that have an unpaired electron on the outer shell which makes the molecules unstable and thus reactive. Free radicals cause detrimental reactions that lead to cell injury and subsequently to inflammation and degenerative diseases. They are formed in almost every cell in the human body and react mostly with membranes of cells or organelles. An important group of free radicals is the reactive oxygen species (ROS). This group contains superoxide ($O_2^{-\circ}$), hydrogen peroxide (H_2O_2), and molecules that can be formed with these two products [122], [123].

There are two types of antioxidants: antioxidative enzymes and small antioxidant molecules. There are three important antioxidative enzyme classes in the human body: s*uperoxide dismutases, catalases,* and *peroxidases*. Collectively, these enzymes convert superoxide ($O_2^{-\circ}$) into water and O_2 or an alcohol [122]–[124]. Some of these enzyme activities depend on the availability of antioxidant minerals like manganese, copper, zinc, and selenium. When there is a shortage of one of these minerals, the activity of the enzymes can decrease [122].

The aforementioned enzymes are very efficient in removing most ROS molecules in the cells. However, they lack the ability to efficiently defend the body against molecules like singlet oxygen and hydroxyl radicals. To protect the cells against these molecules, the body depends on small antioxidant molecules inside the food. The most important antioxidative molecules coming from food are mentioned in Table 2.5 [122].

Table 2.5: Important small antioxidant molecules and their function [122], [125]

Antioxidant	Function
Vitamin E (Tocopherol)	Most important antioxidant in membranes Scavenges peroxy radicals
Vitamin C (Ascorbic acid)	Reduces free radicals, forming dehydroascorbate Regenerates vitamin E
Carotenes	Fat-soluble pigments, precursors for vitamin A Radical trapping agents mostly for peroxyl and hydroxyl radicals
Flavonoids (e.g. anthocyanins)	Water-soluble, polyphenolic antioxidants Scavengers of singlet oxygen, superoxide and peroxyl radicals
Glutathione	Substrate for *glutathione peroxidase* Can scavenge singlet oxygen, superoxide, and hydroxyl radicals
Uric acid	Water-soluble free radical scavenger, reacts with hydroxyl radical
Taurine	A free radical scavenging aminosulfonic acid that can be found in cells that can produce much oxidants

The activity of the antioxidative enzymes show a dose-dependent increased activity in chronic irradiated *Brachypodium distachyon* (50-300 Gy, 35 days) and wheat plants (50 Gy, 2, 4 and 6 weeks), and in acutely irradiated rosemary (5-20 Gy, 0.23 Gy/s) [109], [110], [126]. Research on *Arabidopsis* showed that only the *glutathione peroxidase* enzymes were affected when the plant was irradiated with absorbed doses ranging from 3.9 to 58.8 Gy [127]. Another research showed an increase in antioxidative enzyme activity in *Lemna minor* plants that received a dose rate of 120-232 mGy/h. This research also observed an increase in ascorbate and glutathione when the plants received a dose rate of 232 mGy/h [128]. For Barley plants that experienced microgravity was found that this treatment had no effect on the activity of the antioxidative enzymes, but that the antioxidative capacity of the plants was reduced [129].

To determine the total antioxidative capacity of the plants in this thesis, the ferric reducing antioxidant power (FRAP) assay is used. This is an electron transfer-based assay that uses the reduction of ferric-tripyridyltriazine ($Fe(III)(TPTZ)_2$) to the blue coloured ferrous-tripyridyltriazine (Fe(II)(TPTZ)) to determine the amount of antioxidants in a plant extract [130], [131].

2.4.4 Lignin

Lignin (Figure 2.4) is a complicated phenolic molecule that forms a network via cross-links and attaches cell wall components which causes the tissue to become rigid [132]. Kariuki et al. [133] observed a dose-dependent increase in lignin in rice plants that received a dose rate of 27-400 mGy/h. Wakabayashi et al. [132] showed that microgravity causes a reduction in lignin content

in rice shoots and thus suppresses the strengthening of the cell walls. This decrease in lignin is also observed in other researches among which the analysis of dwarf wheat [70], [112].

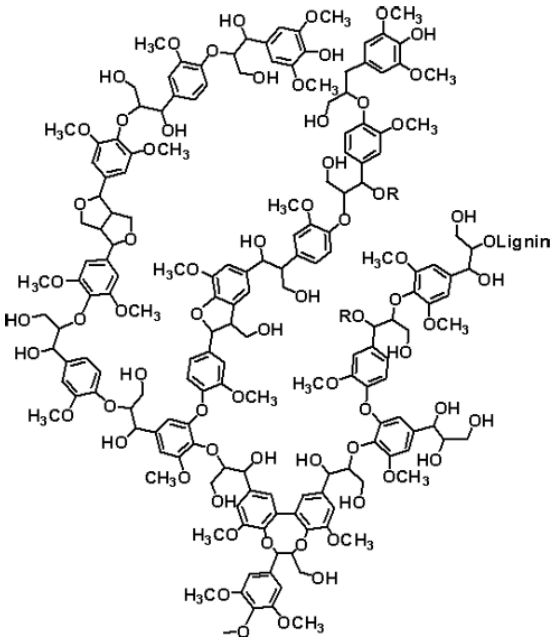

Figure 2.4: Structure lignin [134]

The amount of lignin can be quantified using acetyl bromide. This method is based on the formation of acetyl derivatives and the substitution of the alcohol groups in α- carboxylic acids with bromide [135], [136]. This method can be used for tissues with low amounts of lignin without affecting the recovery and the recovery rates remain similar between different types of tissues [135].

2.4.5 Photosynthetic pigments

Two important groups of pigments in plants are chlorophylls and carotenoids, these pigments are vital for the photosynthesis [137]. The most important chlorophylls in higher plants are chlorophyll a and b (Figure 2.5). Chlorophyll a functions as a photoreceptor and captures light energy which is converted to chemical energy. Chlorophyll b and carotenoids are accessory pigments that absorb light at different wavelengths as chlorophyll a and transfer the energy through the reaction centre where the light energy is converted to chemical energy [138], [139]. When the plants receive high intensity light, carotenoids will absorb the access light that is not used for the conversion to chemical energy and dissipate it. This prevents the light from causing oxidative stress in the plants [140].

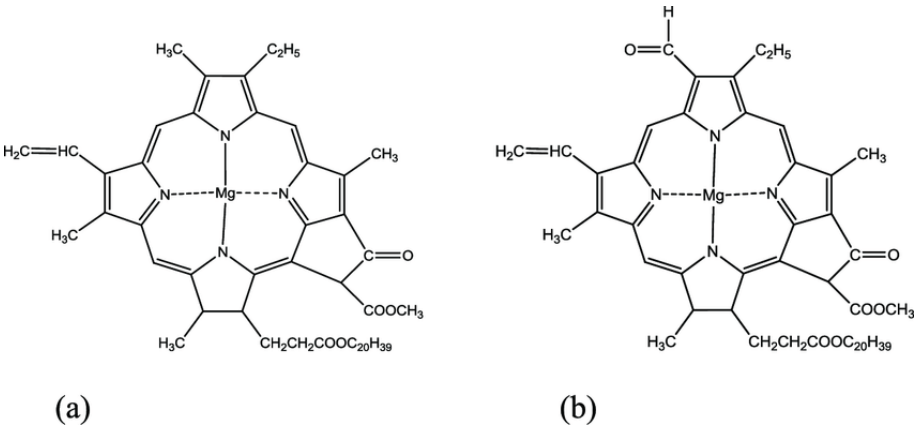

Figure 2.5: Structures of (a) chlorophyll a and (b) chlorophyll b [141]

Research on *Brachypodium distachyon* plants that were exposed to chronic gamma radiation (50-300 Gy, 35 days) showed a decrease in photosynthetic pigments when the absorbed dose increased [126]. Zhao, Li and Liu [142] also showed that simulated microgravity affects strawberry and carnation seedlings differently. While the strawberry seedling showed a decreased amount of chlorophyll, the chlorophyll quantity in the carnation seedlings increased slightly.

Photosynthetic pigments can easily be extracted and measured using N,N-dimethylformamide (DMF) and a spectrometer. No additional reagents have to be added to induce colour formation because the pigments absorb several wavelengths with high efficiency. These wavelengths can be used to determine the amount of pigments in the plant tissue [138], [143].

2.5 Objectives

Now that the different nutritional factors are explained and the concept of microgravity and space radiation is clarified, this knowledge can be used to predict the outcome of the analyses. When the plants experience microgravity, it would be expected that the lignin quantity and antioxidative capacity reduce. Gamma radiation treatment is however expected to increase the antioxidative capacity and lignin content along with the carbohydrates and proteins.

This leads to the next objective, namely the analysis of several microgreen plants that are treated with gamma radiation, that experienced microgravity or plants that experienced a combination of these factors. These analyses will help compare the nutritional values of the differently treated plants and determine the effect of these treatments on the plants.

3 Material and methods

This chapter first describes how the plants were grown and treated. After this, the different analyses that were used to determine the parameters influencing the nutritional value of the plants are explained.

3.1 Cultivation and harvesting

Based on the literature [11] and the commercial availability 3 plants were selected for the current pilot study. The seeds of the selected plants, red arrow radish (*Raphanus sativus* L.), red cabbage (*Brassica oleracea* L. var. *capitate*), and opal basil (*Ocimum basilicum* L.), were commercially obtained from the Mountain Valley Seed Company. These seeds were sown in a box of 13 cm x 13 cm with air holes (below, on the side and in the top corners) to ensure airflow in and out of the box (Figure 3.1). The top part of the box also contained a clear plastic petri dish to allow the illumination of the plants. For the analysis of the photosynthetic activity a different growth system was used which is described in paragraph 3.2.7.

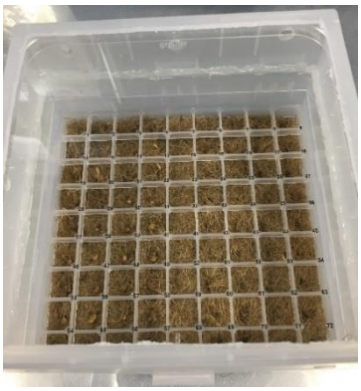

Figure 3.1: Growth box (13 cm x 13 cm) used for cultivation of the selected plants

The plants were grown on a hemp gaze which was sterilized for 120 min at 120°C in an autoclave (Astell AMB 220 BT autoclave). The nutrition for the plants was a Hoagland High Phosphate nutrient solution (the composition is given in Annex A) of which 100 mL was added to the hemp gaze before the seeds were sown. After 4 days, another 50 mL of nutrient solution was added to provide enough nutrients for further growth. This nutrient solution was sterilized for 15 min at 120°C (Tuttnauer 2540 EL autoclave) prior to administration. After sowing, the boxes were covered with aluminium foil and the plants were grown in the dark for 3 days at room temperature. This stimulates the stem growth which leads to a longer stem [144]. After this the plants were illuminated with light with an intensity of ± 150 µmol/m².s (PAR) as measured inside the boxes and a day/night cycle of 14 h/10 h at room temperature. The plants were harvested after 7 or 14 days depending on the plant species by cutting them of at the base of the stem, measuring the fresh weight and freezing them in liquid nitrogen and then stored at -80°C until further analyses.

To determine the effect of chronic radiation on the plants, they were placed at a distance of 2.5 m from a caesium-137 source which emits gamma radiation of moderate energy (662 keV) [102], [145]. This led to a mean absorbed dose of 15.1 mGy/h. This absorbed dose is measured by placing four dosimeters at the front and the back of the growth boxes and irradiating them for one hour.

The effect of microgravity was examined by cultivating the plants in the Desktop Random Positioning Machine (RPM) system of the Dutch Space B.V. (see Figure 2.1). The boxes in which the seeds were sown, were placed in between the frames and attached to the platform using rubber bands. Because plants that are grown in space experience a combination of increased radiation and microgravity, the red arrow radish and red cabbage plants are also grown in an RPM while being irradiated. The opal basil plants could not be treated with the combination of these factors because of a limited amount of RPMs available to investigate the effects on red arrow radish and red cabbage.

To determine if there was a difference between the plants that were irradiated and/or experienced microgravity, and the normal quantities present in microgreens, control plants were grown simultaneously with the treated plants. These control plants were grown in a separate room under the same circumstances as the treated plants but hence did not experience microgravity or were not irradiated.

Table 3.1: Overview of the treatments of the cultivated plants and the time after which the plants were harvested

Plant	**Treatment**	**Harvested after**	**Analyses**
Red arrow radish (*Raphanus sativus* L.)	Microgravity (μ*g*) Gamma radiation (γ) Gamma radiation + microgravity (γ + μ*g*) Control	1 week	Proteins Antioxidative capacity Pigments Sugar and starch Lignin Photosynthetic activity
Red cabbage (*Brassica oleracea* L. var. *capitate*)	Microgravity (μ*g*) Gamma radiation (γ) Gamma radiation + microgravity (γ + μ*g*) Control	1-1.5 week	Proteins Antioxidative capacity Pigments Sugar and starch
Opal Basil (*Ocimum basilicum* L.)	Microgravity (μ*g*) Gamma radiation (γ) Control	2 weeks	Proteins Antioxidative capacity Pigments

3.2 Analyses

3.2.1 Chemicals

The following substances were purchased from Sigma-Aldrich: Disodium EDTA, EDTA, acetone (>99.5%), iron(III)chloride, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Trolox, acetyl bromide (99%), iron sulphate heptahydrate, molybdic acid and Sigma 7-9 (Tris(hydroxymethyl)aminomethane). Sodium hydroxide, ethanol (>99.9%), glycerol (>99.5%), chloroform (99-99.4%), perchloric acid (70-72%), N,N-dimethylformamide (DMF) (>99.5%) and sodium acetate were bought from Merck. DC Protein Assay Reagent A, DC Protein Assay Reagent B, DC Protein Assay Reagent S and BSA protein standard were obtained from Bio-Rad. Hydrochloric acid (37%) was purchased from VWR. Followed by 95-97% sulfuric acid which was bought from J.T. Baker. And finally, 99.8% acetic acid which was bought from Acros organics.

3.2.2 Protein extraction and assay with the Folin reagent

To extract the proteins from the plant tissue, the frozen plant tissue (~20 mg) was first cold shredded with ~20 chrome steel beads (Ø 2.3 mm) using the Retsch MM400 Mixer Mill. After this, 1 mL extraction buffer containing 0.12 M Tris-HCl, 0.004 M EDTA, 4 m% Sodium dodecyl sulphate (SDS), and 10 v% glycerol was added. The samples were then centrifuged in the Eppendorf Centrifuge 5424 R for 20 minutes at 12 000 rpm and 4°C. 500 µL of the supernatant was then transferred in a clean Eppendorf tube after which the protein extraction samples were 2 and 4 times diluted in extraction buffer. Then, the BioRad DC Protein Assay kit was used to quantify the proteins that were present in the extracts. This kit consists of an alkaline copper tartrate solution (reagent A) that is mixed with 5-10% SDS (reagent S) and a diluted Folin reagent (reagent B). For each mL of reagent A, 20 µL reagent S was added, this solution is called reagent A'. 5 µL of the extraction samples and their dilutions were pipetted into a polystyrene 96-well plate. 25 µL of reagent A' was added to each well after which 200 µL reagent B was added. The reaction was allowed to run for 15 minutes after which the absorbance of the solutions was measured at 750 nm using the BioTek Powerwave XS2 Platereader (Annex B).

To determine the quantity of proteins in the samples, a calibration curve was established for each 96-well plate. A bovine serum albumin (BSA) protein standard was used to make a dilution series of 1.5, 1, 0.75, 0.5, 0.25, 0.125 and 0 mg/mL. The extinction coefficient of this series equals to ~0.233 L/(g·cm).

3.2.3 Determination of the total antioxidative capacity using the FRAP assay

To determine the total antioxidative capacity, the ferric reducing antioxidant power (FRAP) assay was used. This method relies on the reduction of ferric-tripyridyltriazine ($Fe(III)(TPTZ)_2$) to the blue coloured ferrous-tripyridyltriazine (Fe(II)(TPTZ)).

First, the frozen plant tissue (~100 mg) was cold shredded with ~20 chrome steel beads (Ø 2.3 mm) using the Retsch MM400 Mixer Mill. After this, 1 mL 0.01 N NaOH – 1 mM Na-EDTA extraction buffer was added after which the samples were thawed. The samples were then centrifuged for 30 minutes at 15 000 rpm and 4°C in the Eppendorf Centrifuge 5424 R. The supernatant was collected in a new Eppendorf tube and contained the hydrophilic fraction of

the antioxidants. The pellet was then resuspended in 1 mL 80% acetone after which the sample was incubated on ice for 1 hour. These samples were then centrifuged for 15 minutes at 15 000 g and 4°C. The supernatant that contained the lipophilic fraction of the antioxidants was also transferred in a new Eppendorf tube. 10 µL of the samples was pipetted in the wells of a polystyrene 96-well plate and diluted 5 times by adding 40 µL extraction buffer. On each well-plate was a dilution series of Trolox (1000 µM, 500 µM, 250 µM, 125 µM, 62.5 µM, 31.25 µM, 15.625 µM and 0 µM) added to establish a calibration curve. The extinction coefficient of this standard curve equals to ~0.003 L/(µmol·cm). 50 µL of each standard of the dilution series was pipetted on the 96-well plate. Then, 150 µL FRAP reagent was added in each well and the reaction was allowed to run for 10 minutes after which the absorbance of the samples was measured at 593 nm using a BioTek Powerwave XS2 Platereader. The FRAP reagent was prepared by bringing 150 µL 100 mM TPTZ, 150 µL 200 mM iron(III)chloride and 17.6 mL Na-acetic buffer in a 50 mL Falcon Tube. The used method is based on the protocol for the determination of the "Total antioxidative capacity according to the FRAP assay for plant material" (Annex C).

