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CHIAMAKA MARIA-GORETTI ADINNU

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HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES
INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY
DEPARTMENT OF MEASUREMENTS AND PROCESS CONTROL

MONITORING OF THE GROWING PROCESS OF SPROUTING PLANTS UNDER TEMPERATURE AND HUMIDITY FACTORS BY NEAR-INFRARED SPECTROSCOPY

CHIAMAKA MARIA-GORETTI ADINNU

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Student: Chiamaka Maria-Goretti Adinnu

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Supervisors Dr. Zoltan Kovacs, full professor
Juan Pablo Aguinaga Bósquez, PhD student

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Dr. László Baranyai
Head of the Thesis Production Site

Dr. Zoltán Kovács
Supervisor

Juan Pablo Aguinaga Bósquez
Supervisor

Dr. Csilla Mohácsi-Farkas
Responsible for the Study Programme

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1. INTRODUCTION

Agriculture, as the backbone of food systems, is under constant pressure to innovate and adapt to meet the rising demand marked by the increasing global population and numerous environmental challenges. Adequate crop management plays a crucial role in safeguarding the food security of the earth's 8 billion inhabitants. Sprouts, which are young living vegetables, are nutritionally-dense and of high economic importance due to their ubiquitousness, versatility, and ease in terms of crop management (Reed et al., 2018; Waliat et al., 2023; Yang et al., 2013).

As the early stages of a plant's life, sprouting represents a critical point that significantly influences subsequent growth and yield potential due to the impact of abiotic stress factors (Dolferus, 2014). Temperature and humidity are major sources of environmental stress factors that influence the yield of economically important crops (Amin et al., 2021; Saibo et al., 2009). As crucial determinants of growth, modern-day invention employs the use of environmentally-controlled chambers to control these environmental variables and provide the optimal growth conditions for crops of commercial importance.

Many analytical techniques exist for monitoring the state of plants, such as gas chromatography, liquid chromatography, spectrophotometry, and sensor-based techniques. However, these methods are largely expensive, labour-intensive, time-consuming, invasive, reagent-reliant, and without real-time capabilities (Bester, 2008; Rahimi et al., 2020).

Near-infrared spectroscopy (NIRS) is a widely recognized fast and non-destructive correlative analytical technique for crops and food analysis (Abbas et al., 2018). It is a cutting-edge tool for monitoring and optimizing plant growth. Unlike other traditional methods, the NIRS has real-time capabilities that offer a unique window into the physiological processes of plants (Pang et al., 2023). Its multivariate data analytical process involves algorithms for the interpretation of spectral information, which provides rapid assessment of plant states and compositions (de Carvalho Lopes & Steidle Neto, 2018; Zahir et al., 2024), enabling scientists and farmers alike to make data-driven decisions for enhanced agricultural productivity, resource efficiency, and ultimately, global food security.

Hence, non-commercial climate chambers were employed to simulate diverse atmospheric conditions in which sunflower and pea plants (economically important sprouting plants) were cultivated. The growth dynamics and responses of the plants to some specified temperature and

humidity environmental variables were systematically observed and documented using NIRS. The need to monitor the growing conditions of these sprouting plants is substantiated in the face of the current escalating demands for global sustenance and the imperativeness of optimizing these conditions to ensure a robust and secure food supply for the expanding global populace.

This study focuses on the applications of NIRS in monitoring the growing process of sunflower sprouts under temperature and humidity factors.

2. AIMS AND OBJECTIVE

2.1 General Objective

- The general objective of the study is to evaluate the suitability of NIRS to monitor the growing process of commercially valuable sprouting plants under different temperature and humidity levels.

2.2 Specific Objective

The specific objective of the study includes:

- To determine the effect of different stress conditions such as optimal temperature and humidity on the growth of sunflower sprouts.
- To determine the applicability of NIRS in combination with chemometric tools for monitoring sunflower sprout growth under different conditions.
- To test the suitability of NIRS for the discrimination sprout samples under different stress conditions.
- To assess the capacity of NIRS for predicting brix, dry matter, height, and other parameters using Partial Least Squares Regression models.

3. LITERATURE REVIEW

3.1 Importance of Sprouts in the Food and Health Industry

Sprouts are young living vegetables germinated from the seeds of different plants such as vegetables, spices, grasses, and some legumes (Sirimuangmoon, 2018). The sprouting process involves germinating seeds, grains, or legumes in water, typically until tiny shoots emerge. During this process of transformation from seeds to sprouts, complex biochemical changes occur, enhancing the bioavailability of nutrients in sprouts and impacting health benefits for consumers when consumed (Hübner & Arendt, 2013; Sirimuangmoon, 2018).

Figure 1. Sprouts (Internet 1)

Due to their health-impacting attributes, sprouts have become a common dietary food source, serving as healthy choices, especially among health-conscious consumers, elderly individuals, and other vulnerable classes. In many parts of the world, various varieties of sprouts are consumed, such as alfalfa sprouts, broccoli sprouts, clover sprouts, radish sprouts, mung bean sprouts, lentil sprouts, soybean sprouts, sunflower sprouts, fenugreek sprouts, chia sprouts, quinoa sprouts, kale sprouts, etc. Each specific sprout variety is unique in its own composition of complex vitamins and minerals, phytochemicals, antioxidants, and unique health-enhancing constituents. For instance, some sprouts have functional properties that boost the immune system and metabolism

of the body (Paśko et al., 2009). Others, like wheatgrass, have a high content of chlorophyll and are therefore, particularly effective for body cell detoxification, oxygenation, antioxidation, and anti-inflammation (Akbas et al., 2017; Viacava & Roura, 2015). Additionally, microgreen sprouts like broccoli and radish sprouts are rich in dietary fiber which improves the digestive system and aids in weight management (Zieliński et al., 2007).

Sprouts are classified based on their sources. These sources include vegetables, fruit, cereals, and spices. These classifications reflect the variety of plant species that undergo the sprouting process and the unique nutritional profiles and flavors that different sprouts have, as shown in **Table *1.*** Classification of sprouts on the basis of the sources and their phytochemical constituents (Waliat et al., 2023)**Error! Reference source not found.**.

Table 1. Classification of sprouts on the basis of the sources and their phytochemical constituents (Waliat et al., 2023)

Sources	Sub-class	Phytochemicals	Reference
Vegetable	Cabbage	Total phenolics and kaempferol	Sola et al., 2020
	Kale	Flavonoids (quercetin and kaempferol derivatives)	Neugart et al., 2018
	Spinach	Ascorbic acid and total phenolic content	Bantis, 2021
	Broccoli	Polyphenol, vitamin C, and glucosinolates	Di Bella et al., 2020
	Mustard	Anthocyanins and carotenoids	Lenzi et al., 2019
Fruit	Almond	Phenolic acids and flavonoids	Prgomet et al., 2017
	Apricot	Polyphenols, carotenoids, and free radical scavengers	Hasib et al., 2002
	Peanut	Resveradiol	De La Lastra and Villegas, 2005; Wang et al., 2005
Cereals	Quinoa	Anthocyanins and total phenolics	Pasko et al., 2009
	Soybean	Phenolic compounds (isoflavone, genistein, and daidzein)	Kim et al., 2006
	Oat grain	Total fatty acids (linoleic acids and oleic acids)	Perreli et al., 2018
	Mung bean	Flavone, isoflavone, flavonoids, and isoflavonoids	Prokudina et al., 2012; Wang et al., 2008
	Buckwheat	Flavonoids (orientin, vitexin, rutin, and their isomers)	Kim et al., 2008; Nam et al., 2018
Spices	Turmeric	Phenolics, terpenoids, and curcuminoids	Retana-Cordero et al., 2021
	Fenugreek	Coumarin, nicotinic acid, trigonelline, saponins, sapogenins, phytic acid, fenugreekine, scopoletin	Billaud, 2001
	Ginger	Paradols, gingerols, shogaols, terpenoids	Ma et al., 2021

In today's food industry, sprouts are of great interest due to their easy and rapid cultivation and established health benefits through their high content of secondary metabolites (Reed et al., 2018). The rapid growth cycle of sprouts enables efficient and continuous cultivation suitable for both small-scale and large-scale production. Their cultivation places emphasis on eco-friendly and resource-efficient food production practices, thus bearing economic significance.

Nutritionally, sprouts constitute an excellent source of phytochemicals encompassing micro and macronutrients, and secondary metabolites, in levels higher than their mature counterparts, with prominent biological activities that help to promote health (Waliat et al., 2023; Yang et al., 2013). These compounds have antioxidant ability, neutralizing toxic free radicals in the body and protecting the body cells from antioxidative stress. Beyond this, phenolic compounds found within sprouts may contribute to various health benefits and reduce the risk of some chronic diseases by acting as anticarcinogenic, anti-ulcer, anti-inflammatory, anti-atherogenic, ant-thrombotic, anti-allergenic, anti-microbial, immunomodulating, cardioprotective and analgesic agents. Sprouts are also high in vitamins (vitamin B, vitamin C, and K), and minerals (phosphorus, iron, magnesium, and potassium), along with amino acids which play crucial roles in the body (Finley, 2005; Judd, 2007). These nutrients enhance digestive health, bone health, immune system support, and energy metabolism.

As a fresh product, sprouts have gained interest in the food industry not only because of their high secondary metabolites content and short production time, but also because of their popularity for specialized nutrition and gourmet cooking (Abellán et al., 2019; Sangsukiam & Duangmal, 2017). Recognized globally as nutritional powerhouses, sprouts have versatile applications due to the health-consciousness of modern-day consumers who are more attentive and involved in the selection and consumption of healthy foods. Modern consumers typically employ them as natural and accessible culinary ingredients to enhance the nutritional profile of various dishes, featuring in dishes such as salads, sandwiches, wraps, stir-fries, and more. Additionally, the use of sprouts as culinary ingredients in the food industry is reported to be the safest way to ingest sprouts because they undergo food processing and hence, reduce the incidence of foodborne diseases (Miyahira et al., 2021). For instance, several studies, as highlighted in **Appendix *1*** have demonstrated the potential of sprouts for food product improvement, fortification, and formulation in the food industry.

Likewise, sprouts have gained global recognition for their immense health-promoting bioactive compound which are applied in the food industry. Their various phenolic and nonphenolic compounds have demonstrated antioxidant properties, as well as other health-benefiting properties. Many studies, as shown in **Appendix *2*** have reported the health-enhancing attributes of sprouts, including their anti-diabetic potential, anti-carcinogenic, cardioprotective, cholesterol-reducing, and immunomodulating activities (Miyahira et al., 2021). Sprouts have also been identified to enhance glucose regulation and increase insulin immunoreactive levels (Yao et al., 2008), including anti-hyperglycaemic activity and hypolipidemic activity (Mendoza-Sánchez et al., 2019). Research by (Ho et al., 2006) and Yanaka et al. (2009) has also demonstrated the antibacterial activity of sprouts like broccoli and pea sprouts against Helicobacter pylori, which is linked to gastric cancer.

Today, several health institutes have approved sprout consumption as a health-promoting diet to aid in the prevention of chronic diseases and illnesses (Sangronis & Machado, 2007).

3.1.1 Sunflower Sprouts and Health Benefits

Sunflower (Helianthus annuus L.) is an oilseed crop that originated from North America (Adeleke & Babalola, 2020). It is reportedly one of the most extensively cultivated oil-producing plants (Yegorov et al., 2019). It ranks in the world's top three leading oilseed crops along with rapeseed and soybean, and is distinguished from the other two oilseed crops as a major source of premium edible oil with significant importance for culinary purposes (Pal et al., 2015). Sunflower cultivation and growth require viable seeds, moderate rainfall, and fertile soil (Adeleke & Babalola, 2020).

As sunflower germinates, its original composition transforms to increase the bioavailability of bioactive compounds and minerals (Márton et al., 2010). A study by (Tiyayon & Duangmal, 2018) found that antioxidant activities, phenolic compounds, and other nutrients like crude fibre, crude protein, and minerals were significantly improved by germination in sunflower sprouts. Being a natural source of antioxidants and bioactive compounds once germinated, sunflower sprouts are renowned for its immense health benefits. Several studies have reported the antimicrobial, anti-inflammatory, antioxidant, antihypertensive, anti-cardiovascular, and wound-healing benefits derived from the inherent compounds such as flavonoids, phenolics, vitamins, and polyunsaturated

fatty acids in sprouts (Fowler, 2006; Guo et al., 2017a), as discussed in **Table *2*.** Biological activities and compounds of sunflower sprouts (Guo et al., 2017a). Additionally, a study by (Bashir, 2015) highlighted their application in ethnomedicine for the treatment of disease conditions like pulmonary, laryngeal infections and bronchial infections, heart disease, and whooping cough (Bashir, 2015). Another study by (Guo et al., 2017a) reported on the culinary benefits of sprouts, including the use of sunflower seed as a snack, salad garnish, and in some bakery products (Guo et al., 2017a). These remarkable culinary and nutritional benefits has resulted in the growing popularity of sunflower constituents and spouts worldwide. However, the nutritional content found in these plants depend on the plant growing conditions.

Table 2. Biological activities and compounds of sunflower sprouts (Guo et al., 2017a)

Biological activities	**Biological compounds**
Antioxidant effects	Tocopherols, L-ascorbic acid, antioxidant enzymes catalase, glutathione dehydrogenase, guaiacol peroxidase, glutathione reductase, carotenoids
Antimicrobial effects	Tannins, saponins, glycosides, alkaloids, phenolic compounds
Antidiabetic effects	Chlorogenic acid, glycosides, phytosterols, caffeic acid, quinic acid
Antihypertensive effects	11S globulin peptides
Anti-inflammatory effects	α-tocopherol, triterpene glycosides, helianthoides
Wounds healing effect	Linoleic acid, arachidonic acid

3.2 Plant Growth Conditions and Environmental Stress Factors Affecting the Nutritional Quality of Sprouts

Plants' growth and development are influenced by environmental factors such as sunlight, temperature, relative humidity, air, soil, water, among others (Kwack et al., 2015). These factors do not only affect plant germination, growth, and development but also the phytochemical composition (Li & Kubota, 2009; Pérez-Balibrea et al., 2008).

3.2.1 Temperature as an Environmental Stress Factor

Temperature is at the core of how climate influences the development, growth, and yield of sprouting plants. The growth and development rate of plants is intricately regulated by air or soil

temperature, and different plant species may exhibit specific temperature requirements (Wheeler et al., 2000). Heat affects plants in various ways including influencing germination and stomatal development and opening. Severe low temperatures can hinder germination, while excessively high temperatures may induce heat stress, affecting overall plant development. Similarly, high ambient temperature impedes stomatal production and poses the risk of thermal injury and dehydration to plants (Driesen et al., 2020).

Conversely, the effects of temperature variations on plants are crucial for their growth and development. Seeds respond to temperature variations uniquely, as the fluctuations towards extreme temperatures affect the survival of plants. Under extreme conditions, plants undergo thermal or temperature stress, which limits their performance compared to optimal conditions of temperature (Wheeler et al., 2000).