3.2.4 Pigment extraction and analysis using dimethylformamide

The pigments in the plants were extracted and measured using the protocol for "Pigment analysis in leaves – Plate reader method" (Annex D).

First, the intact plant tissue was put in a clean 2 mL Eppendorf tube (for samples up to 80 mg) or in a 15 mL Falcon tube if the fresh weight of the tissue exceeded 80 mg. DMF was added to each tube in a ratio of 0.5 mL per 20 mg fresh weight. To extract the pigments from the plant tissue the tubes were then covered with aluminium foil and left in the cold storage room (4°C) for 24 hours.

Before the samples were taken out of the cold storage room, the absorbance of the empty polypropylene 96-well plate was measured at 664 nm, 647 nm and 480 nm. These values were subtracted from the absorbances of the samples later on. The samples were then measured in groups of 4 because the colour of the pigments is not stable in time at room temperature. First, 100 µL DMF was pipetted in 2 columns of the 96-well plate. Then, 200 µL of the samples was added to another column of the well-plate. The samples were then diluted 2 and 4 times by pipetting 100 µL of the undiluted sample in the first column with 100 µL DMF. This solution was then mixed by pipetting after which 100 µL of this dilution was brought into the second column that contained 100 µL DMF. After mixing this solution, 100 µL of the 4 times diluted sample was discarded (see Figure 3.2).

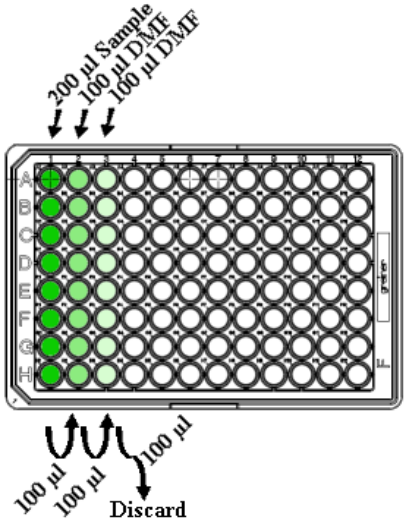

Figure 3.2: Preparation of the dilutions of the samples for the pigment analysis (Annex D)

The absorbance of the samples and their dilutions was then immediately measured at 664 nm, 647 nm and 480 nm using the BioTek Powerwave XS2 Platereader. The following formulas were used to determine the chlorophyll a (C_a), chlorophyll b (C_b) and carotenoid (C_{x+c}) quantities [146]:

$$C_a = 11.65 \cdot A_{664} - 2.69 \cdot A_{647} \quad (2)$$

$$C_b = 20.81 \cdot A_{647} - 4.53 \cdot A_{664} \quad (3)$$

$$C_{x+c} = \frac{1000 \cdot A_{480} - 0.89 \cdot C_a - 52.02 \cdot C_b}{245} \quad (4)$$

The absorbances that were used in these formulas, were corrected by first subtracting the absorbance of the empty well from the absorbance of the sample, then multiplying the absorbance with the dilution factor and finally divided by 0.294 to correct the absorbance to a 1 cm light path.

3.2.5 Sugar and starch determination with Anthrone reagent

To determine the amount of soluble sugars and starch in the plants, they first had to be dried by placing the frozen plants of 150-200 mg in a drying oven at 70°C. Then, the samples were ground by adding 4 chrome steel beads (Ø 2.3 mm) and placing them in the Retsch MM400 Mixer Mill. Before the sugars could be extracted, the interfering pigments had to be extracted by adding 100% acetone. After this, the soluble sugars were extracted by adding 2.5 mL 80% ethanol. These samples were then centrifuged using the Eppendorf Centrifuge 5424 R. The supernatant was then brought in a clean tube and 5 mL of 1.1% HCl was added to the pellet. This solution was heated in a water bath at 100°C for 30 minutes after which they were diluted to 10 mL with milliQ water.

The anthrone reagent was prepared by dissolving 1 g anthrone in 500 mL 72% sulfuric acid. 1 mL sample is pipetted in a 10 mL test tube and cooled on ice after which 5 mL ice cold anthrone reagent is added. This solution is heated at 100°C for 11 minutes and cooled on ice,

then the absorbance of the solution is measured at 630 nm using the BioTek Powerwave XS2 Platereader.

Standard glucose and starch solutions (0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) are used to establish a calibration curve. The extinction coefficient for the glucose samples equals 2.81 L/(g·cm) and the extinction coefficient for the starch samples equals 2.16 L/(g·cm). This procedure is executed using the protocol for the "Analysis of starch and soluble sugars with Anthrone reagent" (Annex E).

3.2.6 Lignin measurement using acetyl bromide

To determine the lignin quantity in the plants, the protocol based on the measurement of lignin using acetyl bromide is used (Annex F).

First, the frozen plants (150 - 200 mg) were thawed and dried by placing them in a drying oven on 70°C. 20 mg of dried plant tissue was put in a clean Eppendorf tube after which the plant tissue was then ground by adding 4 chrome steel beads and putting the tubes in the Retsch MM400 Mixer Mill. After this, 1 mL milliQ H_2O was added to extract a part of the total pectic carbohydrate and the samples were incubated for 30 minutes on 98°C while they were shaken at 750 rpm. The samples were then centrifuged for 3 minutes at 14 000 rpm after which the supernatant was removed and 1 mL ethanol was added to extract the proteins and cell organelles. The samples were then again incubated for 30 minutes at 76°C and shaken at 750 rpm. After centrifuging for 3 minutes at 14 000 rpm, the supernatant was discarded and 1 mL chloroform was added to remove the hydrophobic compounds followed by another incubation step of 30 minutes at 59°C and 750 rpm. The samples were then centrifuged for 3 minutes at 14 000 rpm and the supernatant was yet again removed. Then 1 mL acetone was added to dry the material, after which the samples were incubated for 30 minutes at 54°C and 750 rpm, the samples were centrifuged for 3 minutes at 14 000 rpm and the supernatant was removed. Finally, the pellet was dried for 24 hours at room temperature [147].

When the pellet is dry, 5 mg was transferred in a new Eppendorf tube and 0.1 mL 25% acetyl bromide in glacial acetic acid and 4 µL 60% perchloric acid was added. The samples were then incubated for 30 minutes at 70°C and 850 rpm. After centrifuging the samples for 15 minutes at 14 000 rpm, the supernatant was brought in a new Eppendorf tube and 0.5 mL glacial acetic acid was added to the pellet. The pellet was then vortexed and centrifuged for 3 minutes at 14 000 rpm after which its wash was added to the supernatant. The Eppendorf tube containing the supernatant and the wash of the sample was then filled till 2 mL with glacial acetic acid. After letting this solution rest for 20 minutes, the absorbance of the samples is measured at 280 nm using the Nanodrop 1000 of Thermo Fisher Scientific. The concentration is determined using the Beer-Lambert law (Extinction coefficient = 23.35 L/(g·cm)). For the incubation steps, the Eppendorf Thermomixer C is used to heat and shake the samples.

Due to limited amount of samples lignin content could only be determined in red arrow radish. However, due to a yet unknown problem, the absorbances of the samples were too high to be possible, resulting in over 100% lignin in some cell wall residues. As all samples were used

and time was lacking to start a new experiment, it was not possible to redo or optimise this analysis within the framework of this thesis.

3.2.7 Photosynthetic activity with Li-Cor

In order to determine the photosynthetic activity of the plants, seven seeds are sown in a hemp gaze which was placed in round vessels (Ø 6 cm, Figure 3.3) and provided nutrients with Hoagland high phosphate nutrient solution. The plants are cultivated for a similar timeframe, conditions and treatments as the plants that are used for the other tests (Table 3.1). After the cultivation period, the vessel with the plants is directly placed inside the leaf chamber of the Li-Cor 6400 XT for 20 minutes and the photosynthetic activity is measured. This method is based on the difference in carbon dioxide and water in a gas flow before and after passing the plants. The protocol for operating the Li-Cor is given in Annex G.

Figure 3.3: Vessel (Ø 6 cm) for the cultivation of the plants that are used to determine the photosynthetic activity. 4 of these vessels are placed in a growth box like the one shown in Figure 3.1 to provide for replicates

3.2.8 Statistical analysis

To determine if the results were significantly different from each other, the results were analysed with the program R 3.6.0. First, a Shapiro-Wilk test and a Bartlett test was conducted to determine if the results were normally distributed and if they had the same variance. If this was the case, an analysis of variance (ANOVA) in combination with the Tukey post-hoc test could be conducted. If the results were not normally distributed or if the variance was not the same, first, a Kruskal-Willis test was conducted with a pairwise Wilcox post-hoc test. The standard error (SE) is also determined. For the statistical analysis of the results of the starch quantity in red cabbage was a Kruskal-Wallis rank sum test conducted with a pairwise comparison using Tukey and Kramer (Nemenyi) test as post-hoc test.

4 Results

The following paragraphs show the results of the analyses that are conducted on the selected plants. These analyses are: the fresh weight of the plants, the protein quantity, the total antioxidative capacity, the amount of pigments, and the sugar and starch quantity. The results of the photosynthetic activity of the radish plants showed no significant differences due to the standard error being too high. Because of this, these results are not shown in the next paragraphs. Further, the results of the lignin analysis were not realistic with values exceeding 100% lignin. Due to the lack of time and materials to repeat these tests within the time span of this thesis, the analysis could not be redone. The comparison of the results to the control plants can also be found in Annex I.

4.1 Fresh weight

The fresh weights of the different plants (Figure 4.1) mostly show the same response to the different treatments. The plants that are irradiated all show a decreased fresh weight and the red cabbage and opal basil plants that experienced microgravity have increased fresh weights. Also, the red cabbage plants that experienced the combination of microgravity and radiation had significantly decreased masses with reference to the control plants.

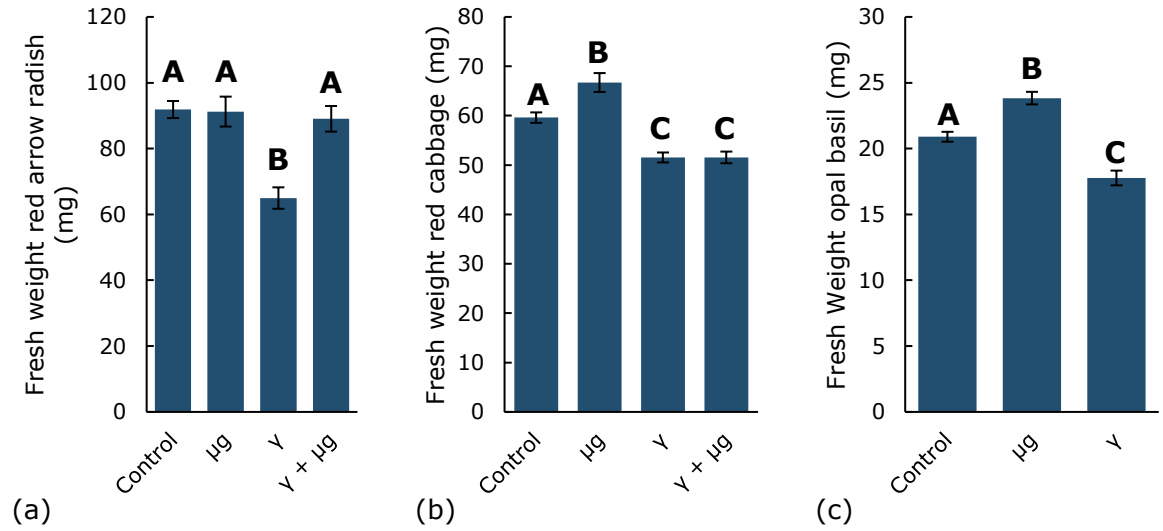

Figure 4.1: Fresh weight of (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (Wilcoxon sum test, $p < 0.05$, (a) $n = 32$, (b) $n = 30$, (c) $n = 51$))

The radish plants grew the fastest and had the highest fresh weight which is advantageous when they are used as a food source during space missions. The opal basil plants were still small when they were harvested after 2 weeks, which is double of the growth period of the radish plants. So, when the opal basil plants would be used as food for astronaut's, more plants should be grown simultaneously.

4.2 Protein quantity

Most stresses in plants induce the production of specific stress-related proteins like heath shock proteins. These function as protectors to maintain normal cellular functions [148].

Like the fresh weight, the response of the amount of proteins in the plants to the different treatments is mostly similar (Figure 4.2). All plants that experienced microgravity show a decrease in protein quantity. Also, the red cabbage and opal basil plants that were irradiated had an increased amount of proteins. Finally, the red arrow radish and red cabbage plants that experienced the combination of microgravity and radiation have significantly higher protein quantities than the control plants.

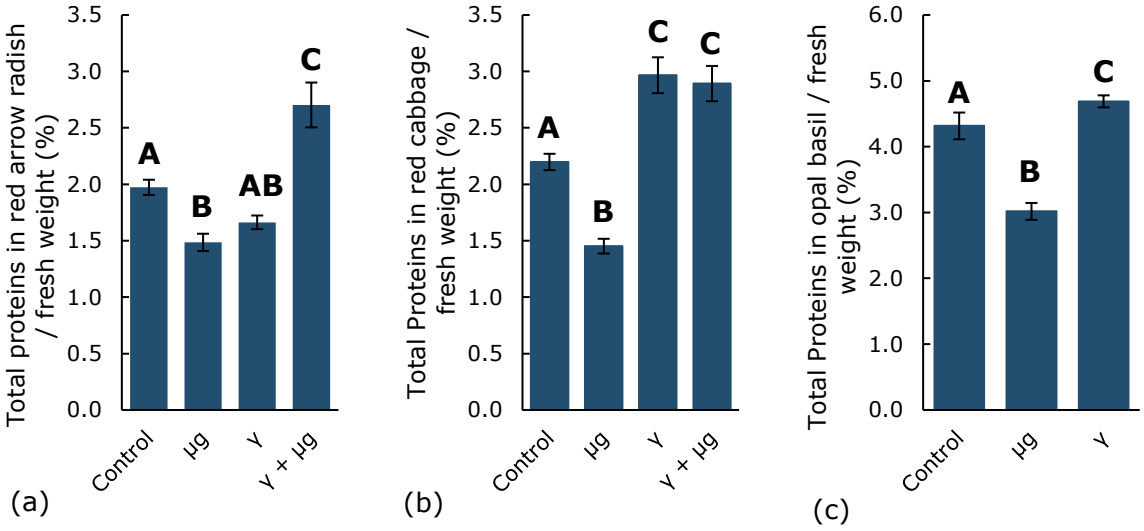

Figure 4.2: Total quantity of proteins in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (two-way ANOVA, $p < 0.05$, $n = 5$).

Further, it can be stated that opal basil has the highest quantity of total proteins while red arrow radish and red cabbage both have approximately the same quantity. Because plants can function as a source for essential amino acids and as a general nitrogen source when they are used as food, a higher amount of proteins can be beneficial.

4.3 Total antioxidative capacity

An important factor when the stress response of plants is investigated, is the antioxidative capacity. An increased production of antioxidants can indicate that the plant is experiencing oxidative stress, which leads to the production of ROS. On the other hand is an increased antioxidant value of the plants beneficiary regarding their use as astronaut's food.

Regarding the hydrophilic antioxidants in Figure 4.3, the red arrow radish plants and the opal basil plants have in general higher amounts of hydrophilic antioxidants than the red cabbage plants. The radish plants that experienced microgravity, gamma radiation or both (Figure 4.3a) show no significant difference in hydrophilic antioxidants with reference to the control samples. This is also the case with the opal basil plants (Figure 4.3c). The irradiated red cabbage plants (Figure 4.3b) show increased levels of hydrophilic antioxidants, while the plants that experienced microgravity have lower levels of antioxidants with reference to the control plants.

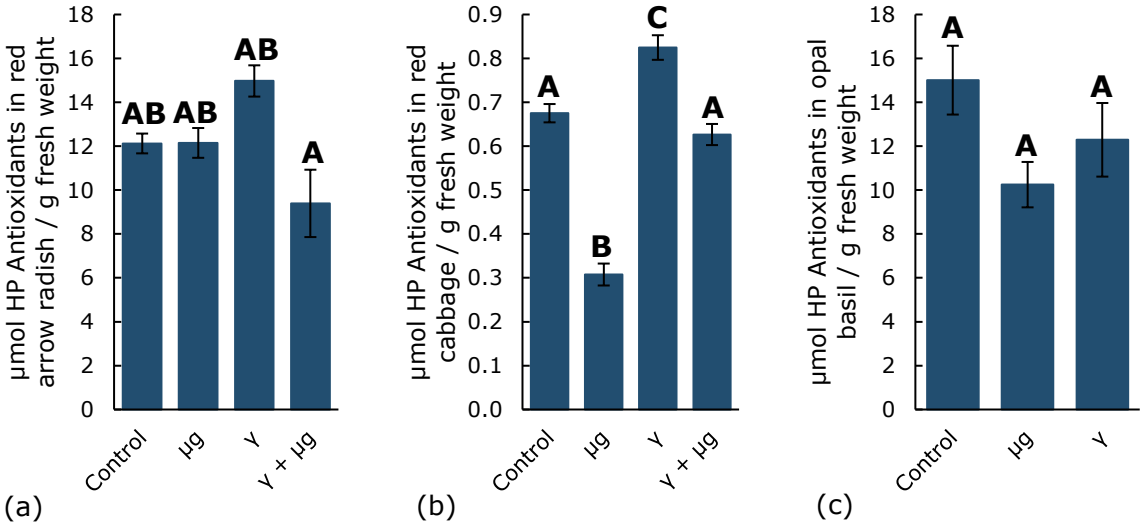

Figure 4.3: Total antioxidative capacity of hydrophilic (HP) antioxidants in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (Wilcoxon sum test, $p < 0.05$, $n = 5$)

The amounts of lipophilic antioxidants in the different plants are shown in Figure 4.4. Opal basil has in general the highest amount of lipophilic antioxidants. All plants respond differently to the different treatments. The irradiated radish plants, for example, show increased levels of lipophilic antioxidants while the irradiated red cabbage plants show no significant difference with reference to the control plants and the irradiated opal basil plants even show a decreased level of lipophilic antioxidants. Further, the red cabbage plants have a decreased amount of lipophilic antioxidants when they experience microgravity or the combination of microgravity with gamma radiation.