Furthermore, nutrient composition and absorption of sprouts as well as other phytochemical accumulations are influenced by temperature (Šamec et al., 2022). Several studies found that sprouts germinated at low temperatures have improved phytochemical properties including the quantity and activity of the antioxidant compounds as compared to sprouts germinated at high temperatures (Calderon Flores et al., 2021; Khayatnezhad, 2011; Kim et al., 2022). A study by (Kim et al., 2022) reported that low temperatures below 4^0C for about 4 days as effective for maintaining sprout growth while improving its antioxidant content and activity(Kim et al., 2022). Different plant phases may necessitate a distinct temperature range. Therefore, maintaining optimal temperatures during plant cultivation is necessary to accelerate metabolic processes within the seed, and facilitate the conversion of stored nutrients into energy.

3.2.2 Humidity as an Environmental Stress Factor

Humidity is an important factor influencing plant growth and development. Relative humidity (RH) refers to the proportion of the water vapour pressure in the air to its pressure at saturation. RH relates to ambient temperature since, at saturation, vapour pressure increases with a rise in air temperature (Driesen et al., 2020). Consequently, high ambient RH during plant growth affects the stomatal closing ability and alters the stomatal size and density (Fanourakis et al., 2013).

On the other hand, plant stomata close at lower relative humidity, hence enabling plants to control fluid loss under high evaporative demand (Merilo et al., 2018) and preventing plant death (Yuan et al., 2019). Beyond these, relative humidity also has implications for sprouts' nutritional

composition. A study by (Amitrano et al., 2020) demonstrated that sprouts germinating under low humidity had higher antioxidant contents, in addition to higher polyphenols and anthocyanins, although they had lower soluble sugar compared to those germinated at higher humidity.

Therefore, knowledge of the vital processes driving plants' responses to relative humidity is critical for the prediction of impacts on plant growth on regional and global scales.

3.2.3 Lighting Conditions as an Environmental Stress Factor

Light is an essential environmental factor whose regulation is crucial for crop production (Driesen et al., 2020). Plants have photoreception systems that respond to light intensity and quality based on the light duration and intermittence, and hence determine plant morphogenetic changes and metabolic processes (Samuoliene et al., 2011). In addition to the proper regulation of their development, plants also require light for photosynthesis, which coordinates stomatal behaviour, that is the opening and closing of the stomata (Bögre & Beemster, 2008). Generally, studies have determined that an increase in light intensity leads to a linear increase in the stomatal (Casson & Gray, 2008; Lake et al., 2001). Moreover, lighting conditions may evoke photooxidative changes in plants, which can alter the plant's antioxidant defense system (Samuoliene et al., 2011). For instance, low-light intensity grown plants are predisposed to phototoxicity than plants grown under high-light intensity hindering the light's capacity for conversion of light energy into chemical energy (Long et al., 1994), whereas high-light intensity grown plants have lower photosynthetic efficiency (Al-Khatib & Paulsen, 1989; Bowes et al., 1971).

The duration and intensity of light influences leaf area and height (Fan et al., 2013). Low light intensity typically leads to expanded specific leaf area and high plant height to maximize light absorption, hence meeting the photosynthetic demand (Steinger et al., 2003). Whereas, high light levels lead to reduced specific leaf area to shield the plant from excessive irradiance. These measures prevent photodamage caused by excessive light damage, ensuring continuous photosynthesis (Givnish et al., 2004; Matos et al., 2009).

Additionally, light intensity and quality also impact on the nutrient profile of sprouts during germination. Several studies have demonstrated that plants germinated under light conditions have the highest phenolic compounds, anthocyanins, and antioxidant activity, especially when combined with low relative humidity germination conditions (Amitrano et al., 2020; Brazaityte et al., 2015; Kyriacou et al., 2016; Qian et al., 2016). For instance, a study by (Tsurunaga et al., 2013)

found that sprouts germinated under Ultraviolet blue light range of >300nm effectively produced buckwheat sprout of higher anthocyanins content, although another study by (Urbonavičiūtė et al., 2009) found that wheat and barley sprouts germinated under amber light at 595nm had no significant increment in the phenolic and ascorbic acid content.

The duration and intensity of light exposure impact the rate of photosynthesis, growth rate, and nutrient composition. Therefore, striking the right balance in light conditions is crucial for cultivating high-yield and nutrient-dense sprouts.

3.2.4 Other Influencing Factors

Various other abiotic and biotic elicitors, including plant genes and hormones, can significantly influence plant growth and nutritional quality. Some of these factors include plant genotype and seed source, and germination conditions like salinity stress, hypoxia stress, and preharvest treatments. A study by (Benincasa et al., 2019) pinpointed the genotype and seed source as the singular most important factor influencing the nutritional content and value of sprouted grains, as this factor characterizes the genetic material of the seed.

Salinity, as a germination condition, has been proven of significant importance due to its ability to cause abiotic stress in plants, particularly during the early phase of seedlings growth, which is a salt-sensitive stage (Benincasa et al., 2019). However, a study by (Lim et al., 2012) noted that the carotenoid and phenolic compounds in buckwheat sprouts increased when treated with salt solution regardless of the growth stage.

Additionally, hypoxia stress alters the gas composition of plants during the germination phase, hence the nutritional content (Benincasa et al., 2019). This stress is associated with acidic culture solutions as germination substrates, which typically leads to oxygen deficiency. In this case, plant tissues stimulate amino acid called as an adaptational mechanism to the stress-indced cytosolic acidosis, caused by the oxygen deficiency (Aurisano et al., 1995).

In general, these factors may have substantial impact on plant growth and development, particularly during sensitive phases such as the early seedling growth phase (Benincasa et al., 2019).

3.3 Modern Agricultural Challenges

Nowadays, modern agriculture is facing a complex web of challenges that demand innovative and sustainable solutions (Lykogianni et al., 2021). The alarmingly high population growth, urbanization, and changing dietary patterns have placed unusual strain on the global food system (United Nations Environment Programme, 2012). In addition, the current climate change issues being faced globally present a profound challenge, introducing uncertainties with extreme and unpredictable weather patterns, and shifts in growing seasons, consequently, impacting crop yields and challenging traditional agricultural practices.

Modern agriculture also faces the challenge of depleting natural resources, such as water and arable land, and these pose a threat to long-term food security (Khatri et al., 2023). Moreover, the use of agrochemicals in modern agricultural practices has raised environmental concerns, with negative implications for water quality, soil health, and biodiversity (Lykogianni et al., 2021).

Beyond these challenges, modern agriculture is influenced by external factors such as the globalization of trade. The globalization of trade introduces interconnected risks, hence making agriculture susceptible to economic and geopolitical shifts. Furthermore, the access to technology, education, and markets has remained uneven in some parts of the world, thus creating disparities in agricultural productivity and income among regions and communities (Thirtle & Wiggins, 2001).

With the global population estimated to exceed 9.7 billion by 2050 and 10.9 billion by 2100, it has become imperative to navigate these challenges and create a resilient and equitable agricultural landscape for the future (United Nations Environment Programme, 2012).

3.4 Controlled Environment Agriculture

In the face of these modern agricultural challenges, the cultivation of plants, especially sprouting, which are fundamental elements of the human diet, undoubtedly necessitates sustainable practices that increase crop yield to provide the food that is needed by a growing population. Controlled Environment Agriculture (CEA), shown in **Figure *2***, has been identified as an approach for adapting to the evolving agricultural landscape and the challenges it presents (Engler & Krarti, 2021).

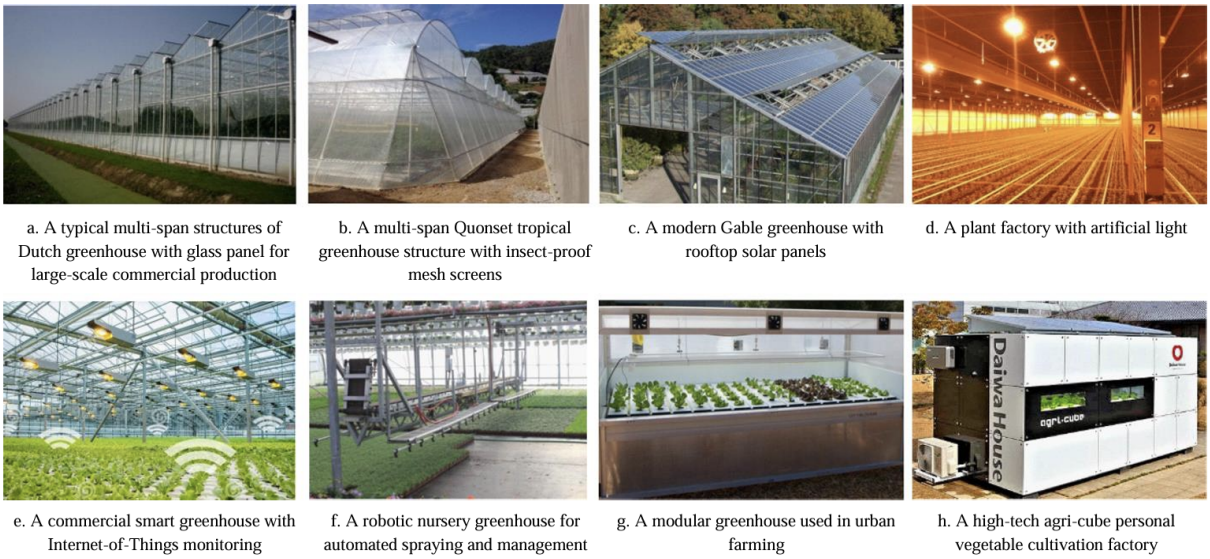

Figure 2. Modern greenhouses and controlled environment agriculture (Shamshiri et al., 2018)

CEA represents a transformative approach to modern farming, where environmental factors like temperature, humidity, light, and nutrient levels are controlled within enclosed structures such as greenhouses, vertical farms, or indoor hydroponic systems. Through the optimization attributes of CEA, it provides an ideal environment for plant growth and development, resulting in rapid plant growth cycles, increased yields, and resource efficiency. The precision which CEA imparts extends beyond climate control, to mitigating environmental impact through hydroponic and aquaponic systems that enables water conservation and nutrient recycling. Furthermore, CEA allows the provision of municipal food production in urban areas, and enhances pest and drought control, biosecurity, perennial crop farming, while minimizing the logistics cost associated with conventional farming (Benke & Tomkins, 2017). CEA also has the potential to maximize land usage in terms of farming, while also providing employment opportunities in urban cities to counter the effects of urbanization and hence, increased national and global economy (Benke & Tomkins, 2017).

3.4.1 Climate Chamber

The rapid expansion and urbanization of modern cities poses threat to sustainable agriculture and food security, through the resource shortage and environmental pollution which it causes (Despommier, 2011; Xie et al., 2017). Despite the tremendous damage that agroecology is

suffering, the nutritional requirements and quality of agricultural products is increasing, which creates a conflicting situation within the agroecology system (Orsini et al., 2013). Therefore, agricultural systems that regulate growth conditions to achieve faster and more efficient production are increasingly sought after. The high interest in such advanced agriculture systems is driven by the need to meet the increasing demands for agricultural produce and the conditions for food security (Eigenbrod & Gruda, 2015). These systems do not only provide opportunities to enhance food supply, but also aid in improving environmental sustainability, social integration, the health of citizens, and the local economy altogether (Orsini et al., 2013).

One such system is the climate chamber. This refers to a controlled environment system that is designed for the precise manipulation of temperature, humidity, light, and other environmental conditions to simulate specific climatic conditions. By such manipulations of the environmental conditions, it optimize plant growth conditions and minimizes the impact of abiotic stress on crop yield. In sprout production, climate-controlled chambers provide a means to fine-tune temperature conditions, ensuring an environment conducive to optimal sprout growth.

3.5 Essential Parameters for Sprouts Quality

Evaluating the quality of sprouts encompasses a multifaceted assessment of parameters crucial for both nutritional content and sensory appeal. Nutrient content is a pivotal aspect, involving the measurement of essential elements such as vitamins, minerals, proteins, fats, and antioxidants (Wang et al., 2022). Additionally, monitoring moisture content is critical, as it profoundly influences both the shelf life and texture of sprouts (Shomodder et al., 2022). Other parameters such as the pH and conductivity are also important indexes to measure, particularly during the sprouting process, due to the antinutritional factors found in grains and cereals which may increase or decrease after sprouting (Purohit et al., 2023).

3.5.1 Moisture Content Determination

The moisture content is a critical parameter in sprout analysis. The volume of water used in hydrolytic reactions may signify the growth phase and can be an indicator of the metabolized constituents (Nugraha et al., 2021). Moisture content represents the proportion of water present in the sprout, which influences the sprout's texture, weight, and shelf-life. Inversely, the moisture

content also relates to the dry matter content, which represents the remaining solid components after removing water. Analysis of the moisture content and dry matter content helps in determining the overall composition and stability of sprouts during storage and processing.

The most commonly used method for water content determination is based on the Association of Official Analytical Chemists (AOAC) method (AOAC, 1995). In this method, the sampling wares (empty dish/crucibles and spatula) are oven-dried at 105^0C for 3 hours, and the equilibrium weight of the wares is recorded. An analytical scale is used in weighing the sprouted sample (3-5g) into the measured container, which undergoes the oven-drying process for another 3 hours. The moisture content is calculated from the mass lost during the oven-drying process (AOAC, 1995).

3.5.2 Ph and Conductivity Determination

Grains and legumes contain toxic and undesirable constituents compared to other plant families. These toxic compounds include phytates, flavonoids, alkaloids, tannins, and trypsin inhibitors that limit their utilization as a protein source (Masood et al., 2014). The pH and conductivity of sprouted plants are determined due to the anti-nutritional factors contained in most grains and legumes, which are affected after the sprouting process. Several studies have highlighted the reduction of anti-nutritional factors, particularly phytic acid, in plants during the sprouting process (Kamal Mohamed et al., 2011; Xue et al., 2016). The reduction of antinutritional components leads to the release of free hydrogen ions, which results in lower pH measurement, and supposedly higher conductivity reading (Nugraha et al., 2021).

Figure 3. pH and electrical conductivity meter (Internet 2)

The common method for the determination of pH and electrical conductivity in sprout extract samples involves using a pH and electrical conductivity meter instrument (**Figure *3***). The electrodes of the meter are dipped into the sprout extract sample contained in a glass beaker and the readings are taken (Nugraha et al., 2021).

3.6 Role and Applications of Near-infrared Spectroscopy in the Food and Agricultural Sector

In recent times, growing public concern over the safety of food has necessitated the development of rapid, robust, and accurate methods and techniques for food safety evaluation and control (Qu et al., 2015). One such advancement is near-infrared spectroscopy (NIRS), a non-destructive and convenient tool, which has proven to be a promising technique for food safety inspection and control due to its significant advantages of speed, non-invasive measurement, ease of use, and minimal sample preparation requirement (Qu et al., 2015).