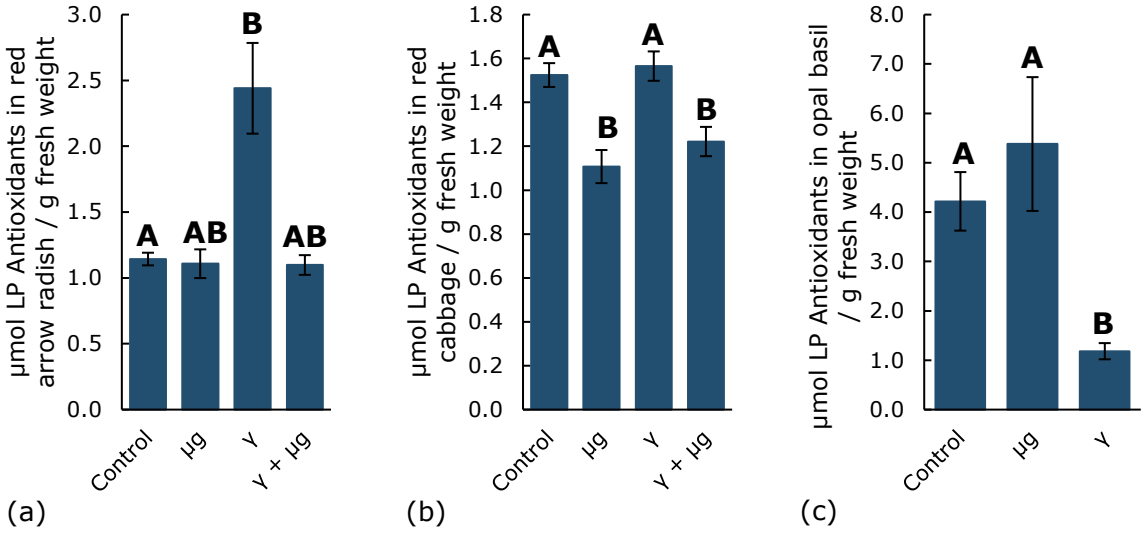

Figure 4.4: Total antioxidative capacity of lipophilic (LP) antioxidants in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (Wilcoxon sum test, $p < 0.05$, $n = 5$)

It can also be stated that the amount of hydrophilic antioxidants in the different plants are higher than the amount of lipophilic antioxidants. In general, the opal basil plants have the highest amount of antioxidants, which can be advantageous when they are used as astronaut's food.

4.4 Photosynthetic pigments

The pigments that were analysed are very important in the photosynthesis. A decreased amount of these pigments could indicate a lower photosynthetic activity, which can lead to slower growth and yield.

Figure 4.5 shows the amount of chlorophyll a in the different plants. It can be seen that red cabbage and red arrow radish have a higher amount of chlorophyll a than the opal basil plants. The effect of the different treatments is species dependent. The only significant difference of the radish plants with the control occurs when the radish plants experienced the combination of microgravity and radiation, this does however not lead to a significant difference in the red cabbage plants. The red cabbage plants that experienced microgravity and radiation even shows and increase in chlorophyll a, however this is not significant. The irradiated red cabbage plants show a significant increase in chlorophyll a. Finally, the opal basil plants have significantly decreased amounts of chlorophyll a when they experience microgravity.

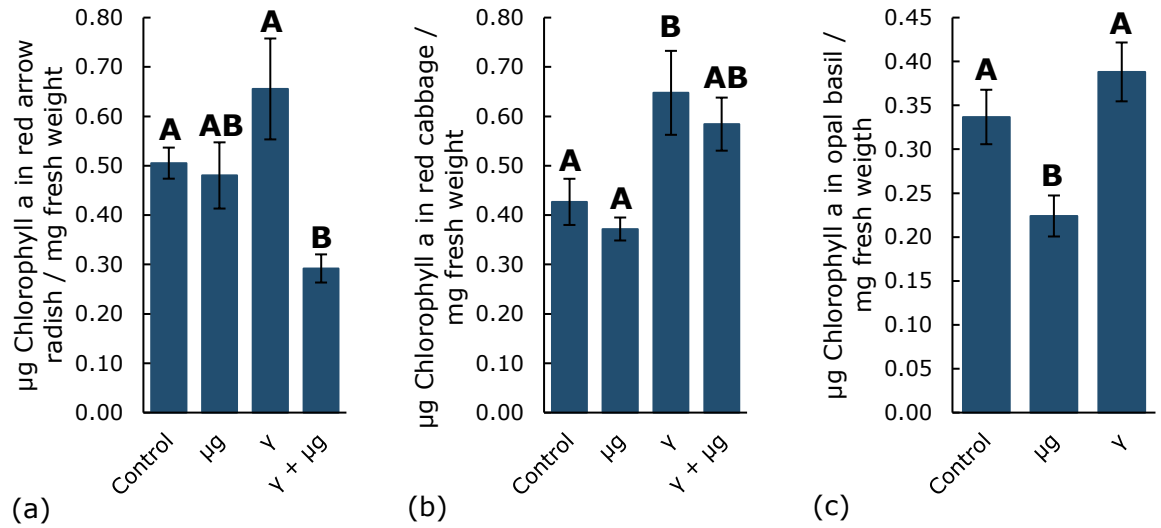

Figure 4.5: Pigment quantity of chlorophyll a in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments ((a)+(c) Wilcoxon sum test, $p < 0.05$, $n = 5$ and (b) two-way ANOVA, $p < 0.05$, $n = 5$)

The amount of chlorophyll b in the plants in shown in Figure 4.6. The results show the same trend as the results for chlorophyll a. However, the opal basil plants that experienced microgravity now show no significant difference with reference to the control samples. The amount of chlorophyll b is also lower than the amount of chlorophyll a in every plant.

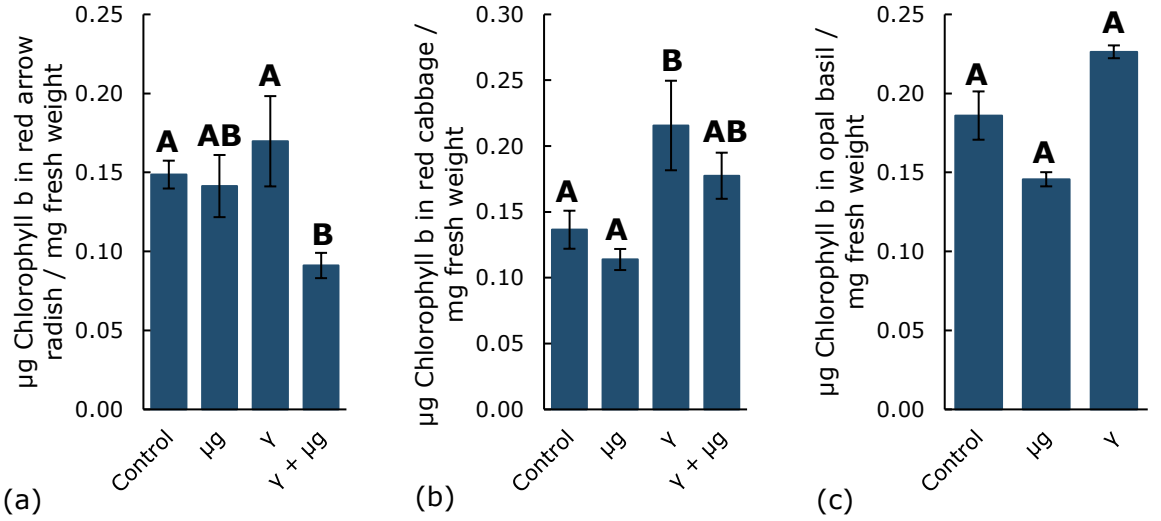

Figure 4.6: Pigment quantity of chlorophyll b in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments ((a)+(b) two-way ANOVA, $p < 0.05$, $n = 5$ and (c) Wilcoxon sum test, $p < 0.05$, $n = 5$)

Figure 4.7 shows the amount of carotenoids in the different plants. These results again show the same trend as the other two pigments.

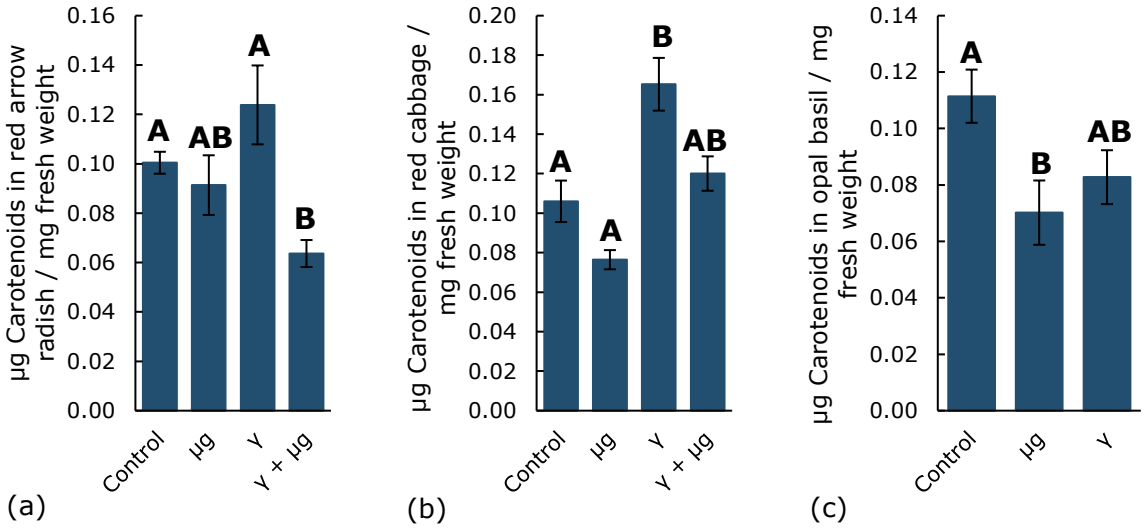

Figure 4.7: Pigment quantity of carotenoids in (a) one-week old red arrow radish microgreens, (b) one and a half-week old red cabbage microgreens and (c) two-week old opal basil microgreens with reference to the fresh weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (two-way ANOVA, $p < 0.05$, $n = 5$)

In general, the reaction of the plants to the different treatments has the same trend. The pigments in radish plants that experienced the combination of microgravity and radiation are significantly lower than the pigments in the control plants. The red cabbage plants show significantly increased levels of pigments when the plants were irradiated. Finally, the opal basil plants that experienced microgravity have lower levels of pigments, but this difference is only significant for chlorophyll a and the carotenoids.

4.5 Sugar and starch

Carbohydrates can be used as energy for the cells and can indicate an increased need for energy. Plants produce soluble sugars during photosynthesis, this is why a decrease in the photosynthetic activity of a plant will lead to a reduction in soluble sugars [107].

The amount of soluble sugars in red arrow radish (a) and red cabbage (b) are shown in Figure 4.8. These results show strong differences between the two species. While the sugar quantity in the radish plants does not change with the different treatments, the red cabbage plants show several significant differences between the results of the different treatments. The amount of sugars in the red cabbage plants that experienced microgravity is almost 5 times lower than in the control plants while the irradiated red cabbage plants show an increased sugar quantity.

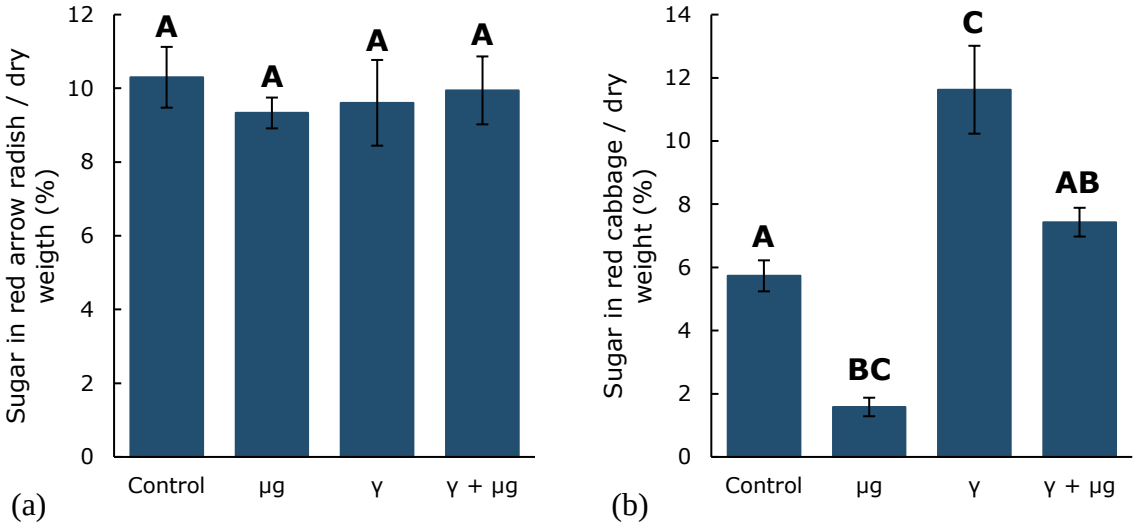

Figure 4.8: Sugar quantity in (a) one-week old red arrow radish microgreens and (b) one and a half-week old red cabbage microgreens with reference to the dry weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments (Wilcoxon sum test, $p < 0.05$, $n = 5$)

Figure 4.9 shows the starch quantity in red arrow radish and red cabbage. These results show the same trend for both the plants, namely a strong decrease in starch when the plants experience microgravity or the combination of microgravity and radiation.

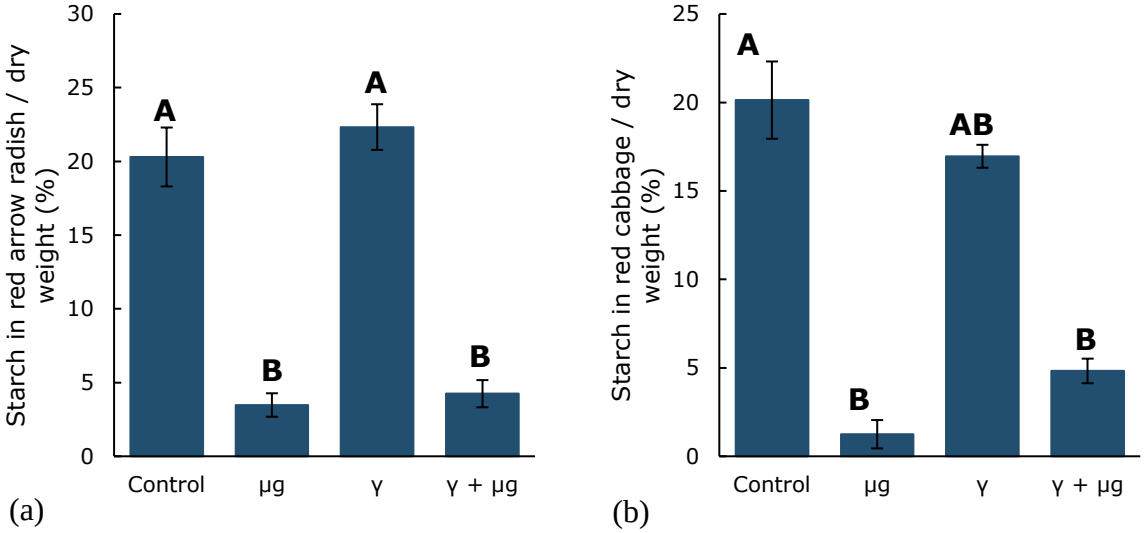

Figure 4.9: Starch quantity in (a) one-week old red arrow radish microgreens and (b) one and a half-week old red cabbage microgreens with reference to the dry weight of the plants after treatment with gamma radiation (γ) or in microgravity (µg) (± SE). Different letters indicate significant differences between treatments ((a)Wilcoxon sum test, $p < 0.05$, $n = 5$, (b) Kruskal-Wallis rank sum test, $p < 0.05$, $n = 5$)

5 Discussion

This chapter discusses the results obtained in this study and shown in the chapter 4. The different possible causes of these differences are listed based on literature describing research conducted on other plants that experienced microgravity or radiation.

Care must be taken when comparing the results obtained in this thesis with reported literature as the experimental conditions (such as dose and duration of treatments) and plants species studied often differ considerably. Additionally, to date researches reporting on the combination between different adverse space conditions (increased radiation + microgravity) are scarce. Another limiting factor for this comparison and discussion is that while many researches are performed on plants that receive acute high-dose gamma radiation, the effect of chronic irradiation (*e.g.* as applied in this study where plants are continuously exposed from germination to harvest) is still poorly understood.

Table 5.1 gives an overview of the significant differences found in the present study in plants exposed to gamma radiation, microgravity or a combination of both treatments, for the fresh weight and for the different tested nutritional parameters. The changes are expressed based with reference to the control samples. The next paragraphs discus the response of the different parameters that were analysed to the different treatments.

Table 5.1: Overview of the effects observed in this study of radiation, microgravity or both on fresh weight and different nutritional values in three microgreen plants red arrow radish, red cabbage and opal basil compared to control plants. ↑ there is a significant increase, ≈ there is no significant change, ↓ there is a significant decrease, / not analysed

				Antioxidants		Photosynthetic pigments			Carbohydrates	
Treatment	**Plant**	**Fresh weight**	**Proteins**	**Hydrophilic**	**Lipophilic**	**Chlorophyll a**	**Chlorophyll b**	**Carotenoids**	**Sugar**	**Starch**
Micro-gravity	Red arrow radish	≈	↓	≈	≈	≈	≈	≈	≈	↓
	Red cabbage	↑	↓	↓	↓	≈	≈	≈	↓	↓
	Opal basil	↑	↓	≈	≈	↓	≈	↓	/	/
Gamma radiation	Red arrow radish	↑	≈	≈	↑	≈	≈	≈	≈	≈
	Red cabbage	↓	↑	↑	≈	↑	↑	↑	↑	≈
	Opal basil	↓	↑	≈	↓	≈	≈	≈	/	/
Micro-gravity and gamma radiation	Red arrow radish	≈	↑	≈	≈	↓	↓	↓	≈	↓
	Red cabbage	↓	↑	≈	↓	≈	≈	≈	≈	↓

5.1 Microgravity

From the response of the different plants to microgravity can be concluded that red arrow radish is the least sensitive of the three plants that were analysed. Red cabbage is the most responsive to microgravity, with six of the nine tested parameters showing significant results compared to the control plants.

Two of the tested microgreen plants (red cabbage and opal basil), grown while experiencing microgravity, showed an increased fresh weight with reference to the control samples. In comparison, the variations in fresh weight of plants grown in microgravity found in the literature show contradictory results. Some researches noticed an increase in fresh weight when plants experienced microgravity [149], while the fresh weight in other researches decreased when the plants experienced microgravity [150]. Claassen and Spooner [151] hypothesised that these contradictory results are caused by the differences in sowing time of the plants. They noticed that in the researches that observed reduced weights, most plants were sown on earth before being send into space. While the plants that were sown in space all showed increased fresh weights. However, this difference in sowing is not of relevance in the current study where we did simulation experiments in the lab and all plants were sown immediately before the experiment.

In contrast to the increase in fresh weight in red cabbage and opal basil all other significant differences in nutrient parameters including total protein were reduced compared to control plants. This indicated that the increase in fresh weight has to be caused by an increase in a parameter that is not analysed most likely an increased uptake of water which also leads to elongation of cells. This can be analysed in further research by determining the ratio of the fresh weight to the dry weight.

The decrease in protein content when the plants experience microgravity, is also shown in an analysis of the proteomic profile of rice plants grown in a satellite [152]. This research revealed that the expression of most proteins in the plants is downregulated during exposure to microgravity. On the other hand, research conducted on *Arabidopsis* seedlings grown in the ISS showed that the expression of specific proteins that are linked with stress responses, is upregulated during spaceflight. This was taken by the authors as an indication that microgravity is a stress inducing factor in these plants [153]. Zupanska et al. [76] found that the expression of heat shock proteins is upregulated during continuous exposure to microgravity. In the latter two researches [153, 77], however, the total protein level was not determined so it is difficult to conclude whether the increase in specific proteins like heat shock proteins is reflected as an increase in the total protein content.

The microgravity also has a negative effect on the amount of antioxidants in radish and red cabbage. Shagimardanova et al. [129] investigated the effect of microgravity on the antioxidative capacity of barley. This research also showed a decrease in antioxidative capacity of the plants. Further, they analysed if the decrease in antioxidative capacity was due to a decrease in phenolic compounds, which was not the case. Finally, they suggested that, because of this decrease of antioxidants in the plants, microgravity probably does not induce oxidative stress but they could not explain the decrease in antioxidative capacity.

The pigments in all tested microgreens show the same tendency to decrease when the plants are grown while experiencing microgravity, but this is only significant in the basil plants. A similar decrease in all photosynthetic pigments was shown in the analysis of Rocket seeds that were grown in an RPM and in the ISS [113]. Further, research has shown that the photosynthetic activity of wheat plants decreased in microgravity, which could be caused by the decrease in photosynthetic pigments [154]. There is, however, also a research that finds no significant differences in photosynthetic pigments in *Brassica rapa* L. [155]. Zhao et al. [142] showed that the effect of microgravity on the photosynthetic pigments depends on the plant species.

A decrease in carbohydrates can be caused by an increased need for energy. Abomohra et al. [156] indicated that this could be a result of the increased usage of ATP by the DNA repair mechanisms in the cells. Colla et al. [113] also noticed a decrease in carbohydrates in rocket seedlings that were grown while experiencing microgravity. The strong decrease in starch is interesting because this compound has a defined function in the process of gravitropism [71]. It can be hypothesised that due to the lack of a fixed gravitational vector when the plant is placed in an RPM, the plant produces less starch to use in the gravitropism process.

In general, it can be stated that microgravity has a negative influence on the analysed parameters. The decrease in nutrients in plants that are grown while experiencing microgravity will reduce the potential value of these plants as a food source and should therefore be further investigated.

5.2 Gamma radiation

While microgravity mostly causes a decrease in nutrients, gamma radiation leads to an increase which is beneficial for the nutritional value.

Comparing the different plant species gamma radiation seems to induce less significant changes in nutritional endpoints in red arrow radish compared to red cabbage and opal basil plants. This could be due to plant-specific differences suggesting that red cabbage is the most radio sensitive of the three tested microgreen plants. Although not further investigated it could also be due to fact that in this study plants were exposed to a fixed dose rate of 15.1 mGy/h but we chose to harvest plants at the two true leaf stage. This resulted in different harvest times for the three used microgreens and hence also in different total absorbed dose. It is conceivable that the red arrow radish plants that received a lower total dose also show less severe radiation-induced changes in nutrient values. To further investigate this it would be interesting to use different dose rate and time point combinations.

Red cabbage and opal basil show a decreased fresh weight, while the fresh weight of irradiated red cabbage increases. Similarly to red cabbage and opal basil, *Arabidopsis* plants irradiated with 2336 µGy/h and a absorbed dose of ~3 Gy [157], red pepper that absorbed 8 and 16 Gy [158] and *Brachypodium distachyon* that received absorbed doses ranging from 50-300 Gy [126] all showed a decrease in fresh weight. Also the fresh weight results are similar to the results of a research where *Arabidopsis* is irradiated with a dose rate of 100 mGy/h for 7 days [159]. This research showed that the *Arabidopsis* plants that were grown for 7 days and then irradiated for 7 days after which they are harvested had higher fresh weights than the control

plants. This is similar to the response of the radish plants in the current thesis which were irradiated for 7 days after which they were harvested. In contrast, the *Arabidopsis* plants that were grown for 10-14 days before being irradiated for 7 days, had lower fresh weights than the control samples. As concluded by Biermans et al. [159] radiation shows plant, age- and dose-dependent responses in fresh weight possibly due to the fact that fresh weight is a very general parameter reflecting different physiological processes including the balance between growth and stress responses.

The radish plants that are irradiated show no significant difference in protein content with the control samples. The irradiated red cabbage and opal basil plants however, have an increased level of proteins. This effect is also shown in a research in which soybean seeds were irradiated with high doses of gamma radiation (10 Gy/min) and then cultivated in normal conditions [160].

The results of the antioxidant analyses show that respectively the amount of hydrophilic antioxidants in the red cabbage plants and the amount of lipophilic antioxidants in radish significantly increase when the plants are irradiated. This is expected because the radiation causes an increase in the production of ROS [110]. Several researches have shown that the activity of antioxidative enzymes and the amount of antioxidants increase when the plant is exposed to gamma radiation [28], [110], [158]. This increase in antioxidants is beneficial when the plants are used as food source because they can protect humans against free radicals. However, in opal basil an unexpected decrease in lipophilic antioxidants was observed possibly indicating some plant specific or time-dependent responses which could be further investigated in the future.

The irradiated red cabbage plants have increased amounts of photosynthetic pigments. This could indicate that the photosynthetic activity of irradiated red cabbage plants is higher than the activity of the control plants, which would lead to the production of more carbohydrates via photosynthesis. The amount of sugar and starch are however not significantly different from the control samples. An increase in photosynthetic pigments is also shown in rosemary plants that received a high absorbed dose of 10-20 Gy [109]. Gicquel et al. [161] showed that when *Arabidopsis* plants that received an absorbed dose of 10 Gy, the transcription of the chloroplast constituent genes is stimulated. Another research that investigated the stress responses of *Arabidopsis* that was exposed to gamma radiation (3.9 – 58.8 Gy, 7 days) found no difference in carotenoids between the irradiated plants and the control. However; carotenoids can be recovered fast by plants. It is possible that the carotenoids in this study already recovered when the plants were harvested [127]. Because of the increase in photosynthetic pigments that occurs in the current study, it would be interesting to determine the photosynthetic activity of irradiated red cabbage plants. Within this study a first attempt was made to design a set-up for this (see 3.1), however, this needs further optimization.

The increase in soluble sugars that is observed in the red cabbage plants is also shown in rosemary by El-Beltagi et al. [109], who showed that the amount of soluble sugars increased with an increasing absorbed dose of gamma radiation.

5.3 Gamma radiation and microgravity

The results of the plants that experienced a combination of microgravity and radiation show a plant specific response, except for the protein content and the fresh weight of the plants. The fresh weight generally decreases compared to the control samples while the protein content increases. The results also show that the response of the plants cannot be derived from the response of the plants to radiation or microgravity alone. The decrease in antioxidants in red cabbage and opal basil, photosynthetic pigments in red arrow radish, and starch in red arrow radish and red cabbage all lead to a decrease in the nutritional value of the plants. This is important to know when the plants are a possible food source for astronauts.

6 Conclusion and perspectives

Before astronauts can be send on long term space missions beyond the reach of the magnetic field of the earth, several problems concerning the food and water supply and the effects of adverse space conditions like reduced gravity and increased radiation on living organisms have to be solved. To help deal with the problem regarding the food and water supply, the research unit Biosphere impact studies of the Belgian nuclear research centre SCK•CEN is investigating the possible use of microgreens as food source for astronauts. This pilot project is the starting point of this investigation and aims to determine the effects of microgravity and radiation on several aspects of the nutritional value of three selected microgreens, red arrow radish, red cabbage and opal basil.

The results of this research showed that the nutritional value of selected microgreens was differently affected by radiation, microgravity or the combination of these factors. Except for the fresh weight and the proteins, most of the tested parameters showed a plant specific response. In general, it can be stated that the fresh weigh of the plants decreases when they are exposed to gamma radiation and to the combination of gamma radiation and microgravity. The radiation treatment also induced in increase in proteins which is possibly caused by the expression of proteins that function in the stress defence, like for example heat shock proteins [148]. Plants that experience microgravity show however a decrease in proteins.

While radish plants that experienced microgravity or radiation alone were not affected, combining these treatments induced a decrease in photosynthetic pigments which could lead to a lower photosynthetic activity and hence in the long term a lower yield. To determine if the combination of these factors leads to a decreased amount of starch, additional samples should be collected to enable repeating the analysis.

The results lead also to the conclusion that the red cabbage plants are more susceptible to changes in antioxidants and pigments both in radiation- and microgravity-exposed plants but show less significant differences when they are treated with the combination of these factors. Hence this pilot study showed that the nutritional value of plants is overall affected by adverse space conditions and this in a plant dependent way. Out of the selected plants, red arrow radish is at the moment the most suitable to be used as food for astronauts because of the short growth period of 1 week and being the least sensitive to the treatments. The red cabbage plants have the advantage that their photosynthetic pigment and protein content increases when they are irradiated increasing their potential yield and nutritional value respectively. The opal basil plants are too small and their growth period of 2 weeks is too long, which is why they probably cannot be used as a food source in space.

The current knowledge is not enough to determine if these plants are appropriate to be used as astronaut's food. To draw a final conclusion, additional research is needed. First, additional plant growth and exposure experiments are needed to obtain more samples. Second, it would be interesting to use different time-points and/or dose rates to better compare the different microgreens. Further, other plants like *e.g.* amaranth, which has a very high nutritional value

[162], could be included in future studies to determine their potential as food source for astronauts. Finally, other nutritional and growth related parameters should be analysed like the photosynthetic activity, carbohydrates, lipid content, the amount of tocopherol, which is the precursor of the lipophilic antioxidant vitamin E, anthocyanin, lignin, fiber content and glucosinolates which can protect humans from cancer in small doses.

Bibliography

[1] National Geographic, “Early Manned Spaceflight,” 2019. [Online]. Available: https://www.nationalgeographic.com/science/space/space-exploration/early-manned-spaceflight/. [Accessed: 17-Mar-2019].

[2] M. Langford, “What is space radiation?,” 2016. [Online]. Available: https://srag.jsc.nasa.gov/SpaceRadiation/What/What.cfm. [Accessed: 25-Mar-2019].

[3] B. Dunbar, “Moon to Mars Overview,” 2019. [Online]. Available: https://www.nasa.gov/topics/moon-to-mars/overview. [Accessed: 20-Mar-2019].

[4] M. Whiting, “5 Hazards of Human Spaceflight,” 2018. [Online]. Available: https://www.nasa.gov/hrp/5-hazards-of-human-spaceflight. [Accessed: 25-Mar-2019].

[5] SCK-CEN, “Ruimtevaart Brochure.” 2011.

[6] B. Allen, “Human Needs: Sustaining Life During Exploration,” *NASA*, 2007. [Online]. Available: https://www.nasa.gov/vision/earth/everydaylife/jamestown-needs-fs.html. [Accessed: 18-Mar-2019].

[7] SCK-CEN, “SCK voorstelling.” [Online]. Available: https://www.sckcen.be/nl/About/Introduction. [Accessed: 25-Mar-2019].

[8] SCK-CEN, “Interdisciplinary Biosciences,” 2019. [Online]. Available: http://science.sckcen.be/en/Institutes/EHS/MCB. [Accessed: 14-Mar-2019].

[9] SCK-CEN, “Biosphere Impact Studies,” 2019. [Online]. Available: http://science.sckcen.be/en/Institutes/EHS/MCB/BIS. [Accessed: 15-Mar-2019].

[10] U. Choe, L. L. Yu, and T. T. Y. Wang, “The Science behind Microgreens as an Exciting New Food for the 21st Century,” *J. Agric. Food Chem.*, vol. 66, no. 44, pp. 11519–11530, 2018.

[11] Z. Xiao, G. E. Lester, Y. Luo, and Q. Wang, “Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens,” *J. Agric. Food Chem.*, vol. 60, no. 31, pp. 7644–7651, 2012.

[12] V. M. Paradiso *et al.*, “Nutritional characterization and shelf-life of packaged microgreens,” *Food Funct.*, vol. 9, no. 11, pp. 5629–5640, 2018.

[13] E. Pinto, A. A. Almeida, A. A. Aguiar, and I. M. P. L. V. O. Ferreira, “Comparison between the mineral profile and nitrate content of microgreens and mature lettuces,” *J. Food Compos. Anal.*, vol. 37, no. 3, pp. 38–43, 2015.

[14] Q. Wang, Z. Xiao, E. E. Codling, G. E. Lester, X. Nou, and Y. Luo, “Microgreens of Brassicaceae: Mineral composition and content of 30 varieties,” *J. Food Compos. Anal.*, vol. 49, pp. 87–93, 2016.

[15] M. C. Kyriacou, S. De Pascale, A. Kyratzis, and Y. Rouphael, “Microgreens as a Component of Space Life Support Systems: A Cornucopia of Functional Food,” *Front. Plant Sci.*, vol. 8, no. September, pp. 8–11, 2017.

[16] S. Frazier, “Real Martians: How to Protect Astronauts from Space Radiation on Mars,” 2017. [Online]. Available: https://www.nasa.gov/feature/goddard/real-martians-how-to-

protect-astronauts-from-space-radiation-on-mars.

[17] K. Mars, “Why Space Radiation Matters,” 2018. [Online]. Available: https://www.nasa.gov/analogs/nsrl/why-space-radiation-matters. [Accessed: 25-Mar-2019].

[18] S. May, “What Is Microgravity?,” 2012. [Online]. Available: https://www.nasa.gov/audience/forstudents/5-8/features/nasa-knows/what-is-microgravity-58.html. [Accessed: 25-Mar-2019].

[19] B. Dunbar, “What Is Microgravity?,” 2009. [Online]. Available: https://www.nasa.gov/centers/glenn/shuttlestation/station/microgex.html. [Accessed: 25-Mar-2019].

[20] J. Z. Kiss, “Plant biology in reduced gravity on the Moon and Mars,” *Plant Biol.*, vol. 16, no. SUPPL.1, pp. 12–17, 2014.