NIRS operates within the wavenumber range between 14,000 cm^{-1} to 4000 cm^{-1} and a wavelength range of 400 - 2,500 nm. It typically constitutes a radiation source, wavelength selector/modulator, sample cell, detector, and signal processor (Agelet et al., 2011). It possesses the following measurement modes: reflectance (specular and diffuse), transmission, interactance, and transflectance. Different NIR signals originate from vibrations caused between bonds of light atoms, such as C, N, O, S, and H single bonds(Lohumi et al., 2015).

For sprouted plants, some studies recognize NIRS as a highly sensitive technique that adequately satisfies the need for quick and effective plant sprout quality checks, especially in large-scale production, where a definite standard, as in the case of mung bean sprouting, has not been established (Caporaso et al., 2018; Nugraha et al., 2021; Wang et al., 2022). Additionally, several studies have extensively employed NIRS for evaluating the internal quality of beans to effectively separate hard seeds from regular seeds (Delwiche et al., 2006; Hacisalihoglu et al., 2010; Haughey et al., 2013; Kaliramesh et al., 2013). Such evaluation is of importance in the seed production process because the hard ones do not germinate during sprouting and thus, easily develop mold and contaminate neighboring seeds. A study by (Phuangsombut et al., 2017) developed a classifying model using Near-infrared hyperspectral imaging in combination with partial least squares discriminant analysis and demonstrated the application of Near-infrared spectroscopy in separating hard mung beans from normal ones.

Beyond this, NIRS has also been utilized for the assessment of the internal quality of seeds, for instance in the evaluation of their nutritional composition (Dong & Qu, 2012). Its non-destructive feature reportedly offers an advantage in terms of preserving seeds' vitality during the determination of seed quality (Phuangsombut et al., 2017).

In analyzing with NIRS, a spectral data pre-treatment step is taken. The pre-treatment involves specific processing procedures performed on raw data to enhance the quality and reliability of analytical results. In addition to improving the accuracy and robustness of quantitative analyses, data pre-processing also helps to improve the interpretability of raw data by transforming the data into a form that is easily understood by users. It also helps in the detection and removal of outliers and trends through the exclusion procedure and also serves as a dimensionality reduction method by the removal of irrelevant and redundant information through feature selection (Lasch, 2012). Common spectral pre-treatments include baseline correction, normalization, smoothing, and derivative transformations. Baseline correction aims at detecting and eliminating spectral outliers or systematic variations in the baseline of a spectrum, while normalization scales the spectra within a similar range, aiding in the comparison of different spectra and consequently improving the accuracy and efficiency of the models (Heraud et al., 2006; Kohler et al., 2006; Liu et al., 2003). Smoothing techniques reduce noise by the removal of unwanted frequencies from the signals to be analyzed, thus enhancing the resolution of spectral features. Derivative transformations minimize background interferences, reduce the baseline drift, and resolve overlapping spectra, consequently amplifying high-frequency signals and providing insights into variations within the spectra (Martens & Næs, 1989).

Beyond the pre-treatment step, the NIRS leverages on multivariate analytical techniques, such as Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and Partial Least Squares Regression (PLSR), as classification and screening tools used for extracting meaningful information from complex datasets (Abdi & Williams, 2010). The primary goal of PCA is dimensionality reduction through the transformation of original variables into a set of uncorrelated principal components, which simplifies data interpretation while retaining essential information (Wold et al., 1987). PCA is a very useful technique to spot hidden patterns in the data and is

therefore mostly used as an initial step of the analysis before classification (Moghaddam et al., 2022)

On the other hand, LDA is a classification technique that aims to maximize separation between predefined groups in a dataset, thus making it beneficial for discrimination and pattern recognition tasks. Due to its high classification attribute, it is typically employed in the detection of food adulteration (Esteki et al., 2018).

Conversely, the PLSR is a pattern recognition tool that identifies an adequate number of latent components with the aim of discriminating between previously established groups (Brereton & Lloyd, 2014). Due to its ability to recognize patterns, it is able to establish relationships between predictor variables and response variables where there is high collinearity among predictors (González-Domínguez et al., 2022).

3.7 Rationale of the Study

The rationale of this study is based on the importance of understanding the dynamics of sunflower sprouts under different environmental conditions. Despite the widely recognized nutritional value and health benefits of sunflower sprouts, limited research has been conducted on the specific environmental factors that influence their sprouting behaviour, and hence, their nutritional value. This study aims to evaluate the use of NIRS for monitoring the growth of sprouting plants under different environmental variables. There is limited literature in developing appropriate models to characterize sprouts grown under different environmental conditions and to predict different quality parameters, therefore, the study sought to address the suitability of NIRS to assess the impact of temperature and humidity on sunflower sprout dynamics and nutritional composition.

4. MATERIALS AND METHODS

4.1 Plant Materials and Growth Conditions

4.1.1 Sunflower Seeds Procurement

The Sunflower seeds (**Figure *4***) used in the research experiment were procured from a reputable organic seed manufacturer and distributor in Budapest, Hungary called Rédei Kertimag Zrt. The seeds are of the same variety and uniform in size, quality, and genetic makeup, hence minimizing genetic variations and suitable for growth in a controlled environment.

Figure 4. Image of sunflower seeds used for the study (Internet 3)

4.1.2 Growth Substrate and Planting Conditions

A growth substrate mix (**Figure *5***) was procured from Agro CS Hungary Kft.

Figure 5. Image of growth substrate used for the study (Internet 4)

Based on the information provided on the labeling, this mix was composed of a balanced combination of organic matter and essential nutrients like Nitrogen, Phosphorus Oxide, and Potassium Oxide. It is composed of peat, sand, modified reaction agent, fine structure, low nutrient content for even planting of seeds and intended for seed germination and grafting of young plants. The constituents of the substrate represent an ideal medium for optimal sunflower sprout development.

4.1.3 Set up of the Climate Chambers

A noncommercial climate chamber (**Figure *6***) was used as the growth environment for the sunflower seeds. The chambers were equipped with in-built temperature and humidity control mechanisms, to provide the optimal growth conditions as required for the experiment. The specifications of the chambers included a 32 x 42cm dimension, a control system powered by STC for the temperature, and Arduino Mega for the humidity. To decrease the temperature, commercial fridges were used, whereas heating plaques (12V) were used to increase the temperature. An ultrasound mist generator was used to increase the humidity to the desired value, while a silica-filled column and fans of 5V were used to decrease the humidity. The temperature and humidity sensors used in the climate chamber was the DHT11-M0 type shown below.

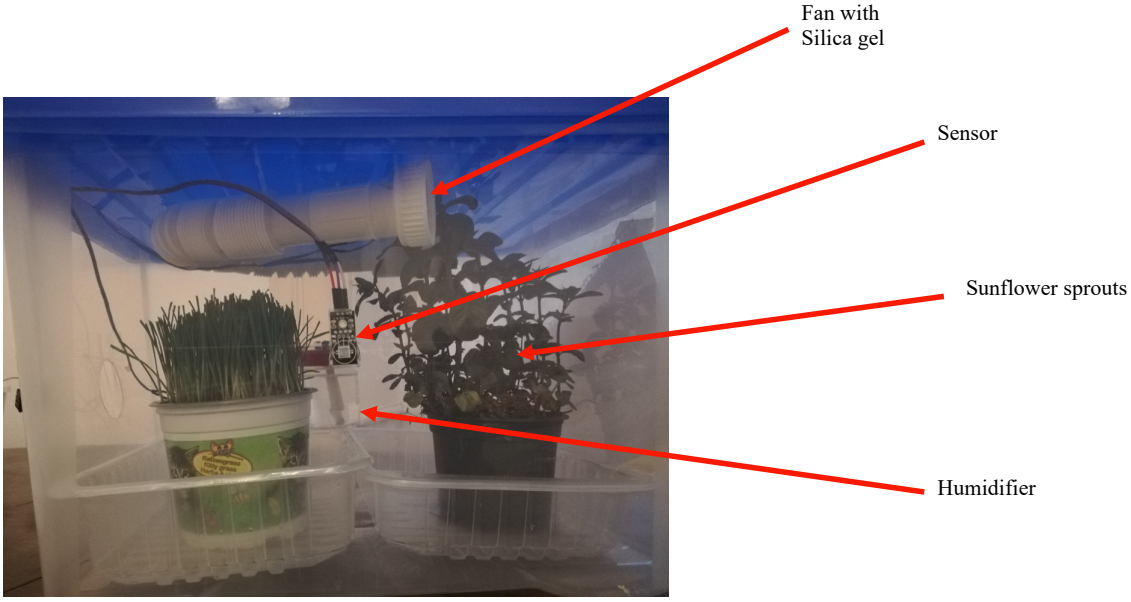

Figure 6. Image of pre-test chamber with the elements of humidity control.

For the experiment, 9 chambers (**Figure *7***) were used to control three different temperatures and humidity.

Figure 7. Image of the 9 chambers used for the study.

The chambers allowed for the regulation of temperature and humidity, the two stress factors of vital interest in this research.

4.2 Experimental Design

The experiment was designed in three phases: The cultivation, the sample preparation and measurements, and the data analysis.

The experiment started with the preparation of the seeds and soil. The process involved an initial pre-germination step (**Figure *8***) of soaking seeds (15g by treatment) for 12 hours. After the pre-germination period, the seeds were washed and rinsed, then covered with dampened paper towel for 24 hours.

After this, the pregerminated seeds were planted into an internal rectangular container containing the growth substrate (**Figure *9***). The sunflower seeds were placed in the prepared rectangular internal container over 2cm of soil and dampened with water. Another 2cm of soil was added to cover the seeds. The seeds were strategically placed to ensure proper spacing and depth for optimal germination. The internal container had holes in the base to prevent water logging. In an external

container, 6ml of water was added which ultimately served as the water source for the seeds to germinate. Next, the prepared containers were placed in the non-commercial growth chambers. The chambers were transferred to the fridge (**Figure *10***).

Figure 8. Image of the pregerminated sunflower seeds.

Figure 9. Image of the plates prepared with the growth substrate.

Figure 10. Image of the non-commercial growth chambers in the fridge.

The planting materials and growth conditions were strictly followed, from planting until 12 days of growing, because the present study aimed to minimize variability and external influences, in order to allow for a more accurate assessment of the impact of stress factors on the growth of sunflower sprouts.

Figure 11. Image of the sunflower sprouts in the fridge

Overall, the present study monitored and evaluated the effect of two stress factors: temperature and humidity on the growth conditions of sunflower sprouts. For this study, a randomized complete block design was used in order to minimize bias and enhance the viability of results. Therefore, the sunflower seed containers were randomly assigned to different treatment groups, and each treatment was represented across multiple blocks. Different treatment groups were exposed to varying temperature and humidity stress levels. The study duration was 12 days. From the planting until the final harvesting, with 5 days of measurement (namely days 4, 6, 8, 10, and 12 where the plants were harvested), carefully chosen to capture the entire lifecycle of sunflower sprouts from germination until the early phase of development, and to allow sufficient time for the expression of stress-induced changes in growth.

Throughout the entire study period, the environmental parameters within the noncommercial chambers were carefully and continuously monitored.

The three temperature conditions were implemented 15°C, 19°C, and 23°C while the three humidity conditions were 70%, 80%, and 90%. In total, nine growth chambers (grouped in 3 sets) were used for the different environmental conditions.

The temperature within the 9 noncommercial chambers was set and maintained at different environmental conditions (highlighted in **Table *3***), each condition is regarding a single climate chamber. After this, the plates containing the plants were placed into the external container, these were put into three plates, accounting for three consecutive repeats.

Table 3. Different temperature and humidity combinations used for the study.

Humidity / Temperature	70%RH	80%RH	90%RH
15°C*	15°C-70%RH (T15H70)	15°C-80%RH (T15H80)	15°C-90%RH (T15H90)
19°C*	19°C-70%RH (T19H70)	19°C-80%RH (T19H80)	19°C-90%RH (T19H90)
23°C**	23°C-70%RH (T23H70)	23°C-80%RH (T23H80)	23°C-90%RH (T23H90)

* Harvesting of plants for evaluation at 6, 8, 10, 12 days after sowing.
** Harvesting of plants for evaluation at 4, 6, 8, 10, 12 days after sowing.

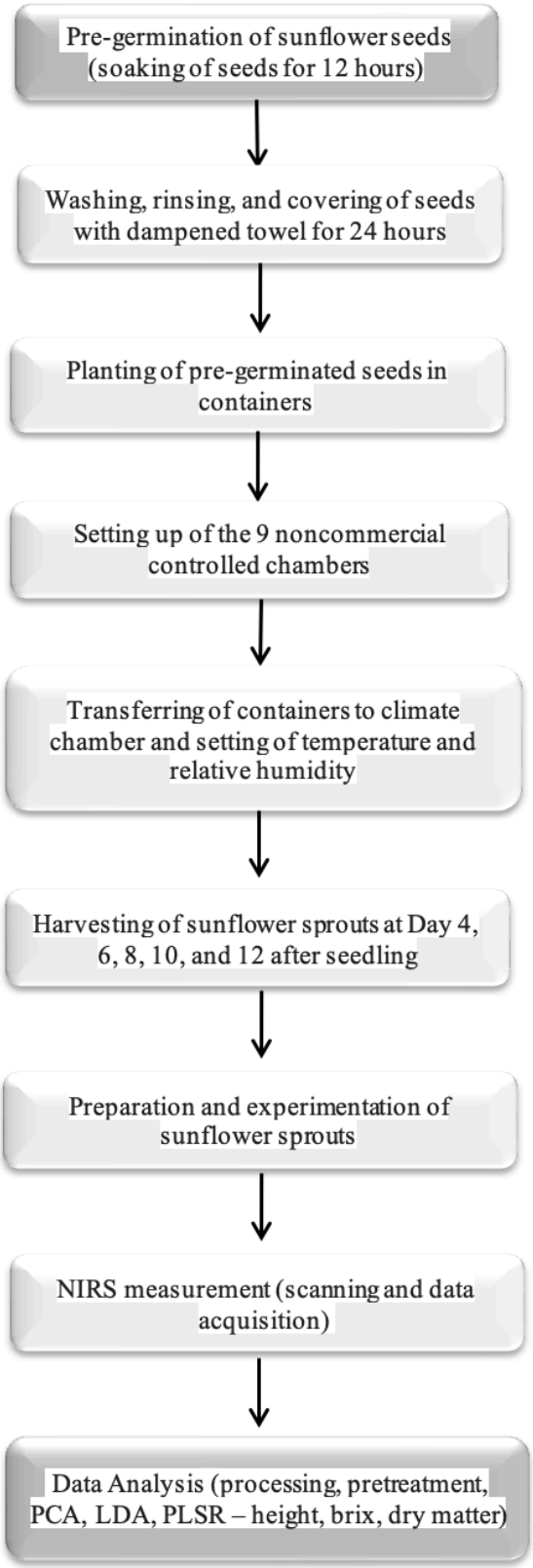

Figure 12. Flowchart of experimental arrangement for the study.