[21] International Atomic Energy Agency, “Radiation in Everyday Life | IAEA,” 2019. [Online]. Available: https://www.iaea.org/Publications/Factsheets/English/radlife. [Accessed: 18-Mar-2019].

[22] J. Rask, W. Vercoutere, B. J. Navarro, and A. Krause, “Space faring: the radiation challenge,” *NASA.gov*, 2008. [Online]. Available: https://www.nasa.gov/audience/foreducators/radiationchallenge/grades9_12/index.html. [Accessed: 18-Mar-2019].

[23] G. Reitz, T. Berger, and D. Matthiae, “Radiation exposure in the moon environment,” *Planet. Space Sci.*, vol. 74, pp. 78–83, 2012.

[24] C. Zeitlin and L. C. Simonsen, “Briefing to NAC HEO/SMD Joint Committee Meeting: Mars Radiation Environment-what have we learned?” 2017.

[25] R. Hampp, E. Hoffmann, K. Schonherr, P. Johann, and L. De Filippis, “Fusion and metabolism of plant cells as affected by microgravity,” *Planta*, vol. 203, no. SUPPL., pp. S42–S53, 1997.

[26] E. L. Kordyum and D. K. Chapman, “Plants and microgravity: Patterns of microgravity effects at the cellular and molecular levels,” *Cytol. Genet.*, vol. 51, no. 2, pp. 108–116, 2017.

[27] N. Caplin and N. Willey, “Ionizing Radiation, Higher Plants, and Radioprotection: From Acute High Doses to Chronic Low Doses,” *Front. Plant Sci.*, vol. 9, 2018.

[28] C. Arena, V. De Micco, E. Macaeva, and R. Quintens, “Space radiation effects on plant and mammalian cells,” *Acta Astronaut.*, vol. 104, no. 1, pp. 419–431, 2014.

[29] E. Preston, “How NASA Is Solving the Space Food Problem,” *Eater*, 2015. [Online]. Available: https://www.eater.com/2015/9/17/9338665/space-food-nasa-astronauts-mars. [Accessed: 25-Mar-2019].

[30] J. Watson, M. Lee, and M. N. Garcia-Casal, “Consequences of Inadequate Intakes of Vitamin A, Vitamin B12, Vitamin D, Calcium, Iron, and Folate in Older Persons,” *Curr. Geriatr. Reports*, vol. 7, no. 2, pp. 103–113, 2018.

[31] G. Galili and R. Amir, “Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality,” *Plant Biotechnol. J.*, vol. 11, no. 2, pp. 211–

222, 2013.

[32] M. C. Kyriacou *et al.*, “Micro-scale vegetable production and the rise of microgreens,” *Trends Food Sci. Technol.*, vol. 57, pp. 103–115, 2016.

[33] C. Piovene *et al.*, “Optimal red: Blue ratio in led lighting for nutraceutical indoor horticulture,” *Sci. Hortic. (Amsterdam).*, vol. 193, pp. 202–208, 2015.

[34] D. A. Kopsell and C. E. Sams, “Increases in Shoot Tissue Pigments, Glucosinolates, and Mineral Elements in Sprouting Broccoli after Exposure to Short-duration Blue Light from Light Emitting Diodes,” *J. Am. Soc. Hortic. Sci.*, vol. 138, no. 1, pp. 31–37, 2013.

[35] J. W. Fahey, Y. Zhang, and P. Talalay, “Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, no. 19, p. 10367, 1997.

[36] M. Renna, F. Di Gioia, B. Leoni, C. Mininni, and P. Santamaria, “Culinary Assessment of Self-Produced Microgreens as Basic Ingredients in Sweet and Savory Dishes,” *J. Culin. Sci. Technol.*, vol. 15, no. 2, pp. 126–142, 2017.

[37] N. Greens, “Different types of microgreens seeds,” 2018. [Online]. Available: https://www.nickgreens.com/single-post/2018/06/09/Different-types-of-microgreens-seeds. [Accessed: 18-Mar-2019].

[38] T. Kwon, J. Alan Sparks, J. Nakashima, S. N. Allen, Y. Tang, and E. B. Blancaflor, “Transcriptional response of arabidopsis seedlings during spaceflight reveals peroxidase and cell wall remodeling genes associated with root hair development,” *Am. J. Bot.*, vol. 102, no. 1, pp. 21–35, 2015.

[39] C. Deng *et al.*, “Effect of modeled microgravity on radiation-induced adaptive response of root growth in Arabidopsis thaliana,” *Mutat. Res. - Fundam. Mol. Mech. Mutagen.*, vol. 796, pp. 20–28, 2017.

[40] K. Koga and Y. Iwasaki, “Psychological and physiological effect in humans of touching plant foliage - using the semantic differential method and cerebral activity as indicators,” *J. Physiol. Anthropol.*, vol. 32, no. 1, pp. 1–9, 2013.

[41] R. Odeh and C. L. Guy, “Gardening for Therapeutic People-Plant Interactions during Long-Duration Space Missions,” *Open Agric.*, vol. 2, no. 1, pp. 1–13, 2017.

[42] R. J. Bula and R. W. Ignatius, “Providing controlled environments for plant growth in space,” *Acta Hortic.*, vol. 440, pp. 13–18, 1996.

[43] L. Zha and W. Liu, “Effects of light quality, light intensity, and photoperiod on growth and yield of cherry radish grown under red plus blue LEDs,” *Hortic. Environ. Biotechnol.*, vol. 59, no. 4, pp. 511–518, 2018.

[44] A. R. Beaman, R. J. Gladon, and J. a Schrader, “Sweet Basil Requires an 500 µmol.m-2.s-1 Irradiancefor Greatest Edible Biomass Production,” *HortScience*, vol. 44, no. 1, pp. 64–67, 2009.

[45] D. I. Duggan-Jones and M. A. Nichols, “Determining the optimum temperature, irradiation and CO2 enrichment on the growth of lettuce and cabbage seedlings in plant factories,” *Acta Hortic.*, vol. 1107, pp. 187–193, 2015.

[46] Johnny’s Selected Seeds, “Radish, Red Arrow - Microgreen Seed,” *Johnny’s Selected*

Seeds, 2019. [Online]. Available: https://www.johnnyseeds.com/vegetables/microgreens/microgreen-vegetables/radish-red-arrow-microgreen-seed-3111M.html. [Accessed: 19-Apr-2019].

[47] S. Mijani, S. Eskandari Nasrabadi, H. Zarghani, and M. Ghias Abadi, "Seed Germination and Early Growth Responses of Hyssop, Sweet Basil and Oregano to Temperature Levels," *Not. Sci. Biol.*, vol. 5, no. 4, pp. 462–467, 2013.

[48] E. Palomo, "Optimum Temperature for Growing Cabbage," *SFGate*, 2019. .

[49] C. L. Mackowiak, L. M. Ruffe, N. C. Yorio, and R. M. Wheeler, "Effect of carbon dioxide enrichment on radish production using Nutrient Film Technique (NFT)," *Tech. Memo.*, vol. 109198, no. 1994, 1994.

[50] T. Ouzounis, E. Rosenqvist, and C.-O. Ottosen, "Spectral Effects of Artificial Light on Plant Physiology and Secondary Metabolism: A Review," *HortScience*, vol. 50, no. 8, pp. 1128–1135, 2015.

[51] K. M. Folta and K. S. Childers, "Light as a growth regulator: Controlling plant biology with narrow-bandwidth solid-state lighting systems," *HortScience*, vol. 43, no. 7, pp. 1957–1964, 2008.

[52] B. G. Bareja, "What is light quality, some effects on plant growth," *Cropsreview.com*, 2011. [Online]. Available: https://www.cropsreview.com/light-quality.html. [Accessed: 16-Apr-2019].

[53] K. R. Cope, K. R. Cope, and B. Bugbee, "Spectral Effects of Three Types of White Light- Emitting Diodes on Plant Growth and Development : Absolute Versus Relative Amounts of Blue Light Spectral Effects of Three Types of White Light-emitting Diodes on Plant Growth and Development : Absolute vers," *HortScience*, vol. 48, no. 4, pp. 504–509, 2013.

[54] I. G. Cummings, "Red to far-red ratio correction in plant growth chambers," *Acta Hortic.*, vol. 907, pp. 145–150, 2011.

[55] K. M. Folta and S. A. Maruhnich, "Green light: A signal to slow down or stop," *J. Exp. Bot.*, vol. 58, no. 12, pp. 3099–3111, 2007.

[56] J. C. Lopez, "Influence of Light on Crop Growth," *PRO-MIX*, 2018. [Online]. Available: https://www.pthorticulture.com/en/training-center/influence-of-light-on-crop-growth/. [Accessed: 16-Apr-2019].

[57] H.-H. Kim, R. M. Wheeler, J. C. Sager, N. C. Yorio, and G. D. Goins, "Light-Emitting Diodes As an Illumination Source for Plants: a Review of Research At Kennedy Space Center," *Habitation*, vol. 10, no. 2, pp. 71–78, 2005.

[58] M. Mõttus, M. Sulev, F. Baret, R. Lopez-Lozano, and A. Reinart, "Photosynthetically Active Radiation: Measurement and Modeling," in *Encyclopedia of Sustainability Science and Technology*, 1st ed., R. Meyers, Ed. New York: Springer, 2012, pp. 7902–7932.

[59] W. C. Randall and R. G. Lopez, "Comparison of Bedding Plant Seedlings Grown Under Sole-source Light-emitting Diodes (LEDs) and Greenhouse Supplemental Lighting from LEDs and High-pressure Sodium Lamps," *HortScience*, vol. 50, no. 5, pp. 705–713, 2015.

[60] W. Schlenker and M. J. Roberts, “Nonlinear temperature effects indicate severe damages to U.S. crop yields under clilmate change,” *Proc. Natl. Acad. Sci.*, vol. 106, no. 37, pp. 15594–15598, 2009.

[61] J. L. Hatfield and J. H. Prueger, “Temperature extremes: Effect on plant growth and development,” *Weather Clim. Extrem.*, vol. 10, pp. 4–10, 2015.

[62] R. Hicklenton and R. D. Heins, “Temperature,” in *Plant Growth Chamber Handbook*, R. W. Langhans and T. W. Tibbitts, Eds. Ames: NC-101 regional committee, 1997, pp. 31–42.

[63] M. F. Cotrufo, P. Ineson, and A. Scott, “Elevated CO2 reduces the nitrogen concentration of plant tissues,” *Glob. Chang. Biol.*, vol. 4, no. 1, pp. 43–54, 1998.

[64] D. R. Taub, B. Miller, and H. Allen, “Effects of elevated CO2 on the protein concentration of food crops: A meta-analysis,” *Glob. Chang. Biol.*, vol. 14, no. 3, pp. 565–575, 2008.

[65] D. R. Taub and X. Wang, “Why are nitrogen concentrations in plant tissues lower under elevated CO2? A critical examination of the hypotheses,” *J. Integr. Plant Biol.*, vol. 50, no. 11, pp. 1365–1374, 2008.

[66] Y. Li, X.-G. Zhu, J. Xu, N. U. Haq, and H. Zhang, “Was low CO2 a driving force of C4 evolution: Arabidopsis responses to long-term low CO2 stress,” *J. Exp. Bot.*, vol. 65, no. 13, pp. 3657–3667, 2014.

[67] F. Wang, X. Cui, Y. Sun, and C. H. Dong, “Ethylene signaling and regulation in plant growth and stress responses,” *Plant Cell Rep.*, vol. 32, no. 7, pp. 1099–1109, 2013.

[68] I. I. Vaseva *et al.*, “The plant hormone ethylene restricts Arabidopsis growth via the epidermis,” *Proc. Natl. Acad. Sci.*, vol. 115, no. 17, pp. E4130–E4139, 2018.

[69] S. Deiters and N. Pailer, *Astronomie*. Cologne: Komet Verslag, 2005.

[70] H. Q. Zheng, F. Han, and J. Le, “Higher Plants in Space: Microgravity Perception, Response, and Adaptation,” *Microgravity Sci. Technol.*, vol. 27, no. 6, pp. 377–386, 2015.

[71] Y. S. Kolesnikov, S. V. Kretynin, I. D. Volotovsky, E. L. Kordyum, E. Ruelland, and V. S. Kravets, “Molecular mechanisms of gravity perception and signal transduction in plants,” *Protoplasma*, vol. 253, no. 4, pp. 987–1004, 2016.

[72] M. Singh, A. Gupta, and A. Laxmi, “Striking the Right Chord: Signaling Enigma during Root Gravitropism,” *Front. Plant Sci.*, vol. 8, pp. 1–17, 2017.

[73] A Dictionary of Biology, “Statolith,” *Encyclopedia.com*, 2004. [Online]. Available: https://www.encyclopedia.com/plants-and-animals/zoology-and-veterinary-medicine/zoology-general/statolith. [Accessed: 08-Apr-2019].

[74] Y. Zhao, “Auxin Biosynthesis and Its Role in Plant Development,” *Annu. Rev. Plant Biol.*, vol. 61, no. 1, pp. 49–64, 2010.

[75] D. J. Betteridge, “What is oxidative stress?,” *Metabolism.*, vol. 49, no. 2 Suppl 1, pp. 3–8, 2000.

[76] A. K. Zupanska, F. C. Denison, R. J. Ferl, and A. L. Paul, “Spaceflight engages heat shock protein and other molecular chaperone genes in tissue culture cells of Arabidopsis

Thaliana," *Am. J. Bot.*, vol. 100, no. 1, pp. 235–248, 2013.

[77] E. Liscum, S. K. Askinosie, D. L. Leuchtman, J. Morrow, K. T. Willenburg, and D. R. Coats, "Phototropism: Growing towards an Understanding of Plant Movement," *Plant Cell*, vol. 26, no. 1, pp. 38–55, 2014.

[78] R. Herranz *et al.*, "Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology," *Astrobiology*, vol. 13, no. 1, pp. 1–17, 2012.

[79] D. Grimm, "Overview of the Different Devices and Techniques for Simulated Microgravity," 2016. [Online]. Available: http://www.grimm-space-research.com/RPM/RPM.html. [Accessed: 18-Mar-2019].

[80] S. Kopp, "Impact of gravity on the actin filament system of the macrophage cell line RAW 264.7," 2011. [Online]. Available: https://www.researchgate.net/figure/C-displays-the-Random-Positioning-Machine-developed-by-Dutch-Space_fig4_225006954. [Accessed: 25-Mar-2019].

[81] Australian Radiation Protection and Nuclear Safety Agency (ARPANSA), "What is non-ionising radiation?," 2019. [Online]. Available: https://www.arpansa.gov.au/understanding-radiation/what-radiation/what-non-ionising-radiation.

[82] Federaal Agentschap voor Nucleaire Controle, "Meeteenheden van radioactiviteit," *FANC*, 2017. [Online]. Available: https://www.fanc.fgov.be/nl/informatiedossiers/wat-radioactiviteit-ioniserende-straling/meeteenheden-van-radioactiviteit. [Accessed: 17-Apr-2019].

[83] J. Rask, W. Vercoutere, B. J. Navarro, and A. Krause, "Space Faring: The Radiation Challenge Radiation educator guide." 2008.

[84] D. C. Giancoli, *Physics for Scientists and Engineers with Modern Physics*, 4th ed. Upper Saddle River: Pearson Education, 2009.

[85] D. R. Fisher and F. H. Fahey, "Appropriate use of effective dose in radiation protection and risk assessment," *Health Phys.*, vol. 113, no. 2, pp. 102–109, 2017.

[86] M. Durante and F. A. Cucinotta, "Physical basis of radiation protection in space travel," *Rev. Mod. Phys.*, vol. 83, no. 4, 2011.

[87] Canadian Space Agency, "How does radiation affect the human body in space?," 2006. [Online]. Available: http://www.asc-csa.gc.ca/eng/sciences/osm/radiation.asp. [Accessed: 20-Mar-2019].

[88] ESA, "Radiation and Life," 2019. [Online]. Available: https://www.esa.int/Our_Activities/Human_and_Robotic_Exploration/Lessons_online/Radiation_and_life.

[89] NASA, "Radiation Belts with Satellites," 2017. [Online]. Available: https://www.nasa.gov/mission_pages/sunearth/news/gallery/20130228-radiationbelts.html. [Accessed: 25-Mar-2019].

[90] NASA, "Orion Radiation Handout," 2014. [Online]. Available: https://www.nasa.gov/sites/default/files/np-2014-03-001-jsc-orion_radiation_handout.pdf. [Accessed: 18-Mar-2019].

[91] N. Mittal and V. K. Verma, “Study of some characteristics of solar energetic particles and associated solar activities during 1996–2016,” *New Astron.*, vol. 69, no. September 2018, pp. 74–87, 2019.

[92] T. Straume, “Medical Concerns with Space Radiation and Radiobiological Effects,” in *Handbook of Cosmic Hazards and Planetary Defense*, 1st ed., J. N. Pelton and F. Allahdadi, Eds. Heidelberg: Springer International Publishing, 2015, pp. 259–292.

[93] G. Sinnis *et al.*, “The Origin of the Galactic Cosmic Radiation,” 2010.

[94] R. Moeller *et al.*, “STARLIFE—An International Campaign to Study the Role of Galactic Cosmic Radiation in Astrobiological Model Systems,” *Astrobiology*, vol. 17, no. 2, pp. 101–109, 2017.

[95] Swinburne University of Technology, “Galactic Cosmic Rays,” *Cosmos*, 2019. [Online]. Available: http://astronomy.swin.edu.au/cosmos/G/Galactic+Cosmic+Rays. [Accessed: 29-May-2019].

[96] R. C. Youngquist, M. A. Nurge, S. O. Starr, and S. L. Koontz, “Thick galactic cosmic radiation shielding using atmospheric data,” *Acta Astronaut.*, vol. 94, no. 1, pp. 132–138, 2014.

[97] Z. Li *et al.*, “Exposure to galactic cosmic radiation compromises DNA repair and increases the potential for oncogenic chromosomal rearrangement in bronchial epithelial cells,” *Sci. Rep.*, vol. 8, no. 1, pp. 1–11, 2018.

[98] V. Manova and D. Gruszka, “DNA damage and repair in plants – from models to crops,” *Front. Plant Sci.*, vol. 6, pp. 1–26, 2015.

[99] M. C. Negritto, “Repairing Double-Strand DNA Breaks,” *Nat. Educ.*, vol. 3, no. 9, p. 26, 2010.

[100] International Atomic Energy Agency, *Effects of ionizing radiation on plants and animals at levels implied by current radiation protection standards*, vol. 332. Vienna, 1992.

[101] J. W. Norbury *et al.*, “Galactic cosmic ray simulation at the NASA Space Radiation Laboratory,” *Life Sci. Sp. Res.*, vol. 8, no. February, pp. 38–51, 2016.

[102] R. Thoraeus, “Cesium 137 and its Gamma Radiation in Teleradiotherapy,” *Acta radiol.*, vol. 55, no. 5, pp. 385–395, 1961.

[103] S. McKenna-Lawlor, P. Gonçalves, A. Keating, G. Reitz, and D. Matthiä, “Overview of energetic particle hazards during prospective manned missions to Mars,” *Planet. Space Sci.*, vol. 63–64, pp. 123–132, 2012.

[104] Biology Online, “Nutritive value,” 2005. [Online]. Available: https://www.biology-online.org/dictionary/Nutritive_value. [Accessed: 13-Mar-2019].

[105] J. M. Berg, J. L. Tymoczko, G. J. J. Gatto, and L. Stryer, “Carbohydrates,” in *Biochemistry*, 8th ed., New York: W. H. Freeman and Company, 2015, pp. 315–340.

[106] R. N. Tharanathan, G. Muralikrishna, P. V. Salimath, and M. R. R. Rao, “Plant carbohydrates-An overview,” *Proc. Plant Sci.*, vol. 97, no. 2, pp. 81–155, 1987.

[107] M. Rosa *et al.*, “Soluble sugars - Metabolism, sensing and abiotic stress,” *Plant Signal. Behav.*, vol. 4, no. 5, pp. 388–393, 2009.

[108] L. Amagliani, J. O'Regan, A. L. Kelly, and J. A. O'Mahony, "Chemistry, structure, functionality and applications of rice starch," *J. Cereal Sci.*, vol. 70, pp. 291–300, 2016.

[109] H. S. El-Beltagi, O. K. Ahmed, and W. El-Desouky, "Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of rosemary (Rosmarinus officinalis L.) callus culture," *Radiat. Phys. Chem.*, vol. 80, no. 9, pp. 968–976, 2011.

[110] M. J. Hong *et al.*, "The effects of chronic gamma irradiation on oxidative stress response and the expression of anthocyanin biosynthesis-related genes in wheat (Triticum aestivum)," *Int. J. Radiat. Biol.*, vol. 90, no. 12, pp. 1218–1228, 2014.

[111] D. G. Mortley *et al.*, "Influence of Microgravity Environment on Root Growth, Soluble Sugars, and Starch Concentration of Sweetpotato Stem Cuttings," *J. Am. Soc. Hortic. Sci.*, vol. 133, no. 3, pp. 327–332, 2019.

[112] G. W. Stutte, O. Monje, R. D. Hatfield, A. L. Paul, R. J. Ferl, and C. G. Simone, "Microgravity effects on leaf morphology, cell structure, carbon metabolism and mRNA expression of dwarf wheat," *Planta*, vol. 224, no. 5, pp. 1038–1049, 2006.

[113] G. Colla *et al.*, "Rocket seedling production on the International Space Station: Growth and nutritional properties," *Microgravity Sci. Technol.*, vol. 19, no. 5–6, pp. 118–121, 2007.

[114] N. Maness, "Extraction and Analysis of Soluble Carbohydrates," in *Plant Stress Tolerance*, 1st ed., R. Sunkar, Ed. New York: Humana Press, 2010, pp. 341–370.

[115] Biocyclopedia, "Methodology for Carbohydrates - Determination of total carbohydrates by anthrone method," *Biocyclopedia*, 2019. [Online]. Available: https://biocyclopedia.com/index/plant_protocols/carbohydrates/total_carbohydrates_by_anthrone.php. [Accessed: 10-Apr-2019].

[116] V. R. Young and P. L. Pellett, "Plant proteins in relation to human protein and amino acid nutrition," *Am. J. Clin. Nutr.*, vol. 59, no. 5 SUPPL., pp. 1203S-1212S, 1994.

[117] A.D.A.M. Inc., "Amino acids: MedlinePlus Medical Encyclopedia," *MedlinePlus*, 2019. [Online]. Available: https://medlineplus.gov/ency/article/002222.htm. [Accessed: 01-Apr-2019].

[118] M. H. Al-Whaibi, "Plant heat-shock proteins: A mini review," *J. King Saud Univ. - Sci.*, vol. 23, no. 2, pp. 139–150, 2011.

[119] J. Wannijn, "DC protein assay (BIO-RAD kit)." 2015.

[120] Thermo Fisher Scientific, "Chemistry of Protein Assays," *Thermo Fisher Scientific*, 2019. [Online]. Available: https://www.thermofisher.com/be/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/chemistry-protein-assays.html. [Accessed: 10-Apr-2019].

[121] Sigma-Aldrich, "47641 Folin-Ciocalteu' s phenol reagent." Sigma-Aldrich, 2015.

[122] V. M. Sardesai, "Role of Antioxidants in Health Maintenance," *Nutr. Clin. Pract.*, vol. 10, no. 1, pp. 19–25, 1995.

[123] J. M. Berg, J. L. Tymoczko, G. J. J. Gatto, and L. Stryer, "Oxidative Phosphorylation," in *Biochemistry*, 8th ed., New York: W. H. Freeman and Company, 2015, pp. 523–564.

[124] J. M. Berg, J. L. Tymoczko, G. J. J. Gatto, and L. Stryer, “The Calvin Cycle and the Pentose Phosphate Pathway,” in *Biochemistry*, 8th ed., New York: W. H. Freeman and Company, 2015, pp. 589–616.

[125] J. M. Berg, J. L. Tymoczko, G. J. J. Gatto, and L. Stryer, “Metabolism: Basic Concepts and Design,” in *Biochemistry*, 8th ed., New York: W. H. Freeman and Company, 2015, pp. 423–448.

[126] D. Y. Kim, M. J. Hong, C. S. Park, and Y. W. Seo, “The effects of chronic radiation of gamma ray on protein expression and oxidative stress in Brachypodium distachyon,” *Int. J. Radiat. Biol.*, vol. 91, no. 5, pp. 407–419, 2015.

[127] N. Vanhoudt, N. Horemans, J. Wannijn, R. Nauts, M. Van Hees, and H. Vandenhove, “Primary stress responses in Arabidopsis thaliana exposed to gamma radiation,” *J. Environ. Radioact.*, vol. 129, pp. 1–6, 2014.

[128] A. Van Hoeck *et al.*, “Characterizing dose response relationships: Chronic gamma radiation in Lemna minor induces oxidative stress and altered polyploidy level,” *J. Environ. Radioact.*, vol. 150, no. 2015, pp. 195–202, 2015.

[129] E. Sharimardanova *et al.*, “Oxidative Stress and Antioxidant Capacity in Barley Grown under Space Environment,” *Biosci. Biotechnol. Biochem.*, vol. 74, no. 7, pp. 1479–1482, 2010.

[130] I. F. F. Benzie and J. J. Strain, “The ferric reducing ability of plasma (FRAP) as a measure of ‘antioxidant power’: The FRAP assay,” *Anal. Biochem.*, vol. 239, no. 1, pp. 70–76, 1996.

[131] R. Apak, S. Gorinstein, V. Böhm, M. Özyürek, K. Güçlü, and K. M. Schaich, “Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report),” *Pure Appl. Chem.*, vol. 85, no. 5, pp. 957–998, 2013.

[132] K. Wakabayashi *et al.*, “Suppression of hydroxycinnamate network formation in cell walls of rice shoots grown under microgravity conditions in space,” *PLoS One*, vol. 10, no. 9, pp. 1–17, 2015.

[133] J. Kariuki *et al.*, “The responses and recovery after gamma irradiation are highly dependent on leaf age at the time of exposure in rice (Oryza sativa L.),” *Environ. Exp. Bot.*, vol. 162, no. February, pp. 157–167, 2019.

[134] Western University and Institute for Chemicals and Fuels From Alternative Resources, “What is Lignin?,” *Lignoworks*, 2016. [Online]. Available: http://www.icfar.ca/lignoworks/content/what-lignin.html. [Accessed: 01-May-2019].

[135] F. C. Moreira-Vilar *et al.*, “The acetyl bromide method is faster, simpler and presents best recovery of lignin in different herbaceous tissues than klason and thioglycolic acid methods,” *PLoS One*, vol. 9, no. 10, 2014.

[136] M. Gielen, “Acetylbromide measurement (lignin).” 2014.

[137] N. Sumanta, C. I. Haque, J. Nishika, and R. Suprakash, “Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents,” *Res. J. Chem. Sci.*, vol. 4, no. 9, pp. 63–69, 2014.

[138] J. M. Berg, J. L. Tymoczko, G. J. J. Gatto, and L. Stryer, “The Light Reactions of Photosynthesis,” in *Biochemistry*, 8th ed., New York: W. H. Freeman and Company,

2015, pp. 565–588.

[139] Encyclopedia Britannica, “Chlorophyll,” *Encyclopedia Britannica*, 2019. [Online]. Available: https://www.britannica.com/science/chlorophyll. [Accessed: 21-May-2019].

[140] B. Demmig-Adams, A. M. Gilmore, and W. W. Adams, “In vivo functions of carotenoids in higher plants,” *FASEB J.*, vol. 10, pp. 403–412, 1996.

[141] M. A. M. Al-Alwani, A. B. Mohamad, N. A. Ludin, A. A. H. Kadhum, and K. Sopian, “Dye-sensitised solar cells: Development, structure, operation principles, electron kinetics, characterisation, synthesis materials and natural photosensitisers,” *Renew. Sustain. Energy Rev.*, vol. 65, no. July, pp. 183–213, 2016.

[142] Q. Zhao, J. Li, and M. Liu, “Effects of simulated microgravity on characteristics of photosynthesis in plant seedling,” *Sp. Med. Med. Eng.*, vol. 15, no. 2, pp. 79–83, 2002.

[143] R. Moran, “Formulae for Determination of Chlorophyllous Pigments Extracted with N,N-Dimethylformamide,” *Plant Physiol.*, vol. 69, pp. 1376–1381, 1982.

[144] P. Burke, *Year-Round Indoor Salad Gardening: How to Grow Nutrient-Dense, Soil-Sprouted Greens in Less Than 10 Days*, 1st ed. Vermont: Chelsea Green Publishing, 2015.

[145] National Center for Biotechnology Information, “Cesium-137,” *PubChem Database*. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/Cesium-137. [Accessed: 06-May-2019].

[146] A. R. Wellburn, “The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution,” *J. Plant Physiol.*, vol. 144, no. 3, pp. 307–313, 1994.

[147] C. E. Foster, T. M. Martin, and M. Pauly, “Comprehensive Compositional Analysis of Plant Cell Walls (Lignocellulosic biomass) Part I: Lignin,” *J. Vis. Exp.*, no. 37, p. e1745, Mar. 2010.

[148] S. Ghosh, P. Sarkar, P. Basak, S. Mahalanobish, and P. C. Sil, “Role of Heat Shock Proteins in Oxidative Stress Tolerance,” in *Heat shock proteins and stress*, 15th ed., A. A. A. Asea and P. Kaur, Eds. Cham: Springer, 2018, pp. 109–126.

[149] J. Aarrouf, N. Darbelley, C. Demandre, N. Razafindramboa, and G. Perbal, “Effect of horizontal clinorotation on the root system development and on lipid breakdown in rapeseed (Brassica napus) seedlings,” *Plant Cell Physiol.*, vol. 40, no. 4, pp. 396–405, 1999.

[150] C. M. Rivera *et al.*, “Influence of simulated microgravity on growth, yield, and quality of leafy vegetables: Lettuce and rocket,” *Eur. J. Hortic. Sci.*, vol. 71, no. 1, pp. 45–51, 2006.

[151] D. E. Claassen and B. S. Spooner, “Impact of Altered Gravity on Aspects of Cell Biology,” *Int. Rev. Cytol.*, vol. 156, no. C, pp. 301–373, 1994.

[152] Y. Ma, Z. Cheng, W. Wang, and Y. Sun, “Proteomic analysis of high yield rice variety mutated from spaceflight,” *Adv. Sp. Res.*, vol. 40, no. 4, pp. 535–539, 2007.

[153] C. Mazars *et al.*, “Microgravity induces changes in microsome-associated proteins of Arabidopsis seedlings grown on board the international space station,” *PLoS One*, vol.

9, no. 3, 2014.

[154] S. A. Wolff, L. H. Coelho, M. Zabrodina, E. Brinckmann, and A. I. Kittang, “Plant mineral nutrition, gas exchange and photosynthesis in space: A review,” *Adv. Sp. Res.*, vol. 51, no. 3, pp. 465–475, 2013.

[155] M. E. Musgrave, A. Kuang, L. K. Tuominen, L. H. Levine, and R. C. Morrow, “Seed Storage Reserves and Glucosinolates in Brassica rapa L. Grown on the International Space,” *J. Am. Soc. Hortic. Sci.*, vol. 130, no. 6, pp. 848–856, 2005.

[156] A. E.-F. Abomohra, W. El-Shouny, M. Sharaf, and M. Abo-Eleneen, “Effect of Gamma Radiation on Growth and Metabolic Activities of Arthrospira platensis,” *Brazilian Arch. Biol. Technol.*, vol. 59, no. 0, pp. 1–12, 2016.

[157] H. Vandenhove, N. Vanhoudt, A. Cuypers, M. van Hees, J. Wannijn, and N. Horemans, “Life-cycle chronic gamma exposure of Arabidopsis thaliana induces growth effects but no discernable effects on oxidative stress pathways,” *Plant Physiol. Biochem.*, vol. 48, no. 9, pp. 778–786, 2010.

[158] J. H. Kim, Y. C. Byung, J. S. Kim, and G. W. Seung, “Effects of in planta gamma-irradiation on growth, photosynthesis, and antioxidative capacity of red pepper (Capsicum annuum L.) plants,” *J. Plant Biol.*, vol. 48, no. 1, pp. 47–56, 2005.

[159] G. Biermans *et al.*, “Arabidopsis thaliana seedlings show an age-dependent response on growth and DNA repair after exposure to chronic γ-radiation,” *Environ. Exp. Bot.*, vol. 109, pp. 122–130, 2015.

[160] S. Alikamanoglu, O. Yaycili, and A. Sen, “Effect of gamma radiation on growth factors, biochemical parameters, and accumulation of trace elements in soybean plants (Glycine max L. Merrill),” *Biol. Trace Elem. Res.*, vol. 141, no. 1–3, pp. 283–293, 2011.

[161] M. Gicquel, L. Taconnat, J. P. Renou, M. A. Esnault, and F. Cabello-Hurtado, “Kinetic transcriptomic approach revealed metabolic pathways and genotoxic-related changes implied in the Arabidopsis response to ionising radiations,” *Plant Sci.*, vol. 195, pp. 106–119, 2012.

[162] E. Park, Q. Wang, G. E. Lester, Z. Xiao, R. A. Saftner, and Y. Luo, “Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens,” *Postharvest Biol. Technol.*, vol. 110, pp. 140–148, 2015.

Annexes

Annex A: Composition Hoagland High Phosphate nutrient solution

Table A1: Composition Hoagland High Phosphate nutrient solution

Macro Elements	**10 x conc**	**g / 2 L**
KNO_3		20.4
$Ca(NO_3)_2 \cdot 4\ H_2O$		14.16
$MgSO_4 \cdot 7\ H_2O$		9.8
Phosphate solution	**10 x conc**	**g / 2 L**
$NH_4H_2PO_4$		4.6
Fe solution	**1667 x gec**	**g / 250 mL**
$FeSO_4 \cdot 7\ H_2O$		1.9
EDTA-$Na_2 \cdot 2\ H_2O$		1.25
(dissolve in 4/5 volume 70 °C (brown color) stirring, cool down and dilute)		
µ-Elements	**1000 x gec**	**g / 1 L**
H_3BO_3		2.86
$MnCl_2 \cdot 4\ H_2O$		1.81
$CuSO_4 \cdot 5\ H_2O$		0.08
$H_2MoO_4 \cdot H_2O$		0.09
$ZnSO_4 \cdot 7\ H_2O$		0.22

High phosphate Hoagland (<u>for 10 L</u>)		
macro	100	ml
P	50	ml
Fe	0.6	ml
µ	1	ml

Add to the bottle and autoclave the solution.