4.3 Sample Preparation and Experiment

The measurement period for the analysis lasted for a week from May 26 to June 2, 2023. For each measurement, samples were randomly taken from the chambers in the fridges. The selected samples (15 plants for each treatment) were cut, washed, rinsed, and left to dry on paper towels at room temperature for 30 minutes. All the parts were then arranged together in the NIR curvette and measured with the XDS.

For the height, 15 plants by treatment were measured using a ruler, and the values were recorded for the average value. Similarly, 15 plants were weighted using the analytical balance in two repeats, the values were recorded and an average value was determined from the two repeats.

In determining the dry matter content, the samples/plants that were weighed were cut into several parts, including the stem and the leaves. They were transferred to a labelled foil paper and taken to the oven for drying at 105°C for 3 hours.

The pH, conductivity, and ascorbic acid were determined by transferring 2g from the 15 plants from Parallel 1 to a labelled Ziploc bag. The brix was first measured by crushing the plants in a mortar (**Figure *13***) and recovering the liquid part, which was recovered using distilled water in the ratio of 1:10 (2g in 20ml DW). The mixture was then filtered using the Whatman filter paper.

Figure 13. Image of the sunflower sprouts crushed for brix measurements

4.4 NIRS Scanning and Acquisition of NIR Spectra

The NIRS instrument utilized in this research was the Metrohm XDS RapidContent Analyzer (**Figure *14***). It is an analytical instrumentation tool designed for swift and accurate content analysis in various industries. In the food industry, it enables the rapid, nondestructive analyses of solid powders, coarser granules, pellets or flakes. Hence, it is an indispensable instrument for the spectral measurement of every possible sample type in the visible and near-infrared wavelength range (400 - 2,500 nm) (Metrohm, 2023). With the XDS Rapid Content Analyzer, the testing for material or composition identification is performed either in the laboratory or at-line, on samples contained in their original bags or packaging. The XDS RapidContent Analyzer is also used in the analysis of sprouts, to determine the composition.

Figure 14. NIRS XDS RapidContent Analyzer (Internet 3)

It has real-time features that characterize its efficiency and enables users to make informed decisions swiftly, once receiving the real-time data.

Prior to the measurements, the instrument was calibrated to ensure and assure the reliability of data obtained from the measurements. The sunflower sprouts were prepared as described in section 4.3 and the cut samples were carefully arranged in two layers and covered with the lid (0.50MM) in the NIRS XDS curvette (with the inner diameter of 43.20mm) to ensure uniform exposure to the NIRS instrument.

The NIRS scanning and spectra of sunflower sprouts were measured over the range of 800-2500nm, with a resolution of 0.5nm. The duration for each NIRS scan was 30 seconds, which is sufficient for the acquisition of comprehensive spectral data. The data was collected using the diffuse reflectance mode, allowing the spectra of the materials to be collected by measuring the amount of light reflected by the sample. Each spectrum was the average of 32 scans recorded at 40°C. The spectra for each sample analyzed were recorded in three consecutive scans to account for the instrumental or sampling variability. Before each series of measurements, the background spectrum was also acquired as a precautionary measure against instrument-related variations and drifts, hence to generally enhance the accuracy and reliability of the spectral data obtained from the sample by providing a baseline reference.

4.5 Data Analysis

4.5.1 Processing and Pretreatment

The analysis of the generated spectra was performed with R-project software (specs) using the aquap2 package (Tsenkova et al., 2018). Prior to the multivariate data analysis, the following pretreatments were applied in order to extract the significant information contained in the spectral data: Savitzky-Golay pretreatment for smoothing (with 2nd order of polynomial, p = 2; number of data points in the filtering window, n =35; and order of differentiation applied to the polynomial, m=2) and the Multivariate Scatter Correction (MSC) to correct scattering. The wavelength selection for the data analysis was 1100-1800nm.

4.5.2 Multivariate Data Analysis

Following the pretreatment and processing step, the dataset was examined for its overall distribution using the principal component analysis (PCA) for pattern recognition of the samples. The environmental factors (temperature and humidity) and treatment/repetitions were used to colour the PCA scores to observe the patterns of major significance to the study. Outlier detection and removal were made based on PCA scores to ensure that the principal components accurately represent the variance within the data, hence preventing skewed results due to extreme observations. The new independent variables (PCA scores) generated by the PCA served as input for the LDA classification models, also referred to as PCA-DA models. In line with a study by

Kovacs et al. (2011), a three-fold cross-validation technique was utilized for the PCA-DA models for the inclusivity of samples in every evaluation set, hence resulting in three PCA-DA models. For the PCA-DA models, there were databases each according to three different levels of temperature (15°C, 19°C, and 23°C) and humidity (70%RH, 80%RH, 90%RH). These datasets (that is the temperature and humidity dataset) were utilized for the PCA-DA models and the classification was done according to sprouting days. From the three models, an average is calculated to report. In the calculation of the models, it is maximized the between-class distance and minimized the within-class distance.

Partial Least Squares Regression (PLSR) was employed in constructing and validating the chemometric models for quantitative evaluation of the composition of the sunflower sprouts based on their spectral characteristics.

An initial cross-validation, precisely the K-fold cross-validation, was performed where the sample set/observations inclusive of the consecutive scans were randomly but equally divided into ten subsets. Of these, one part of the subsets was used for cross-validation while the rest were used for building and training the model.

5. RESULTS AND DISCUSSION

5.1 Visual Inspection of the NIR Spectra

The NIR raw spectra of the sunflower sprout samples, coloured by temperature, in the full NIR range (700 - 2500nm) is shown in **Figure *15***. The raw spectra offer an initial broad view of the sample's molecular absorptions and interactions. The plot below was coloured by temperature because it showed a better distinction of the spectra compared to the other coloring possibilities (humidity, day). In general, it can be observed from the plot that T15 has a lower absorbance than T19 and T23.

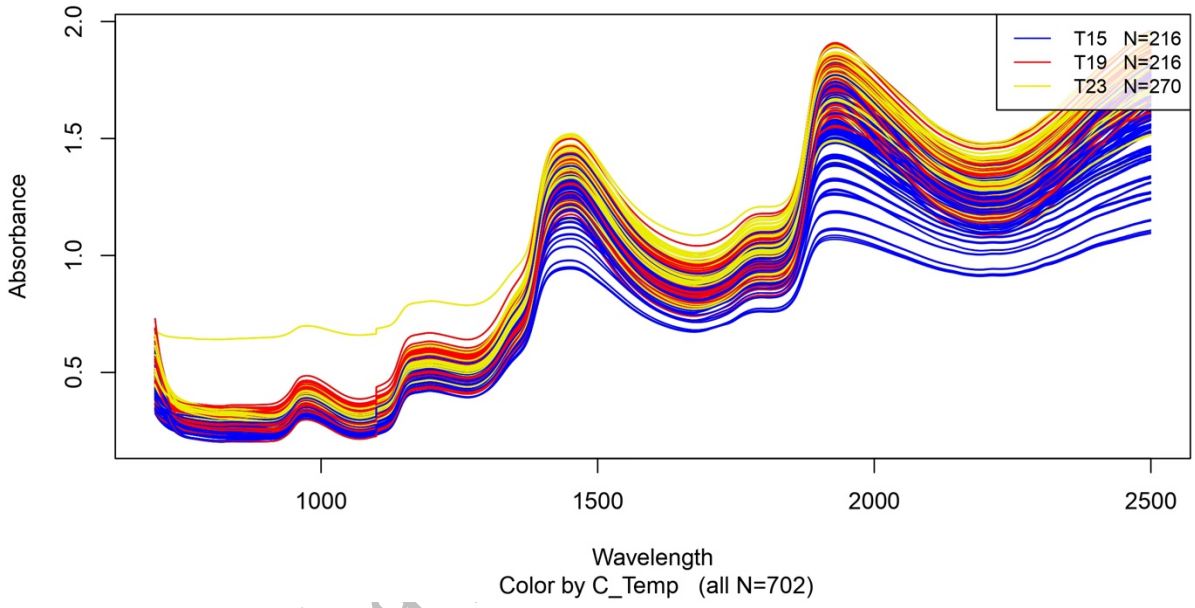

Figure 15. The raw spectra of the sunflower sprouts acquired by the XDS in the full range of 700-2500nm region.

Additionally, the loading spectra of the multivariate analysis within the full electromagnetic regions of 1100 - 1800nm were identified as the regions of high correlation, whereas the VIS/NIR regions (400-1099nm) and the far shortwave infrared regions (1801-2499.5nm) (Hennessy et al., 2020) fail to do so, hence these regions were excluded from multivariate analysis. Additionally, this wavelength range was selected because, at 1100nm, there is a shift of the spectra for the change of another detector in the spectrometer, which could be mistaken for a peak, hence the need to select the 1100 - 1800nm range.

Therefore, truncation of the spectra was necessary to isolate the regions of interest for a more detailed analysis and extraction of meaningful information from the NIR spectra. As such, the acquired spectra were truncated to a specific wavelength range, and the Savitzky-Golay second derivative was applied to the preprocessed spectra for noise reduction and to highlight spectral variations that correspond to characteristics of importance or peaks of interest.

The second derivatives of the spectra for the sunflower sprout samples in the range of 1100-1800nm are shown below coloured by temperature (**Figure *16***) variable.

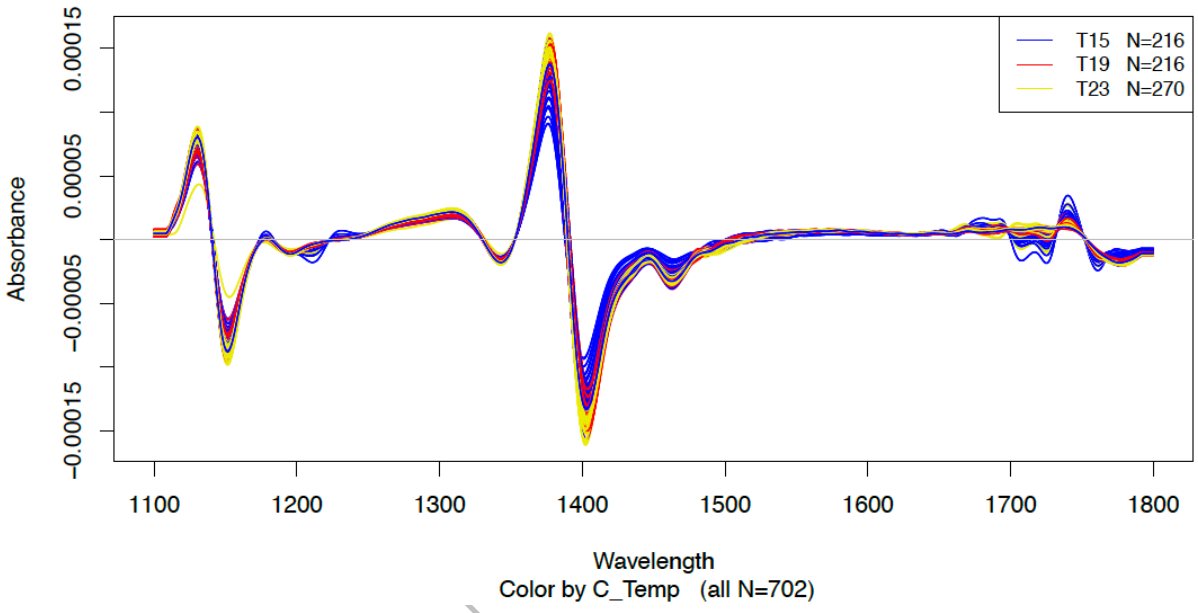

Figure 16. The 2nd derivative of Savitzky-Golay (2nd order polynomial and 35 points) of the sunflower sprouts NIR spectra in the 1100-1800nm region colored by temperature

From the NIR spectra of **Figure *17***, the spectra show broad and distinct characteristic absorbance bands at 1125, 1150, 1350, 1370, and 1400nm. According to the study by (Usman et al., 2010), the wavelength range 1125-1150nm shows absorption associated with the first overtone of Carbon-Hydrogen (C-H) stretching vibrations, while 1150-1250nm denotes absorptions occurring due to the second overtone vibration of C-H stretching (Paradkar et al., 2002). Peaks within this region are indicative of the presence of organic compounds containing C-H bonds such as fats, oils, carbohydrates etcetera. (Guo et al., 2017b) demonstrated that sunflower plants are rich sources of oil and polyunsaturated fatty acids, hence the absorption peaks at the above-mentioned wavelengths could be associated with the lipids and fats present which contribute to the nutritional profile of sunflower sprouts.

Peaks around the wavelength range of 1350-1400 are typically associated with a combination of C-H stretching and bending vibrations, including the first overtone of O-H stretching in water (Paradkar et al., 2002), confirming the naturally high-water content of sprouted plants.

Additionally, the raw spectra showed a noticeable separation between the spectra, likely attributed to scattering, hence further statistical analysis and data treatment like the multiplicative scatter correction (MSC) for baseline shift correction was applied to reveal a clearer tendency, as shown in **Figure *17***.

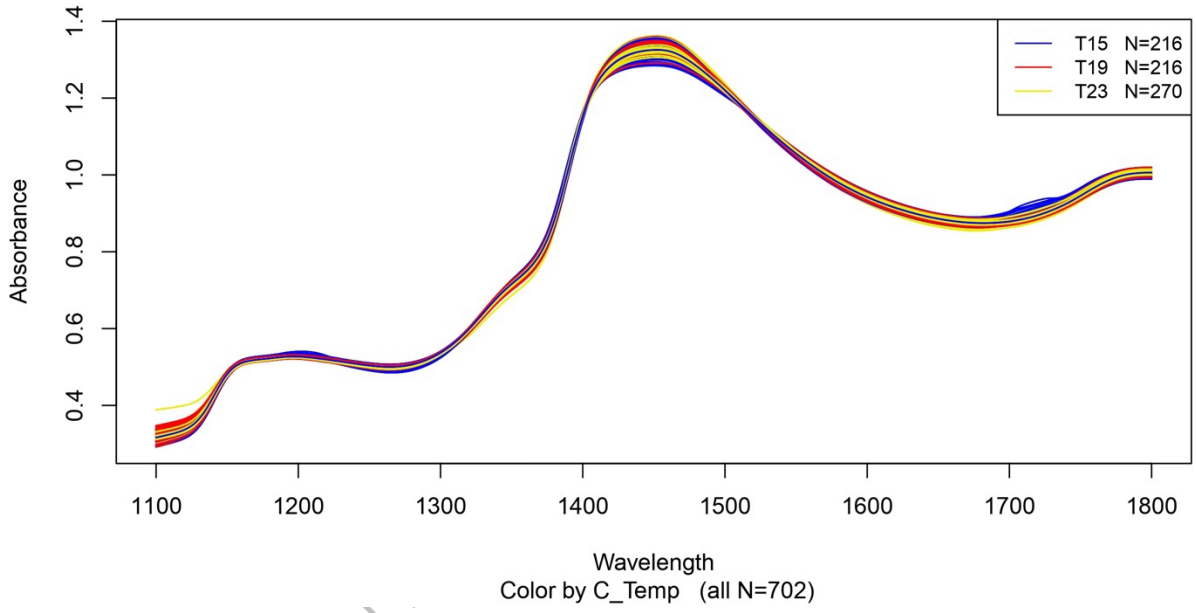

Figure 17. NIR spectra of the sunflower sprouts after Multiplicative Scatter Correction (MSC) pretreatment in the 1100-1800nm region, coloured by temperature.

The MSC spectra revealed a certain degree of overlap between the three temperatures, with no noticeable separation, hence the need for further statistical analysis like the PCA for pattern recognition of the variables.

5.2 Exploratory data evaluation of the NIR Spectroscopy results with PCA

The following figures (**Figure 18**, **Figure *19***, **Figure *20***, **Figure 21**, and **Figure *22***) show the results of the PCA on the entire Near-Infrared Spectroscopy (NIRS) on the smoothed (Savitzky-Golay filter) and MSC-corrected spectra of the sunflower sprouts.

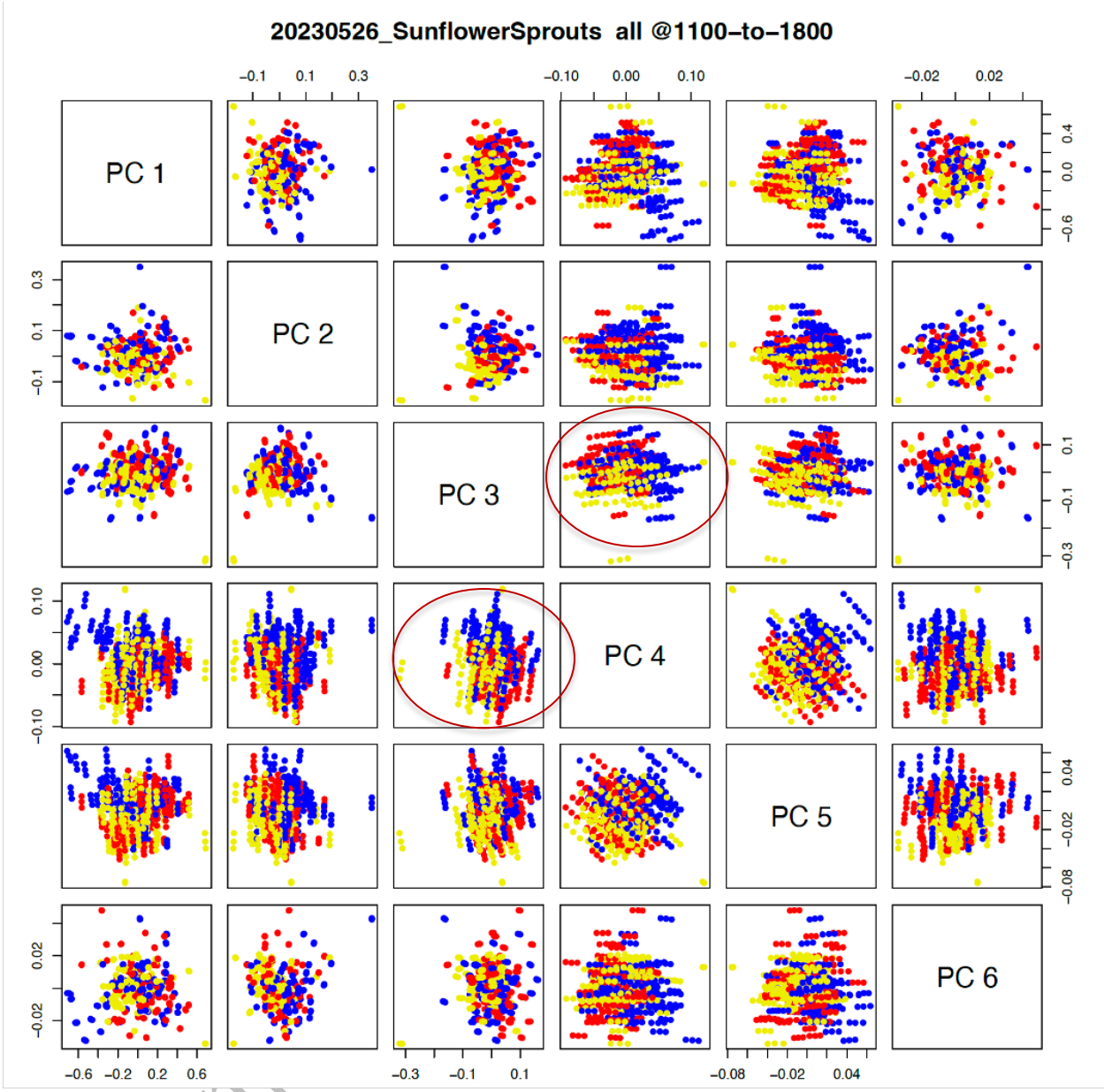

Figure 18. Principal component analysis (PCA) scores on the full NIRS spectral data of sunflower sprouts after smoothing (Savitzky-Golay filter) and MSC pretreatments, in the 1100-1800nm range. PCA Score dots coloured by temperature: temperature 1 – 15^{0}C (coloured in blue dots), temperature 2 - 19^{0}C (coloured in red dots), and temperature 23^{0}C (coloured in yellow dots). Total Number of Observations, N = 702.

From **Figure 18**, most PCs exhibited some overlapping, however, there is an observable trend showing more dispersion for temperatures 15°C and 19°C compared to temperature 23°C. This can be explained as temperatures 1 and 2 are low levels of temperature, and thus with lesser impact in terms of variability when compared to temperature 3. This is in line with the study by temperature (Driesen et al., 2020) which demonstrated high differences in plants grown at excessively high

temperatures due to thermal shock. Additionally, there is an observable pattern of separation for the different temperature variables that is apparent in the principal components PC3 and PC4, highlighted in the figure.

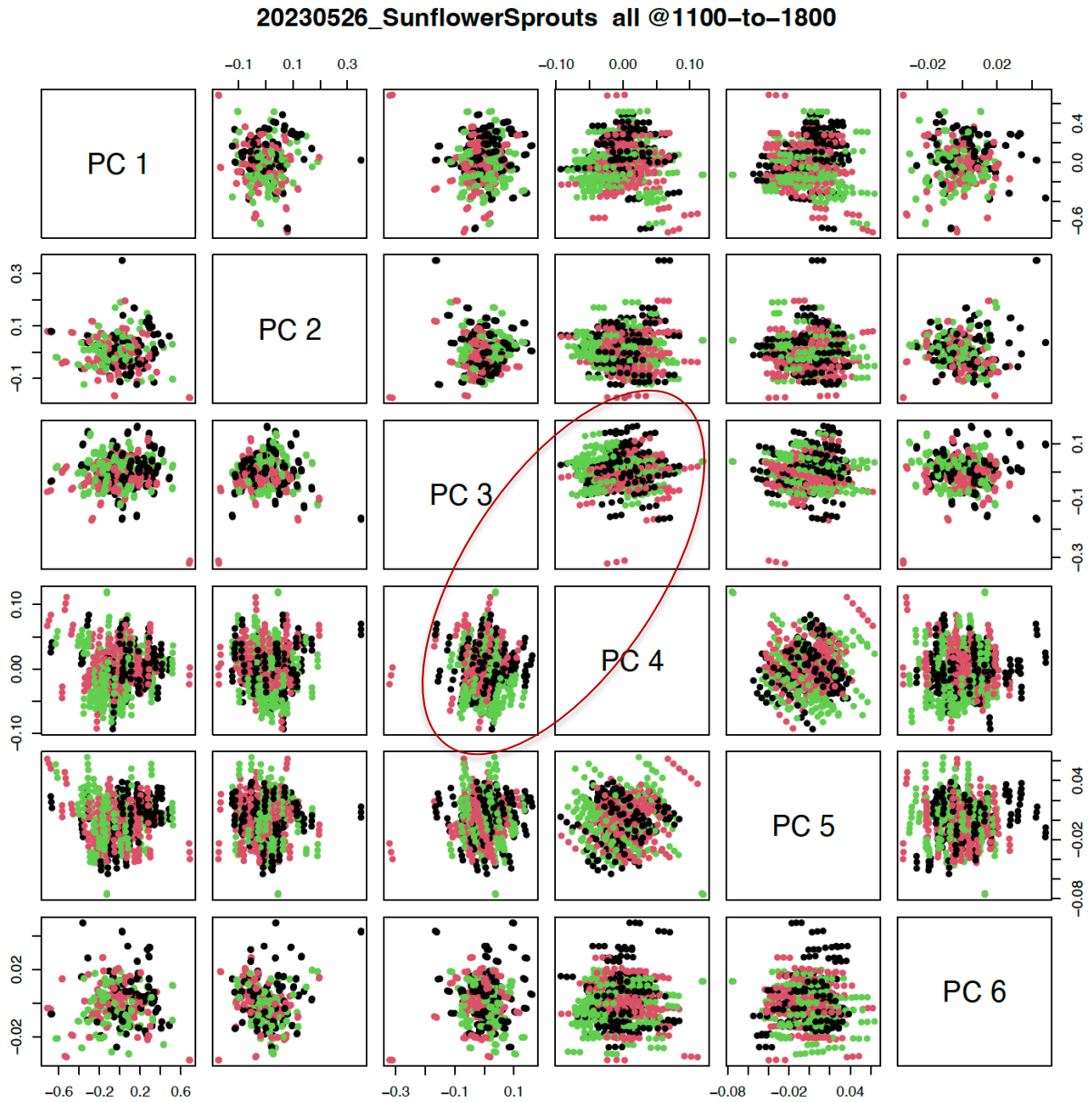

Figure 19. PCA scores on the full NIRS spectral data of sunflower sprouts calculated by the three different humidity levels after the XDS smoothing (Savitzky-Golay filter) and MSC pretreatments in the range 1100-1800nm. PCA Score dots coloured by humidity 70% (coloured in black dots), humidity 80% (coloured in red dots), and humidity 90% (coloured in green dots). Total Number of Observations, N = 702.

Similar to **Figure 18**,**Error! Reference source not found.** the PCs in **Figure *19*** exhibited a degree of overlapping and broad dispersion for the first and second levels of humidity (70% and 80% respectively), unlike the third level of humidity (90%RH). PC3 and PC4 also showed a good level of separation between the humidity levels than the other PCs which showed more overlapping.

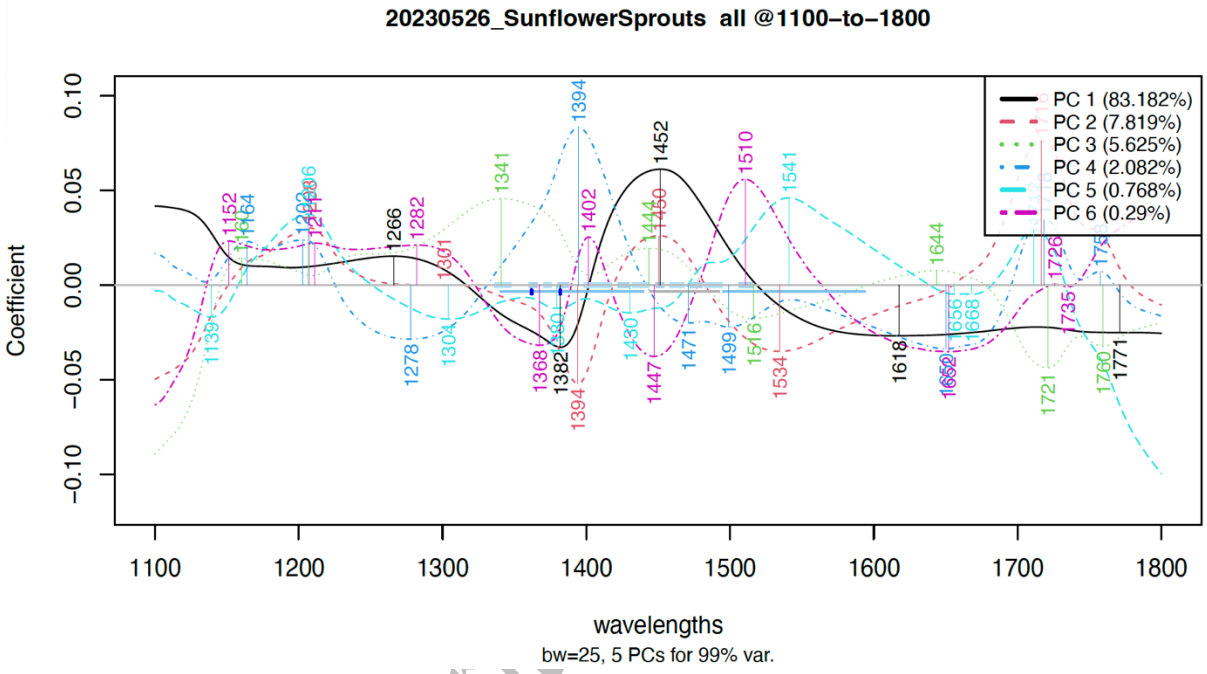

Figure 20. PCA loading vectors on the full NIRS spectral data of sunflower sprouts after smoothing (Savitzky-Golay filter) and MSC pre-treatments, in the 1100-1800nm range.

The corresponding loadings plot shown in **Figure *20*** showed 83.182% of the total variance described by the first two PCs (PC1 and PC2) and the most contributing wavelengths to the two PCs were found at 1266, 1382, 1394, 1450, 1452, 1534, 1618, and 1771.