Annex B: Protocol protein extraction

Equipment and reagents

Machine/Product	Reference
Centrifuge	Eppendorf Centrifuge 5424 R
Tris	
Hydrochloric acid	
EDTA	
Sodium dodecyl sulphate	
Glycerol	

Procedure

Preparation solutions

Tris-HCl (1 M)

- Weigh 12.114 g Tris
- Dissolve the Tris in 100 mL HCl (38%)

EDTA (0.25 M)

- Weigh 4.653 g EDTA
- Dissolve and dilute it in 50 mL milliQ H_2O

SDS (10 m%)

- Weigh 10 g SDS
- Dissolve and dilute the SDS in 100 mL milliQ H_2O

Glycerol (50 v%)

- Weigh 63.065 g glycerol
- Dilute it to 100 mL using milliQ H_2O

Preparation extraction buffer

Put following solutions in a flask (100 mL):

- 12 mL 1 M Tris-HCl
- 1.6 mL 0.25 M EDTA
- 40 mL 10 m% SDS

- 20 mL 50 v% glycerol

Dilute this solution to 100 mL with milliQ H2O (+23.7 mL)

Extraction proteins

- Add 1 mL extraction buffer to the plant tissue and mix well
- Centrifuge the samples at 12 000 g and 4°C for 20 minutes
- Transfer the supernatant in a clean Eppendorf tube and store the samples at -20°C

Annex C: Protocol protein quantification

Title protocol: DC Protein assay (Bio-Rad kit)

Author: Jean Wannijn

Last modified: 01/04/2015

Principle

Measurement of protein content in plant or animal extracts. The method is a commercial and stable variety of the Lowry method. The assay is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. As with the Lowry assay, there are two steps which lead to colour development: The reaction between protein and copper in an alkaline medium, and the subsequent reduction of Folin reagent by the copper-treated protein. Colour development is primarily due to the amino acids tyrosine and tryptophan, and to a lesser extent, cystine, cysteine, and histidine. Proteins effect a reduction of the Folin reagent by loss of 1, 2, or 3 oxygen atoms, thereby producing one or more of several possible reduced species which have a characteristic blue colour with maximum absorbance at 750 nm and minimum absorbance at 405 nm.

Lowry, O.H., Rosebrough, N.J., Farr, A. L. and Randall, R.J., "Protein Measurement with the Folin Phenol Reagent" Journal of Biological Chemistry, 193 (1951): 265-275.

Equipment and Reagents

Machine/Product	**Reference**
Microplate reader set to 750 nm	BioTek Powerwave HT with Gen 5 software
Extraction buffer	See SLP-BIS-059
96-well plate	Greiner PS-Microplate
1.5 mL Eppendorf tubes	3x per sample + 7 for Standard Curve
2.0 mL Eppendorf tube	For reagent preparation
Reservoirs for working reagent	
Crushed Ice	
DC Protein Assay Kit: DC™ Protein Assay Reagent A (250 ml) *alkaline copper tartrate solution* DC™ Protein Assay Reagent B (2x 1 l) *dilute Folin Reagent* DC™ Protein Assay Reagent S (5 ml) *sufficient for 500 standard assays or 10000 microplate assays*	Bio-Rad reagent package (Cat# 500-0116)  Bio-Rad Cat# 500-0113  Bio-Rad Cat# 500-0114  Bio-Rad Cat# 500-0115
BSA Protein Standard II, bovine serum albumin	 Bio-Rad Cat# 500-0007

Procedure

Safety Considerations

Eye protection and gloves should be worn while using these products. Consult the MSDS for additional information.

Standard Curve

- Prepare 1 mL of a 2 mg/mL stock solution of BSA or protein standard in the buffer used during protein extraction.
- This buffer can be the one described in **SLP-BIS-060**_PROTEIN EXTRACTION FROM ARABIDOPSIS THALIANA or it can be the buffer used for enzyme extraction as described in **SLP-BIS-032** _ENZYME ANALYSIS IN PLANTS - PLATE READER METHOD
- Keep at room temperature

- Prepare a dilution series of the BSA stock solution in extraction buffer
- Recommended concentration range: 2 - 1 – 0.5 – 0.25 – 0.125 – 0.0625 – 0 $mg.mL^{-1}$
- Keep at room temperature.

Sample preparation

- Thaw samples on ice, flip the Eppendorf tubes (do not vortex)
- Aliquot 50 µL of each sample in a new Eppendorf tube.
- Make 1/2 and 1/4 dilutions in extraction buffer of each sample by pipetting 25 µL from the 1/1 aliquot onto 25 µL of buffer, and repeating this step with the 1/2 dilution thus obtained.
- Let the sample aliquot and dilutions stabilize at room temperature.

Assay

- Calculate [(#samples * 3)+ 30] * 25 µL = Volume Reagent A required.
- Round this number up to the nearest whole ml and pipet the corresponding amount of Reagent A into an Eppendorf or falcon tube
- Add 20µL Reagent S to the tube for each ml of Reagent A. Mix by pipetting. This is Reagent A'
- Pipet 5 µL of each standard, sample and sample dilution onto the 96-well plate. Provide three replicates for the standard curve.
- Pipet 25 µL of Reagent A' into each well. Make sure no bubbles are created.
- Pipet 200 µL of Reagent B into each well. Make sure no bubbles are created.
- Shake the plate at slow speed in the spectrophotometer. Remove bubbles
- Let the reaction run for 15 minutes.
- Measure absorbance at 750 nm on the spectrophotometer
- The absorbance remains stable for 1 hour.

Storage of Solutions and Standards

- REAGENT A, REAGENT B, and REAGENT S should be stored away from direct sunlight at room temperature. (Reagent A and B may also be stored in the refrigerator) All reagent are good for 6 months from date of purchase.

- Lyophilized preparations of protein standard (I and II) should be refrigerated upon arrival. These lyophilized preparations have a shelf life of 1 year at 4°C. Rehydrated and stored at 4°C, the protein solutions should be use within the 60 days. Rehydrated and stored at -20 °C, the protein should be used within 6 months.

Annex D: Protocol FRAP assay

Title protocol: Total Antioxidative Capacity according to the FRAP assay for plant material

Author: Hanne Vercampt

Original protocol/article: Penarrieta J. M., J. A. Alvarado & B. Akesson (2008) – Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (Chanopodium pallidicaule): An Andean pseudocereal. Mol. Nutr. Food Res. 52: 708-171.

Kerchev P. & S. Ivanov (2008) – Influence of extraction techniques and solvents on the antioxidant capacity of plant material. Biotechnol. & Biotechnol. Eq. 22(1): 556-559.

Last modified: 02/04/2014

Equipment and reagents

Machine/Product	
Liquid nitrogen	
Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid)	
TPTZ (2,3,5-Trifenyltetrazoliumchloride)	
Iron(III)Chloride and Sodium Hydroxide	
Ethanol	
Hydrochloric acid	
Na_2-EDTA ((Ethylenedinitrilo)tetraacetic acid disodium salt) and sodium acetate	
Acetic acid	
Plate reader	BioTek Powerwave XS2 Platereader
Centrifuge	Eppendorf Centrifuge 5424 R
Eppendorf tubes	Eppendorf Safe-Lock Tubes; 2.0 mL
Reagent basin	
96-well plates	Greiner PS-Microplate

Procedure

Preparation of extraction and reagent solutions

Extraction medium – 0.01 N NaOH solution with 1 mM Na-EDTA (make fresh when sediments arise)

- Stock solution (100x) 100 mM Na-EDTA:
  - Dissolve 399.9711 mg NaOH in milliQ and dilute to 10 mL (1 M NaOH)
  - Put 2 mL of this solution in a beaker of 50 mL
  - Add 1.861 g Na-EDTA and dilute to 50 mL
- Flask 500 mL: little milliQ
- Add 813 µL HCl (38%) and 5 mL 100 mM EDTA-solution and dilute to 500 mL with MilliQ water
  (pH should be around 1.7)

Trolox standard solution (100 mM) (make fresh each day!)

- Weigh ± 10 mg trolox in a collection tube of 1.5 mL
- Part the exactly weighed amount by 25.029
- This is the amount of **ethanol (mL)** you have to add

FRAP reagent

Make 200 µL aliquots of 100 mM TPTZ and 200 mM $FeCl_3$ solution. Per 6 samples you need 2 aliquots of each.

a. 1 ml TPTZ solution (100 mM) (can be stored in -80°C)
   - Weigh ± 31 mg TPTZ in a collection tube of 1.5 mL
   - Part the exactly weighed amount by 31.234
   - This is the amount of **HCl (40%)** (mL) you have to add

b. 1 ml $FeCl_3$ solution (200 mM) (can be stored in -80°C)
   - Weigh ± 32 mg $FeCl_3$ in a collection tube of 1.5 mL
   - Part the exactly weighed amount by 32.442
   - This is the amount of **milliQ** (mL) you have to add

c. Na-acetic buffer (100ml) (can be stored in at 4°C)
   - 329 µL acetic acid (CH_3COOH)
   - 820 mg Na-acetic
   - Bring to pH 3.6 - 4
   - Dilute to 100 mL

Method

- **Extraction of samples (use cotton and nitrile gloves!)**
  - Use the shredder with chrome steel beads to crush the sample
  - Add **1 mL** of **extraction buffer**
  - Crush it carefully with the sample until homogeneous powder
  - Let the sample thaw slowly at room temperature (time period about 20')
  - in the meanwhile, prepare the other samples at the same way
  - Once thawed, put the samples in a collection tube on ice

Centrifuge the samples **30 minutes on 15 000 g, 4°C** (This extract contains the hydrophilic fraction of antioxidants (low molecular protein fraction (GSH, AsA, …))

- **Make/thaw the reagents**
  - Make 100 mM Trolox in ethanol fresh each day (= standard)
  - Thaw 2 aliquots of 100 mM TPTZ in HCl on ice
  - Thaw 2 aliquots of 200 mM $FeCl_3$ in MilliQ on ice

- **Put on the plate reader at least half an hour before the measurement!!**

- **Make a dilution range**

  - Dilute 100 mM Trolox standard solution 100 x until 1 mM Trolox:

  Pipet 100 µL 100 mM Trolox in an Eppendorf tube + 900 µL extraction medium (= 10 mM Trolox)

  Pipet 100 µL 10 mM Trolox in an Eppendorf tube + 900 µL extraction medium (= 1 mM Trolox)

  - Vortex in between!!!
  - Pipet successively 500 µL 1 mM Trolox-solution in a new collection tube and add 500 µL extraction buffer ➔ 500 µM
  - Vortex and repeat this 1:1 dilution until 15.625 µM, according to the scheme:

µM Trolox	nmol Trolox/well
1000	50
500	25
250	12.5
125	6.25
62.5	3.125
31.25	1.5625
15.625	0.78125
0	0

- **Put the 96-well plate on ice with a layer of aluminium foil in between, to prevent denaturation and vaporisation.**
- **Samples**
  - Take the samples out of the centrifuge
  - Collect 500µl supernatant in a new collection tube
  - Discard the remaining supernatant
  - Resuspend the pellet in **1ml acetone (80%)** with a potter (= lipophilic fraction TAC)
  - **Incubate** these samples on ice for **1 hour**!
  - In the meanwhile you measure the hydrophilic fraction

After 1 hour of incubation, **centrifuge** the lipophilic fraction at **15000 g, 15 minutes, 4°C**
➔ Supernatant is used to measure the lipophilic fraction antioxidants (Vitamin E and carotenoids)

- **96-well plate: preparation**
  Pipet the samples and standards on the plate: in total **50 µl sample** or standard solution per well

  - 2 technical replicates of standard: 50 µl
  - 3 technical replicates per sample:
    - 10 µl sample + 40 µl extraction medium (1/5 dilution!)
      (check the dilution for you samples in advance with test samples!)

  Put the plate off the ice. The reactions occur more quickly at room temperature.

- **Make FRAP reagent**
  Add in a tray (for multichannel):
  - 150 µL 100 mM TPTZ-solution
  - 150 µL 200 mM $FeCl_3$-solution
  - 17.6 mL Na-acetic buffer
- **Addition of FRAP reagent**

  Add **150 µL FRAP reagent** to every well with a multi-pipet
  (Notice the blue colouring! When you do not see this, start all over!!)

  Incubate for **10 minutes** and measure at wavelength **593 nm**

  BLANK = 50 µL H_2O + 150 µL FRAP reagent

- **Repeat this procedure (from step 6) for the lipophilic fraction.**

Annex E: Protocol pigment analysis

Title protocol: Pigment analysis in leaves – Plate reader method

Author: Jean Wannijn

Last modified: 01/04/2015

Principle

Chlorophyll A is a specific form of chlorophyll used in oxygenic photosynthesis. It absorbs most energy from wavelengths of violet-blue and orange-red light. This photosynthetic pigment is essential for photosynthesis in eukaryotes, cyanobacteria and prochlorophytes because of its role as primary electron donor in the electron transport chain

Chlorophyll B is a form of chlorophyll. Chlorophyll B helps in photosynthesis by absorbing light energy. It is more soluble than chlorophyll a because of its carbonyl group. Its colour is green, and it primarily absorbs blue light.

Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus some bacteria and at least one species of aphid.

Wellburn, A.R. (1994). *The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant physiol. 144, 307-313.*

Equipment and reagents

Machine/Product	**Reference**
Plate reader	BioTek Powerwave HT with Gen 5 software
96-well plate (DMF-resistant)	Greiner REF 655201 PP-Microplate flat bottom
Reagent Basin 60 ml (DMF-resistant)	Thermo Scientific Cat# 9910027
2 ml Microtubes	Eppendorf Safe-Lock Tubes™, 2.0 ml
DMF (Dimethylformamide) 99.8%	 Merck 1.03053

Procedure

Pigment Extraction

- Put your plant material in a microtube.
- Add DMF (keep a ratio of 0.5 mL DMF for each 20 mg fresh weight)
- Cover with aluminium foil and leave in the dark for 24 hours at 4 °C

Pigment Measurement

- Measure protocol: Shake: Medium for 0:05 | Delay for 0:00:10 | Read: (A) 664, 647, 480
- Measure the empty 96-well plate and record the absorbance for each well, for the 3 wavelengths. These are the well-specific blanks.
- The pigment colour is not stable in time. When analysing a great number of samples, measure in groups of 8 samples.
- Transfer 200 µL of the 8 first samples to a column on the 96 well plate.
- Pipette 100 µL in the wells of the next 2 columns. Use a 100 µL multichannel pipette and a reagent basin.
- Using a multichannel pipette, pipet 100 µL from the sample in the first column to the DMF in the second column. Pipette up-and-down a few times.
- Pipet 100 µL of the mixed solution to the third column. Pipette up-and-down a few times to mix.
- Pipet 100 µL of the mixed solution in the third and discard it.
- This yields a 1:1, a 1:2 and a 1:4 dilution series for each sample.
- Immediately measure the absorbance at 664, 647 and 480 nm with the plate reader.

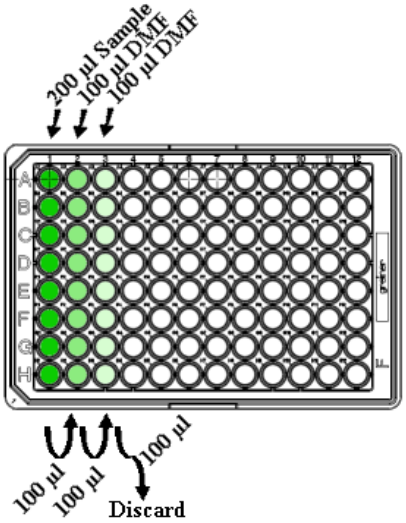

Figure 1: Dilution scheme for 8 samples on a 96-well plate.

Calculations

- Correct the measurements of each sample at each wavelength using the results from the empty plate measurement.
- Multiply the different absorbance values with their relevant dilution factors. This will provide with technical replicates.
- Calculate the light path length of the plate using the formula below and the specifications of the 96-well plate. Other plates can have other dimensions.

$$\text{Light path (cm)} = \frac{\text{Vol (mL)}}{\pi\, R^2} = \frac{0.100}{3.14159\ (6.58\ \div 2)^2} = 0.294 \text{ cm}$$

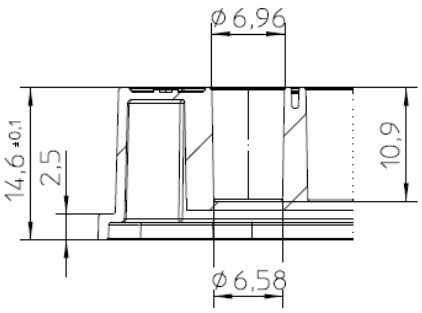

Figure 2: Dimensions of the Greiner bio-one PP-Microplate flat bottom REF 655201

- Divide all Absorbance values by this factor to correct to a 1 cm light path.
- Calculate the different amounts of carotenoid A (Ca) carotenoid B (Cb) and carotenoid X+C (Cx+c) using the equations below:

$$C_a = 11.65A_{664} - 2.69A_{647}$$

$$C_b = 20.81A_{647} - 4.53A_{647}$$

$$C_{x+c} = \frac{(1000A_{480} - 0.89C_a - 52.02C_b)}{245}$$

- This yields a result of µg pigment per ml DMF.
- Calculate the amount of pigment expressed on a fresh weight base (µg/g FW) by multiplying the result with the amount (mL) of DMF used for the extraction and dividing it by the sample fresh weight (g).