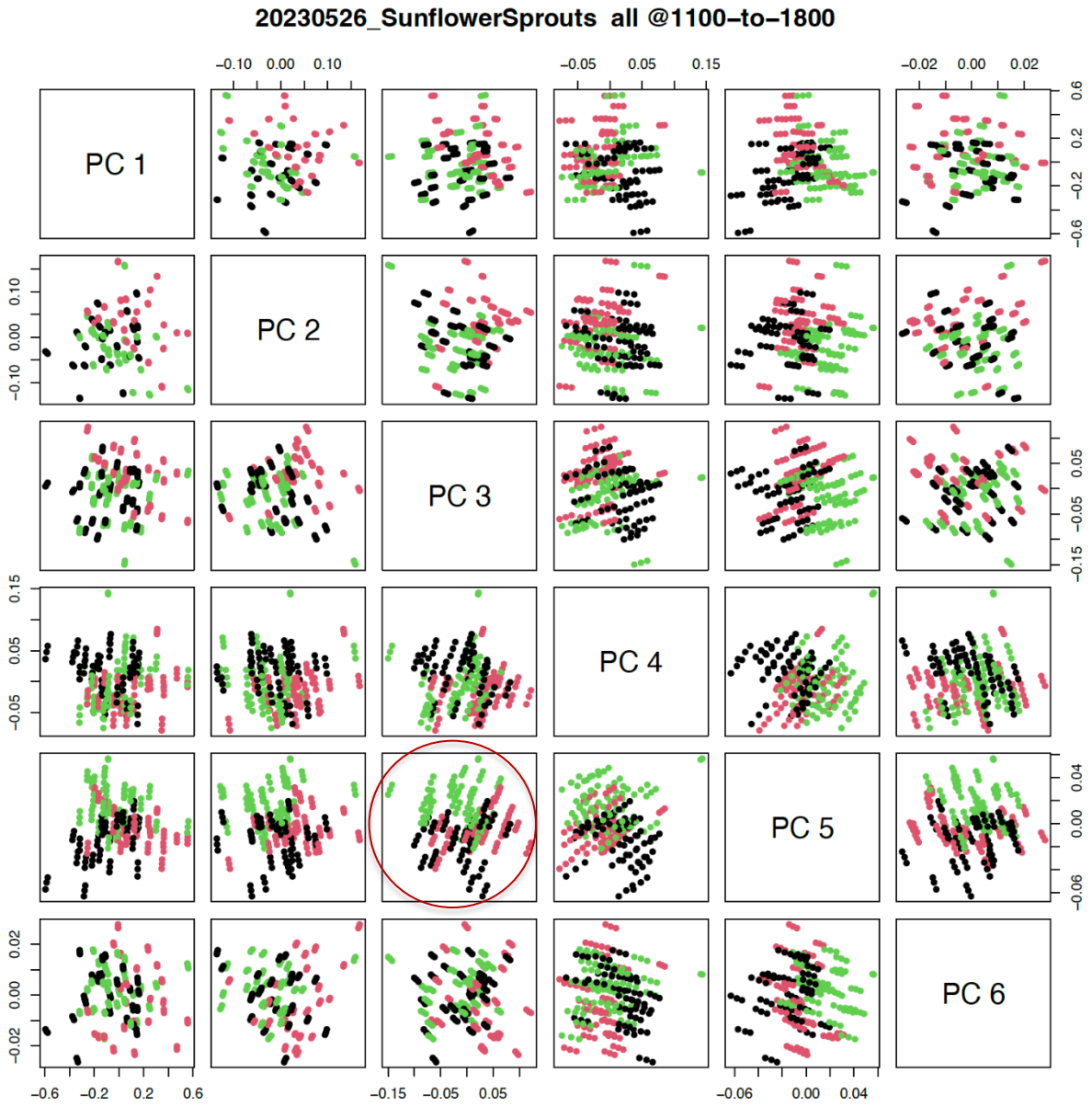

Figure 21. Principal component analysis (PCA) Scores on the full NIRS spectral data of sunflower sprouts calculated by the three levels of temperature against the highest humidity (90%) variable after XDS smoothing (Savitzky-Golay filter) and MSC pre-treatments in the 1100-1800nm range. PCA Score dots coloured by humidity: temperature T15_RH90 (coloured in black dots), temperature T19_RH90 (coloured in red dots), and temperature T23_RH90 (coloured in green dots). Total Number of Observations, N = 234.

In **Figure 21Error! Reference source not found.**, a subset of the full data comprising of only samples exposed to the highest humidity (90% RH) was selected for PCA analysis and calculated by the three different levels of temperature. The result showed a separation trend for the PCA

scores according to the temperature levels and different PCs, as can be seen in the highlighted PC3 and PC5. It can be observed that the spectral characteristics of temperature 3 (23^0C) were dominant and the grouping clearly showed the difference between the different temperature levels. It confirms the previously observed trends from **Figure 18** and **Figure *19*** that the spectral characteristics of the lower levels of temperature were more broadly dispersed and overlapping than the highest level of temperature, which had a distinct dense and dominant spectral.

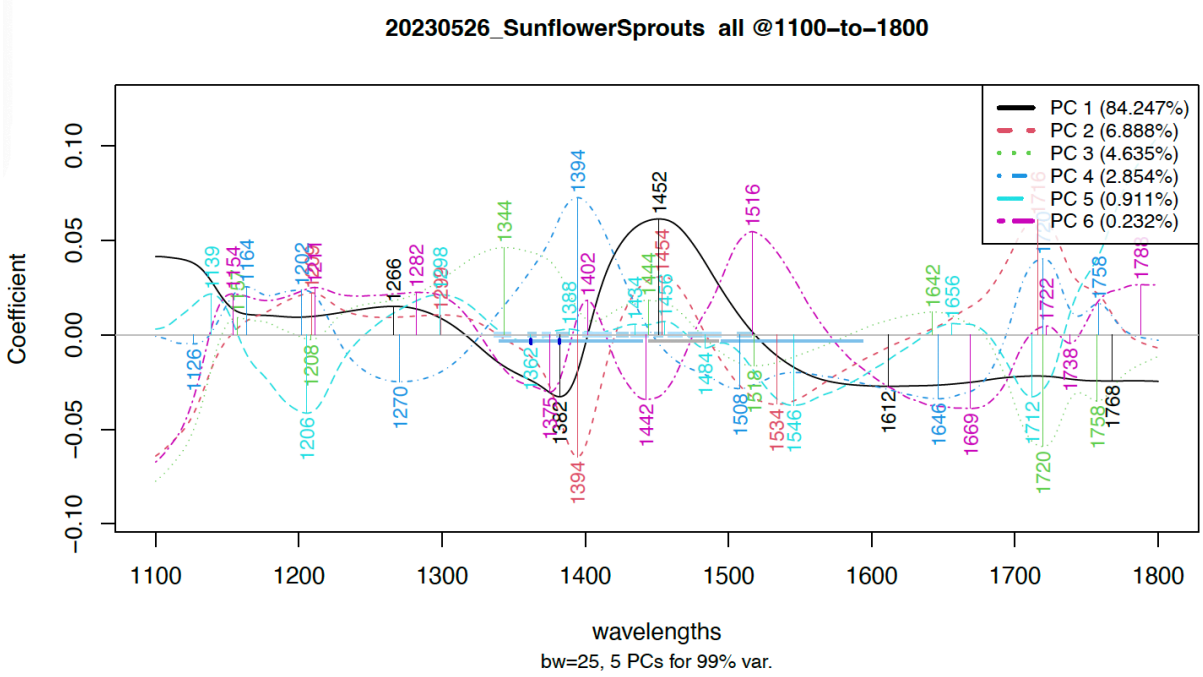

Figure 22. PCA Loading Vectors of the full spectral data of sunflower sprouts calculated by the three levels of temperature against the highest humidity (90%) variable after the XDS smoothing (Savitzky-Golay filter) and MSC pretreatment spectra in the range 1100-1800nm.

Figure *22* shows corresponding loading vectors with 84.247% of the total variance described by the first two PCs (PC1 and PC2). For PC1, 1266, 1382, 1452, 1612, and 1768 were found to be important in its formation whereas 1394, 1454, and 1534 wavelengths contributed more for PC2.

5.3 Classification of sunflower sprouts according to Temperature, Humidity, Treatments, and Day Variables by Linear Discriminant Analysis

The Linear Discriminant Analysis based on the Principal Component Analysis (PCA-DA) model built for sunflower sprouting time classification can be seen in the following figures (**Figure *23***, **Figure *24***, and **Figure *25***).

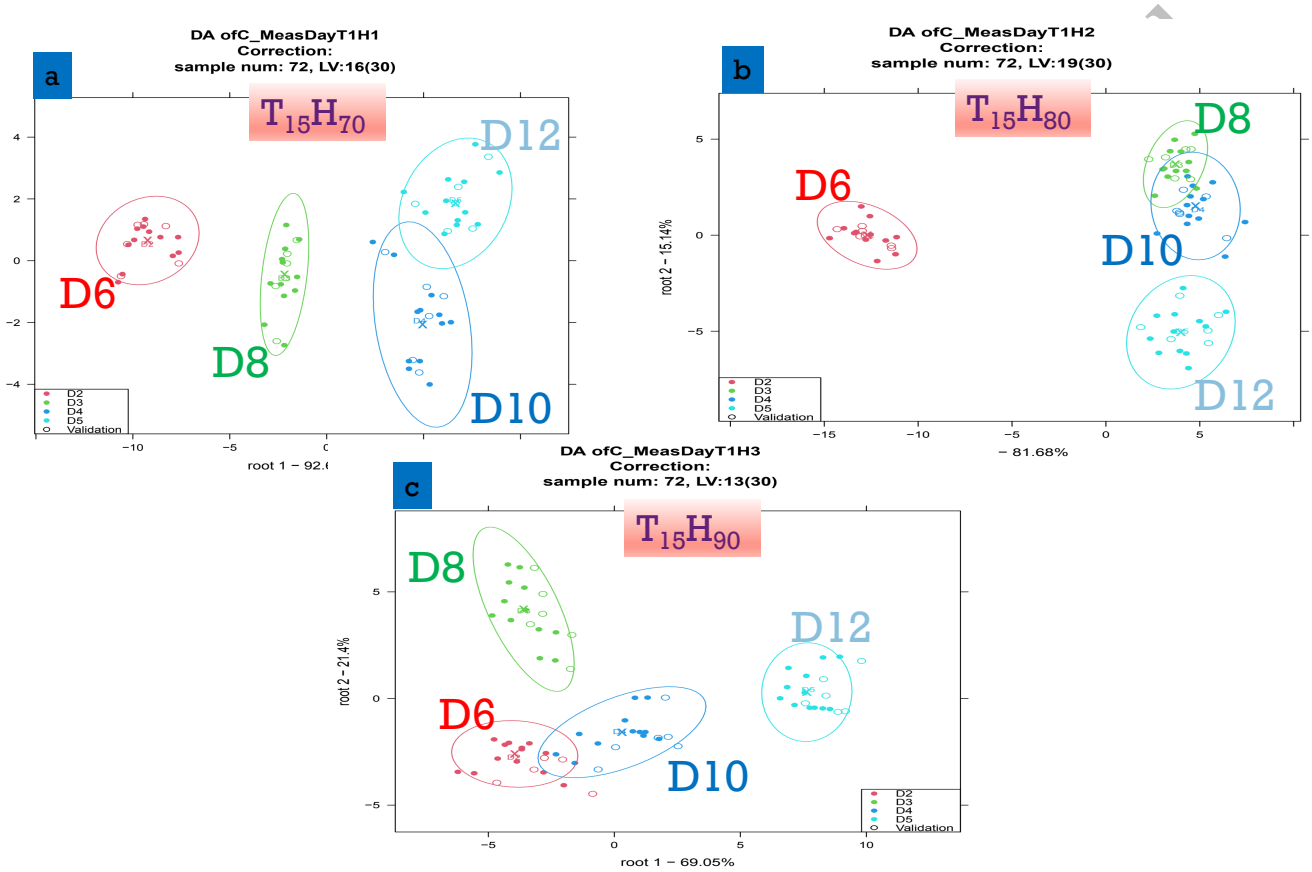

Figure 23. Results of PCA-DA classification for days of sunflower sprouting at the lowest temperature (15^{0}C) and by three levels of relative humidity: (**a**) humidity 70%, (**b**) humidity 80%, and (**c**) humidity 90%, after the XDS smoothing (Savitzky-Golay filter) and MSC spectra pre-treatments, in the 1100-1800nm range. Total Number of Observations, N = 72 each.

Figure *23* highlights three plots from the combination of the lowest temperature (15^{0}C) by the three levels of relative humidity. The LDA results showed only from Day 6, 8, 10, and Day 12 at this first temperature level because the plants at the low-temperature levels (15°C and 19°C) were harvested and measured only on the mentioned days because it was observed that sprouting time

is slow unlike in high temperature when sprouting time is fast and significant changes in growth and development of plants occur immediately as observed in **Figure *25***.

Generally, the three plots showed similar separation tendencies based on root 1, with only slight differences across the three different humidity levels for the five days of observation. All three plots/combinations were correctly classified with 100% accuracy for recognition and prediction, hence signifying that although other roots contributed to the discrimination of the samples, root 1 explains the majority percentage of the variance. Furthermore, the 100% recognition and prediction value signify that there were no false predictions of sunflower sprouting time within the 5-day experimented time interval.

In the first LDA treatment plot (T_{15} plot), it can be observed that the days follow a certain order in the PCA-DA plot, especially for T15H70 treatment where it can be seen in root number 1 (horizontal axis) that the order is Day 6, Day 8, Day 10, Day 12. Root number 1 explains about 92% of the sample variance meanwhile root number 2 (vertical axis) shows a clearer discrimination between Day 4 and Day 5. Root number 2 explains about 4.85% of the variance. Furthermore, Day 2 and Day 3 showed separate plots, which may indicate similar patterns in the relationship between the temperature, humidity, and sprouting time during the initial phases of growth. It may also indicate the high sensitivity of spouts to environmental conditions during early sprouting stages, with different combinations of temperature and humidity variables exerting different effects on the sprouting process. However, Day 10 and Day 12 plots showed slight overlapping, which may indicate that certain temperature and humidity levels/combinations can have similar sprouting outcomes towards the later stages of growth. It can also imply that sprouts' response to environmental factors may stabilize over time, hence lessening the impact of these factors on the sprouting time and process. This is in line with a study by Benincasa et al. (2019) which demonstrated that factors such as temperature and humidity may have a substantial impact on plant growth and development, particularly during sensitive phases such as the early seedling growth phase (Benincasa et al., 2019).

In the second (T15H80) and third treatments (T15H90) plots of the temperature 1 combinations, it can be seen that the intersection was between Day 3 and Day 4 for the T15H80 combination and

Day 6 and Day 10 for the T15H90 combination. However, these plots only give an idea of the discrimination as they show only root 1 and root 2, with other roots not shown, necessitating the recognition and prediction values with their values of misclassification as shown in **Appendix *3*** and **Appendix *4***. Based on their classification values, root number 1 explains about 82% of the sample variance for the second treatment and 69% of the sample variance for the third treatment while root number 2 (vertical axis) shows a clearer explains about 15% and 21% of the variance for the second and third treatment respectively. This unique response signifies that certain combinations of temperature and humidity variables may have a similar influence on sprouting outcomes, regardless of the day or duration of the sprouting process.

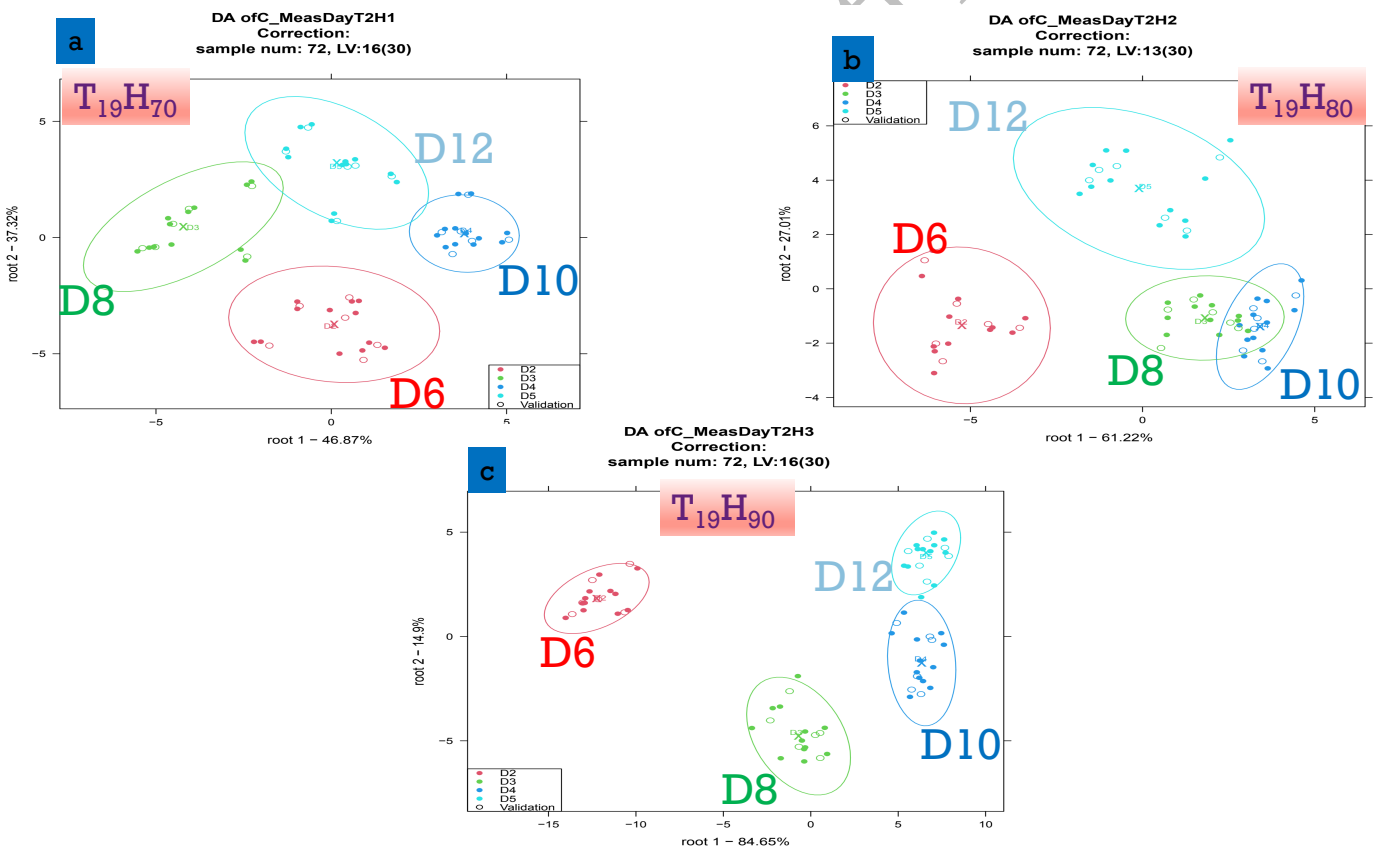

Figure 24. Results of PCA-DA classification for days of sunflower sprouting at the median temperature (19^0C) and by three levels of relative humidity: (**a**) humidity 70%, (**b**) humidity 80%, and (**c**) humidity 90%, after XDS smoothing (Savitzky-Golay filter) and MSC spectra pretreatment, in the 1100-1800nm range. Total Number of Observations, N = 72.

Figure *24* features three plots from the combination of the median temperature (19^0C) by the three levels of relative humidity.

These plots showed similar separation tendencies based on root 1, with only slight differences across the three different humidity levels for the five days of observation. Just as in **Figure *23***, although there were some levels of overlapping, the model presents 100% accuracy for recognition and prediction, which indicates that root 1 explains most of the variance amongst other roots. And that there were no false predictions of sunflower sprouting time within the 4-day experimented time interval.

Just as in **Figure *23***, the different plots for the three combinations under the median temperature (T19H70, T19H80, T19H90 combinations) showed some specific separation and intersection of plots for the duration of the analysis, which can be attributed to the fact that some combinations of temperatures and humidity variables can have similar and even differing influences on sprouting outcomes, irrespective of the stage of the sprouting process.

In the LDA treatment plots of the median temperature, it can be observed that the days follow a certain order in the PCA-DA plot, especially for T19H80H and T23H90H treatment where it can be seen in root number 1 (horizontal axis) that the order is Day 6, Day8, Day 10, Day 12. For the first treatment plot T19H70, Root number 1 explains about 46.87% of the sample variance meanwhile root number 2 (vertical axis) explains about 37.32% of the variance. Meanwhile, in the second treatment plot T19H80, Root number 1 explains about 61.22% of the sample variance meanwhile root number 2 (vertical axis) explains about 27% of the variance. However, the third treatment plot - T19H90 had the highest accurate classification values, with Root number 1 explaining about 84.65% of the sample variance meanwhile root number 2 (vertical axis) explains about 14.9% of the variance.

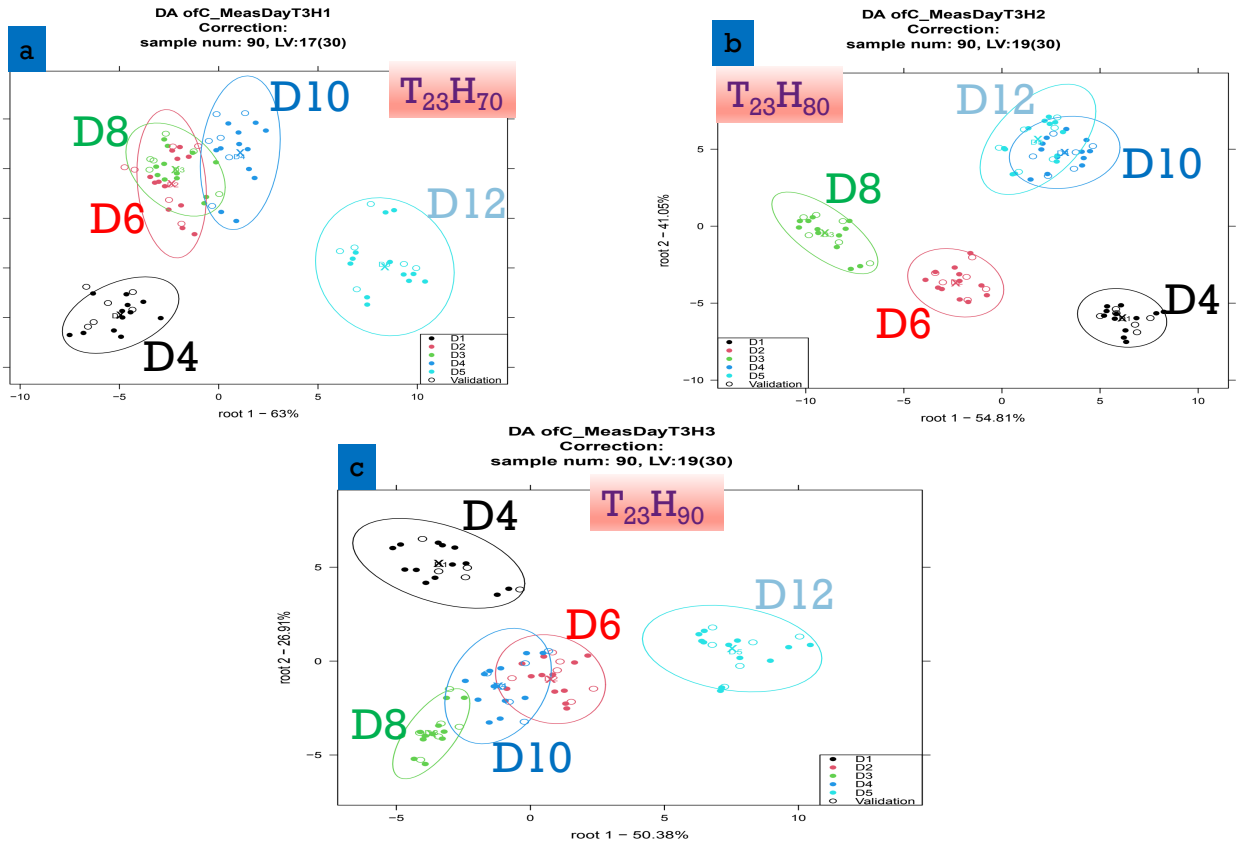

Figure 25. Results of PCA-DA classification for days of sunflower sprouting at the highest temperature (23^0C) and by three levels of relative humidity: (**a**) humidity 70%, (**b**) humidity 80%, and (**c**) humidity 90%, after XDS smoothing (Savitzky-Golay filter) and MSC spectra pretreatment, in the 1100-1800nm range. Total Number of Observations, N = 90.

Figure *25* highlights three plots from the combination of the highest temperature level (23^0C) by the three levels of relative humidity. Interestingly, much unlike other temperature levels, the LDA results showed from Day 1-Day 5. This indicates that at high-temperature levels, significant changes can occur even from the early stages of sprouting which can impact the growth and development of plants.

Just as for **Figure *23*** and **Figure *24***, all three plots/combinations were again correctly classified with 100% accuracy for recognition and prediction, hence signifying that there were no false predictions of sunflower sprouting time within the 5-day experimented time interval.

The classification results of sunflower sprouts are given in **Table *4***. The table below presents the various combinations of the variables used for the Linear Discriminant Analysis (LDA) of sunflower sprouting time, and their corresponding average prediction and recognition values from the LDA models. These combinations of the variables are necessary to predict the performance of the LDA model as well as provide critical insights into how these factors influence the predictive performance of the model.

Table 4. LDA recognition and prediction rates with classification according to days from different variable combinations (temperature and humidity) in the 1100-1800 range.

Levels/Combinations	Recognition Value	Prediction Value	Classification Concept
T15H70	100	100	Dataset from a specific temperature and humidity and classified according to different sprouting days.
T15H80	100	100	
T15H90	100	100	
T19H70	100	100	
T19H80	100	100	
T19H90	100	100	
T23H70	100	100	
T23H80	100	100	
T23H90	100	100	

From ***Table 4,*** it can be observed that the LDA analysis had perfect prediction and recognition values of 100% across all combinations of temperature and humidity.

The lowest prediction and recognition value (60.59% and 60.29% respectively) obtained from this analysis was for all treatments (that is a combination of all temperatures and humidity levels), which remains above average.

Table 5. LDA recognition and prediction rates from specific humidity dataset with classification according to days in the 1100-1800 range.

Levels/Combinations	Recognition Value	Prediction Value	Classification Concept
H70	91.49	89.48	Dataset from a specific humidity level and classified according to different sprouting days.
H80	87.95	85.55	
H90	86.31	85.89	
C_DayAllRH (RH 1, 2, and 3) and Temp (T1, 2, and 3)	71.94	70.41	Dataset from all humidity levels and classified according to different sprouting days

Table 5 also shows high prediction and recognition values of over 70% for dataset from specific humidity levels and classified according to days. However, the modeling with the dataset for all measurement days and all humidity levels had the least accurate recognition and prediction values at 71.94% and 70.41% respectively.

Table 6. LDA recognition and prediction rates from specific temperature dataset with classification according to days in the 1100-1800 range.

Levels/Combinations	Recognition Value	Prediction Value	Classification Concept
T15	92.81	92.59	Dataset from a specific temperature and humidity and classified according to different sprouting days.
T19	92.14	90.73	
T23	90.38	89.62	

Table *6* shows higher prediction and recognition values of over 90% for the dataset from specific temperature levels and classified according to days, with the least accurate recognition and prediction values at 90.38% and 89.62% from the T23°C dataset.

The high values indicate that the LDA model successfully classified and predicted the growing conditions of sunflower sprouts with perfect accuracy, especially for the temperature and humidity conditions tested. The high level of accuracy indicates that these variables strongly influence the growing conditions of plants.

The cross-validation table for the recognition and prediction of some datasets of importance that present misclassification is shown in **Appendix *3*** and **Appendix *4***. In **Appendix *3***, the classification of the sunflower sprouts subjected to different levels of humidity (70%, 80%, and 90%) is shown. The modeling showed high values of accurate classification, above 85% in all cases, but with some misclassifications. The modeling according to relative humidity 70% (RH70%) presented the highest accuracy for recognition and prediction at 91.498% and 89.482% respectively. Although the modeling for RH80% presented a higher accuracy for recognition (87.958%), it had a slightly lower accuracy for prediction (85.557%) compared to the model for RH90% which had an accuracy value of 85.891% for prediction.

Appendix *4* highlights the classification of the sunflower sprouts subjected to different levels of temperature (15˚C, 19˚C, 23˚C). The modeling also showed high values of accuracy in classification, above 70% in all cases, but with some misclassifications. The modeling according to temperature 15˚C (T15˚C) presented the highest accuracy for recognition and prediction at 92.817% and 92.595% respectively, followed by the modeling for T19˚C with accuracy values of 92.144% and 90.732% for recognition and prediction respectively. However, the modeling for T23˚C had the least accurate recognition and prediction values at 90.38% and 89.62% respectively.