Annex F: Protocol quantification sugar and starch

Title protocol: Analysis of starch and soluble sugars with Anthrone reagent

Original protocol/article: Hansen J, Møller IB (1975) Anal Biochem 68: 87-94.

Oren et al. (1988) Oecologia 75: 28-29

Marshall (1986) Plant and Soil 52-54

Principle

Anthrone dissolved in sulphuric acid may be used for the quantitative determination of different carbohydrates. Quantitative determination is only possible where the identity of sugar components is known because colour development varies with the different sugars. Nevertheless, the anthrone method is widely used for the determination of starch and soluble sugars in plant material.

Generally sugars and carbohydrates are extracted from dried and ground plant material. First soluble sugars are extracted with aqueous ethanol, later starch is extracted with an acid. Acidic starch extracts are typically clear, however ethanolic sugar extracts may be green (leaves) or brown (roots). To remove these interfering colours first pre-extract plant material with 100% acetone (Marshall 1986, Oren). Any one of hundreds of extraction procedures may be used to extract soluble sugars not just aqueous ethanol. If methanol/chloroform/water was used it would not be necessary to do first an acetone extraction.

Equipment and reagents

Machine/Product	
Acetone	
Ethanol	
Hydrochloric acid	
Sulfuric acid	
Anthrone	
Glucose	
Centrifuge	Eppendorf Centrifuge 5424 R
Eppendorf tubes	Eppendorf Safe-Lock Tubes; 2.0 mL
96-well plates	Greiner PP-Microplate
Water bath	

Extraction procedure

- Dry and grind leaf material
- Weigh 10 -20 mg of ground leaf into a centrifuge tube or similar
- Extract interfering pigments with 100% acetone, e.g using ultra-turrax and filtering/centrifuging
- Extract sugars with 2 * 2.5 mL aliquots of 80% ethanol
- Filter or centrifuge and keep supernatant for soluble sugar analysis
- To the residue add 5 mL of 1.1% HCl
- Heat in a water bath at 100ºC for 30 min
- Dilute to 10 mL with DI water

Analysis procedure

- Turn on spectrophotometer and let warm up
- Make Anthrone reagent: dissolve 1 g of anthrone in 500 mL of 72% sulphuric acid
- Pipette 1.0 mL of test solution into a 10-mL test tube and cool to 0ºC on ice
- Add 5 mL of ice-cold anthrone reagent. Note: anthrone reagent is quite viscous, particularly when cold
- Heat for **exactly** 11 minutes at 100ºC (in water bath) and cool rapidly to 0ºC on ice
- Read A630 (against water) within an hour

Carry standard starch solutions (0 to 10 mg starch/10 mL solution) and a blank through extraction and analysis procedure.
Note: starch is normally stored in the fridge (0-5ºC) to minimise degradation/hydrolysis.

Annex G: Protocol lignin quantification

Title protocol: Acetyl bromide measurement (lignin)

Author: Marijke Gielen

Last modified: 24/06/2014

Equipment and reagents

Machine/Product	
Ethanol	
Chloroform (trichloromethane)	
Acetone	
Acetyl bromide	
Glacial acetic acid	
Perchloric acid	
Sodium hydroxide	
Thermomixer	Eppendorf Thermomixer C
Nanodrop	Thermo Fisher Scientific ND-1000 Spectrophotometer
Centrifuge	Eppendorf Centrifuge 5424 R
Eppendorf tubes	Eppendorf Safe-Lock Tubes; 2.0 mL
Reagent basin	
96-well plates	Greiner PS-Microplate

Procedure

Cell wall-preparation stem (CWR)

- Dry to frozen stems in an oven at 70°C
- Cut the stems in little pieces (scissors for herbs)
- Weigh +- 20 mg dry stem material
- Ad 1 mL MQ-H2O and incubate 30 min 98 degr. +750 rpm

- Spin down for 5 min 13200 rpm and remove supernatant
- To pellet: ad 1 mL EtOH, vortex and incubate 30 min 76 degr. +750 rpm
- Spin down for 5 min 13200 rpm and remove supernatant
- To pellet: ad 1 mL chloroform, vortex and incubate 30 min 59 degr. +750 rpm
- Spin down for 5 min 13200 rpm and remove supernatant
- To pellet: ad 1 mL acetone, vortex and incubate 30 min 54 degr. +750 rpm
- Spin down for 5 min 13200 rpm and remove supernatant
- Dry pellet by leaving it on the bench for 24h

Acetylbromide

Weigh 5 mg of CWR (balance DK → two decimals)

(From here on work with 10 samples at the time because if they stay for over an hour the ABS changes)

- TAKE A BLANK !!!!!! → max 15 samples + 1 blanco
- Add 0.1 mL 25% acetylbromide in glacial acetic acid (freshly made)
- Add immediately 4 µL 60% perchloric acid
- Incubate for 30 min at 70 degr. while shaking (850 rpm)
- Spin down for 15 min at 14000 rpm
- Supernatant separately in new ep
- To supernatant: add 0.2 mL 2 M NaOH and 0.5 mL glacial acetic acid
- Wash pellet with 0.5 mL glacial acetic acid.
- Centrifuge 5 min 13200 rpm.
- Add the wash of the pellet to the supernatant.
- Fill the eppendorf till 2 mL with glacial acetic acid (+0.7 mL)
- Shake the solution and leave on your bench for 20 min
- Measure the absorbance at 280 nm with the nanodrop (measure each sample 3 times in a row and calculate the average)
- Calculate the concentration with the law of Bougeur-Lambert-Beer A = e x l x c (l=0,1 cm, e =23,35 L/(g x cm)

Annex H: Protocol Li-Cor

Title protocol: Using the Li-Cor 6400 XT

Author: Axel Van Gompel

Last modified: 04/02/2019

Equipment and reagents

Machine/Product	Reference (Company, Type, …)
Li-Cor 6400 XT	For all parts: CATEC
Drierite	Eml: Paul.Hofstede@catec.nl
CO2-scrub	
CO2-capsule	

Using the Li-Cor

Gloves should be worn while using these products. Consult the MSDS for additional information.

Taking Pictures and determine leaf area

See protocol SLP-BIS-068.

Before turning on the Li-Cor

Before turning on the Li-Cor make sure the blue drying agent is still blue or if in doubt change it. (Used drierite can be dried in the oven at 210°C. But it will lose its colormarking over time).

The CO2-scrub should also be checked but this agent will last longer than the drierite.

To change the agents in the tubes: always open them from the bottomend!

Every day of measurement the CO2-capsule should be changed. One capsule lasts 8 hours from the moment it is breached. No matter if the machine is on or not. Always check the black o-ring if it's still intact. The filter can also be changed after about 20 days of measuring.

Now it is almost the time to turn on the Li-Cor. Just a few things left to check. First of all: is the lamp connected to the electricity? Is de leafchamber connected to the Li-Cor? Make sure the leafchamber is airtight. It has to be a closed circuit!

TL:DR: Replace Drierite, CO2-scrub, CO2-capsule. Check all connections. Check closed circuit.

Turning on the Li-Cor

- Turn on the Li-Cor.
- Choose the program: WholePlantChamber RGB EB.xml by pressing F5.
- (If the lamp is not connected it will give an error.)

- Next the Li-Cor will ask if CHAMBER/IRGA is connected. Press Y for Yes.
- You will now enter the start screen.
- **3.4 Set up the Li-Cor**
- At the startscreen press F4 to enter New Measurements.
- Press 2 and then F4 to adjust the temperature in the leaf chamber. Do this by choosing black temperature and press ENTER.
- At target press 25 and ENTER.
- Use F5 to save.
- Use ESCAPE to go back to the start screen.
- Press F3 to go to the calibration menu.
- Choose Flow Meter Zero
- The machine wil stop the pump for 10s. Press Y.
- Wait 10s to see if the voltage is about +/- 0V. Adjust if necessary. Press F5 to save.
- After this enter IRGA and then IRGA ZERO. The Li-Cor will ask if there is new drieriteand CO2-scrub. Press Y. Press Enter and make sure both tubes are on the position SCRUB. Press M to match.
- Wait 20 to 30 minutes for the H2O level to stabilize. Press F2 to autozero.
- Now bypass the drierite by closing the valve. Wait for the CO2-level to stabilize and press F1 to autozero.
- Return to the startscreen.
- Press again F4 for to enter New Measurements and press 2 followed by F3 to adjust the reference CO2 (500). Press F5 to save.
- Press again F5 and choose PAR. Choose target and insert 1500. Press again F5 to save.
  TL:DR: Choose WholePlantChamber RGD EB.xml. Put temperature on 25°C. Autozero for H2O and CO2. Put the CO2 input flow at 500 and the light intesity (PAR) on 1500.

Starting a measurement.

- Press 1 and F1 to open and name a logfile. Press F5 to save. Give a potential remark and ENTER.
- Press 5 and F1 to start an autoprogram. Choose the program Lightcurve2.
- The Li-Cor will ask to add the data to the logfile. Press Y.
- If all the settings are ok press F5. Above the 5 of menu 5 there will be now an asterix visible. This indicates a program is running. If we press F1 we can see at which logpoint the program is running (total of 23 points). PGUP we can see the data of the logstep. If we press] you can look at the graphs.
- When the program is done running press F3 to close the file.
- Make a new logfile for the next plant and repeat.

TL:DR: Make a logfile, choose program Lightcurve2, run it and save logfile.

Transfer data to PC

- Check if the Li-Cor is connected to the PC with USB.
- Press ESCAPE to enter the start screen.
- Press F5 for the utility menu and go to communications. Configure Comm Port and press ENTER. Press again ENTER to turn Lterm on. Press F5 to enter in the FILE EXCHANGE MODE.
- (TURN LTERM AT THE AND ON OFF. The Li-Cor responds very slow when this mode is on.)

- On the PC open LI6400Xterm. For the connection choose RS-232 and press connect. It is possible that you have to go through all the comm ports depending which USB is connected. You will know that you have the right one if the screen on the PC resembles the Li-Cor screen.
- Then select in the menu Windows the menu Files.
- Choose the map / and then USER. The Files will appear.
- Double click on the files you want to extract. Wait for a file to be “Done!” before selecting another one.

TL:DR: Connect USB, turn on file exchange mode, transfer files.

Shutdown

- Delete the logfiles in the PC-menu.
- Turn of Lterm.
- Unplug the lamp.
- Turn of the Li-Cor)

Annex I: Results analyses compared to the control samples

Table I1: Fresh weights of the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (mg) (% of control)	**µ*g* (mg) (% of control)**	**γ radiation (mg) (% of control)**	**γ radiation + µ*g* (mg) (% of control)**
Red arrow radish	91.9 ± 2.6 (A) (100%)	91.2 ± 4.5 (A) (99.3%)	65.0 ± 3.3 (B) (70.7%)	89.0 ± 3.9 (A) (96.9%)
Red Cabbage	59.6 ± 1.1 (A) (100%)	66.7 ± 1.9 (B) (111.9%)	51.5 ± 1.0 (C) (86.5%)	51.5 ± 1.2 (C) (86.5%)
Opal Basil	20.9 ± 0.4 (A) (100%)	23.8 ± 0.5 (B) (114.1%)	17.8 ± 0.6 (C) (85.0%)	

Table I2: Protein quantity in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (% Protein/FW) (% of control)	**µ*g* (% Protein/FW) (% of control)**	**γ radiation (% Protein/FW) (% of control)**	**γ radiation + µ*g* (% Protein/FW) (% of control)**
Red arrow radish	1.97 ± 0.07 (A) (100%)	1.49 ± 0.08 (B) (75.3%)	1.66 ± 0.06 (AB) (84.3%)	2.70 ± 0.20 (C) (137.0%)
Red Cabbage	2.20 ± 0.07 (A) (100%)	1.45 ± 0.07 (B) (66.1%)	2.97 ± 0.16 (C) (135.0%)	2.89 ± 0.16 (C) (131.6%)
Opal Basil	4.32 ± 0.20 (A) (100%)	3.02 ± 0.13 (B) (69.9%)	4.69 ± 0.09 (C) (108.6%)	

Table I3: Hydrophilic antioxidants in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (µmol/g FW) (% of control)	**µ*g* (µmol/g FW) (% of control)**	**γ radiation (µmol/g FW) (% of control)**	**γ radiation + µ*g* (µmol/g FW) (% of control)**
Red Arrow Radish	12.1 ± 0.5 (AB) (100%)	12.2 ± 0.7 (AB) (100.2%)	15.0 ± 0.7 (AB) (123.5%)	9.39 ± 1.5 (A) (77.5%)
Red Cabbage	6.8 ± 0.2 (A) (100%)	3.1 ± 0.2 (B) (45.6%)	8.3 ± 0.3 (C) (122.2%)	6.3 ± 0.2 (A) (92.8%)
Opal Basil	15.0 ± 1.6 (A) (100%)	10.3 ± 1.0 (A) (68.3%)	12.3 ± 1.7 (A) (81.9%)	

Table I4: Lipophilic antioxidants in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (µmol/g FW) (% of control)	**µ*g* (µmol/g FW) (% of control)**	**γ radiation (µmol/g FW) (% of control)**	**γ radiation + µ*g* (µmol/g FW) (% of control)**
Red Arrow Radish	1.14 ± 0.05 (A) (100%)	1.11 ± 0.11 (AB) (96.9%)	2.44 ± 0.34 (B) (213.4%)	1.10 ± 0.07 (AB) (96.0%)
Red Cabbage	5.53 ± 0.05 (A) (100%)	1.11 ± 0.08 (B) (72.6%)	1.57 ± 0.07 (A) (102.7%)	1.22 ± 0.07 (B) (80.1%)
Opal Basil	4.22 ± 0.59 (A) (100%)	5.38 ± 1.35 (A) (127.5%)	1.18 ± 0.16 (B) (28.0%)	

Table I5: Amount of chlorophyll a in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (nmol/g FW) (% of control)	**µ*g* (nmol/g FW) (% of control)**	**γ radiation (nmol/g FW) (% of control)**	**γ radiation + µ*g* (nmol/g FW) (% of control)**
Red Arrow Radish	505 ± 31 (A) (100%)	480 ± 67 (AB) (95.1%)	656 ± 102 (A) (129.7%)	292 ± 28 (B) (57.8%)
Red Cabbage	427 ± 47 (A) (100%)	372 ± 23 (A) (87.1%)	648 ± 85 (B) (151.7%)	584 ± 54 (AB) (136.9%)
Opal Basil	337 ± 31 (A) (100%)	388 ± 23 (B) (115.2%)	224 ± 34 (A) (66.5%)	

Table I6: Amount of chlorophyll b in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (nmol/g FW) (% of control)	**µ*g* (nmol/g FW) (% of control)**	**γ radiation (nmol/g FW) (% of control)**	**γ radiation + µ*g* (nmol/g FW) (% of control)**
Red Arrow Radish	149 ± 9 (A) (100%)	141 ± 20 (AB) (95.1%)	170 ± 29 (A) (114.2%)	91 ± 8 (B) (61.3%)
Red Cabbage	136 ± 14 (A) (100%)	114 ± 8 (A) (83.4%)	216 ± 34 (B) (158.0%)	177 ± 18 (AB) (130.1%)
Opal Basil	186 ± 15 (A) (100%)	146 ± 4 (A) (78.3%)	226 ± 4 (A) (121.7%)	

Table I7: Amount of carotenoids in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (nmol/g FW) (% of control)	**µ*g* (nmol/g FW) (% of control)**	**γ radiation (nmol/g FW) (% of control)**	**γ radiation + µ*g* (nmol/g FW) (% of control)**
Red Arrow Radish	100 ± 4 (A) (100%)	91 ± 12 (AB) (91.0%)	124 ± 16 (A) (123.3%)	64 ± 5 (B) (63.4%)
Red Cabbage	106 ± 10 (A) (100%)	76 ± 5 (A) (72.1%)	165 ± 13 (B) (155.9%)	120 ± 9 (AB) (113.3%)
Opal Basil	111 ± 9 (A) (100%)	70 ± 11 (B) (63.0%)	83 ± 10 (AB) (74.3%)	

Table I8: Amount of sugar in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (% sugar/DW) (% of control)	**µ*g* (% sugar/DW) (% of control)**	**γ radiation (% sugar/DW) (% of control)**	**γ radiation + µ*g* (% sugar/DW) (% of control)**
Red Arrow Radish	10.3 ± 0.8 (A) (100%)	9.3 ± 0.4 (A) (90.6%)	9.6 ± 1.2 (A) (93.3%)	9.9 ± 0.9 (A) (96.5%)
Red Cabbage	5.7 ± 0.5 (A) (100%)	1.6 ± 0.3 (BC) (27.6%)	11.6 ± 1.4 (C) (202.8%)	7.4 ± 0.5 (AB) (129.6%)

Table I9: Amount of starch in the different plants compared to the control plants. Different letters indicate significant differences between treatments (± SE). µg = microgravity

	Control (% starch/DW) (% of control)	**µ*g* (% starch/DW) (% of control)**	**γ radiation (% starch/DW) (% of control)**	**γ radiation + µ*g* (% starch/DW) (% of control)**
Red Arrow Radish	20.3 ± 2.0 (A) (100%)	3.5 ± 0.8 (B) (17.1%)	22.3 ± 1.5 (A) (110.0%)	4.2 ± 0.9 (B) (20.9%)
Red Cabbage	20.1 ± 2.2 (A) (100%)	1.2 ± 0.8 (B) (6.2%)	17.0 ± 0.6 (AB) (84.2%)	4.8 ± 0.7 (B) (24.0%)