5.4 Results of Partial Least Square regression (PLSR) to Predict the Parameters of Sunflower Sprout based on NIR Spectroscopy

To assess the efficiency of NIRS for predicting brix, dry matter, height, and other parameters of sunflower sprouts, different predictive models were built. The figures (**Figure *26***, **Figure *27***, **Figure *28***, **Figure *29***, **Figure *30***, **Figure *31***, **Figure *32***, and **Figure *33***) show the predictive models/ Y-fit graph and their corresponding vectors for sunflower sprouts parameters tested in combination with temperature and humidity. **Figure *29***

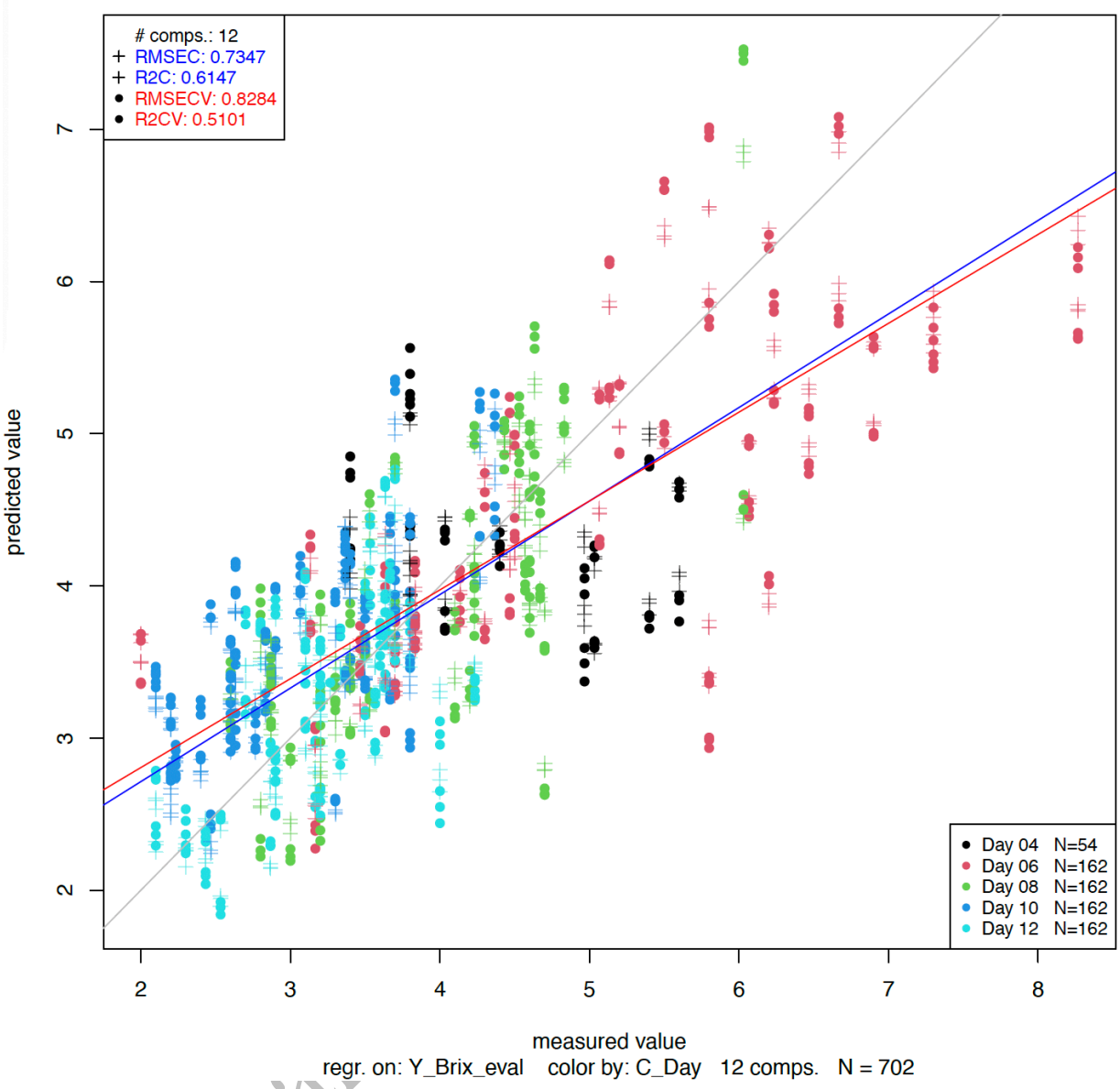

Figure 26. Y-fit graph for the prediction of brix content in sunflower sprout samples after the XDS smoothing (Savitzky-Golay filter) and MSC spectra pre-treatments in the 1100-1800nm region.

Figure 26, shows PLSR for brix content for all the sunflower plants which were growth at different temperature and humidity combinations for the five measurement days across the 12-day sprouting period. It can be observed that the coefficient of determination for calibration (R^2C) was 0.61 with a root mean square error of calibration (RMSEC) of 0.73 ºBx. Following the cross-validation, R^2CV and RMSECV were 0.51 and 0.82 ºBx respectively. From the scatter points, it can be seen that in the lower days (Day 4 colored by black, day 6 colored by red, and day 8 colored by green), there were higher values of brix compared to the brix content for later days, especially day 10 and

day 12. This is in line with a study by (Massa et al., 2015) which demonstrated that brix content decreased with a longer harvest period, indicating lower sugar contents as plants aged.

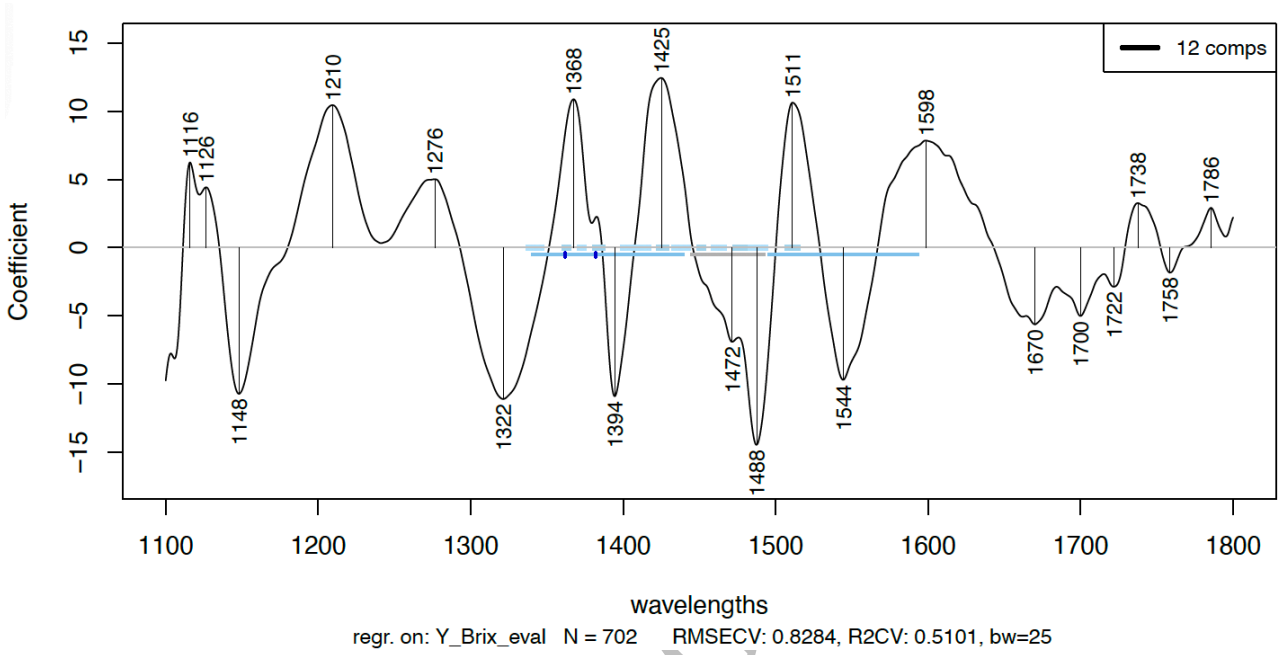

Figure 27. Regression vector of the sunflower sprouts' brix content PLSR model, with total sample number (N) = 702 and 12 components.

Figure *27* shows the corresponding regression vector for the sunflower sprouts' brix content PLSR model. The figure presented a high number of contributing wavelengths. The main contributing wavelengths with the highest peaks included 1116, 1148, 1210, 1322, 1368, 1394, 1425, 1488, 1511, 1544, and 1598. The high absorbance within the region of 1300-1600 indicates a high content of water and a lesser amount of organic compounds in the sunflower sprout samples. Based on previous studies, the wavelength range around 1488 is associated with OH/NH stretching (Slavchev et al., 2015; Tsenkova et al., 2018), while the wavelength range of 1140 (which is the closest band to the 1148 wavelength found in **Figure *27***) is associated with the combination overtone of free water (Tsenkova et al., 2018).

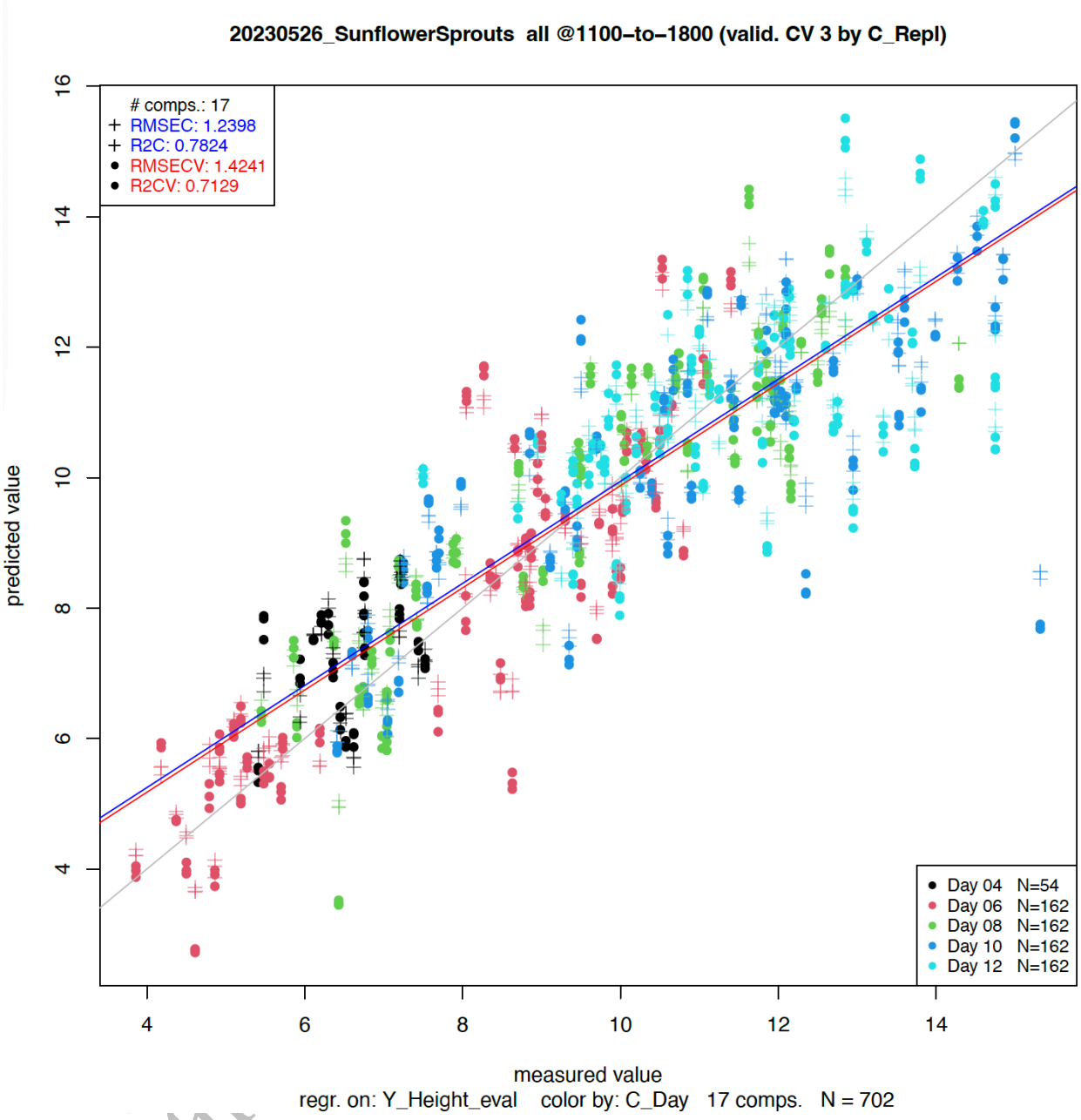

Figure 28. Y-fit graph for the prediction of the sunflower sprout height after the XDS smoothing (Savitzky-Golay filter) and MSC spectra pre-treatments in the 1100-1800nm region.

From **Figure *28***, the coefficient of determination for calibration (R^2C) was 0.78 with a root mean square error of calibration (RMSEC) of 1.23 cm. After the cross-validation, R^2CV and RMSECV were 0.71 and 1.42 cm respectively. The number of components (# comps) established to achieve the best model was seventeen with 702 total samples for the five measurement days across the 12-day sprouting period. From the scatter points, it can be seen that the earlier (Day 4 colored by

black, day 6 colored by red, and Day 8 colored by green) had lower height values compared to the height values for later days, especially Day 10 and day 12.

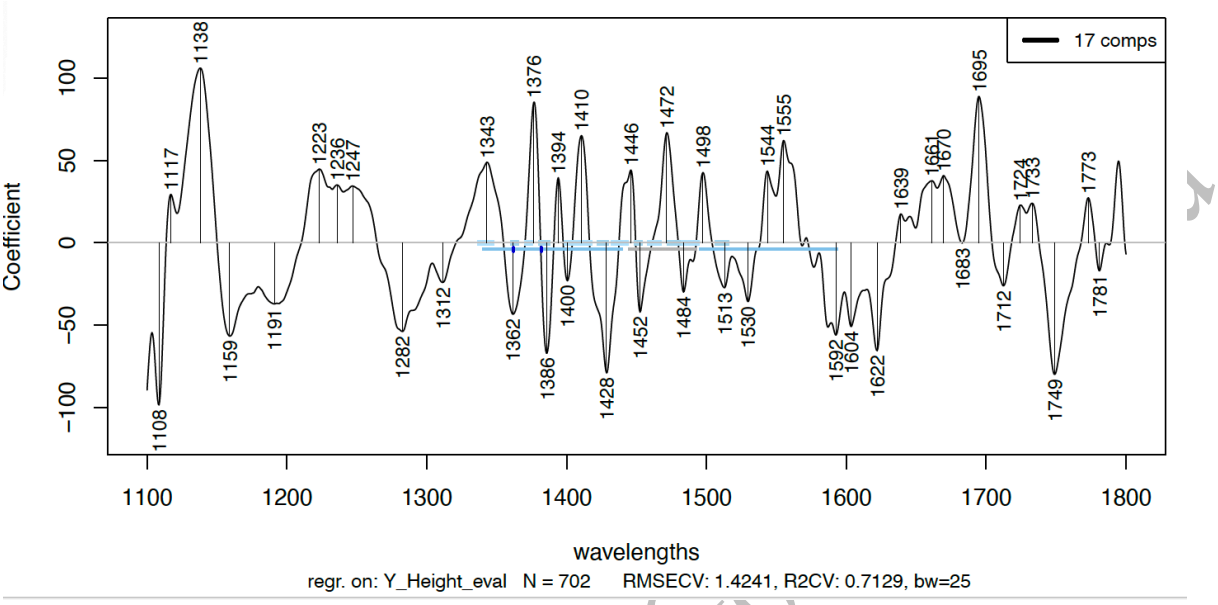

Figure 29. Regression vector of the sunflower sprouts' height PLSR model, with total sample number (N) = 702 and 17 components.

Figure *29* shows the corresponding regression vector for the PLSR model for sunflower sprouts' height values. Similar to **Figure *27***, it presented a high number of contributing wavelengths with the main contributing wavelengths found at 1138, 1376, 1386, 1472, 1484, 1622, 1695, and 1749. The wavelength range of 1484 was previously reported by (Slavchev et al., 2015; Tsenkova et al., 2018) as associated with OH/NH stretching, while the wavelength range of 1140 (which is the closest band to the 1138 wavelength found in **Figure *29*Figure *27***) is associated with the combination overtone of free water (Tsenkova et al., 2018).

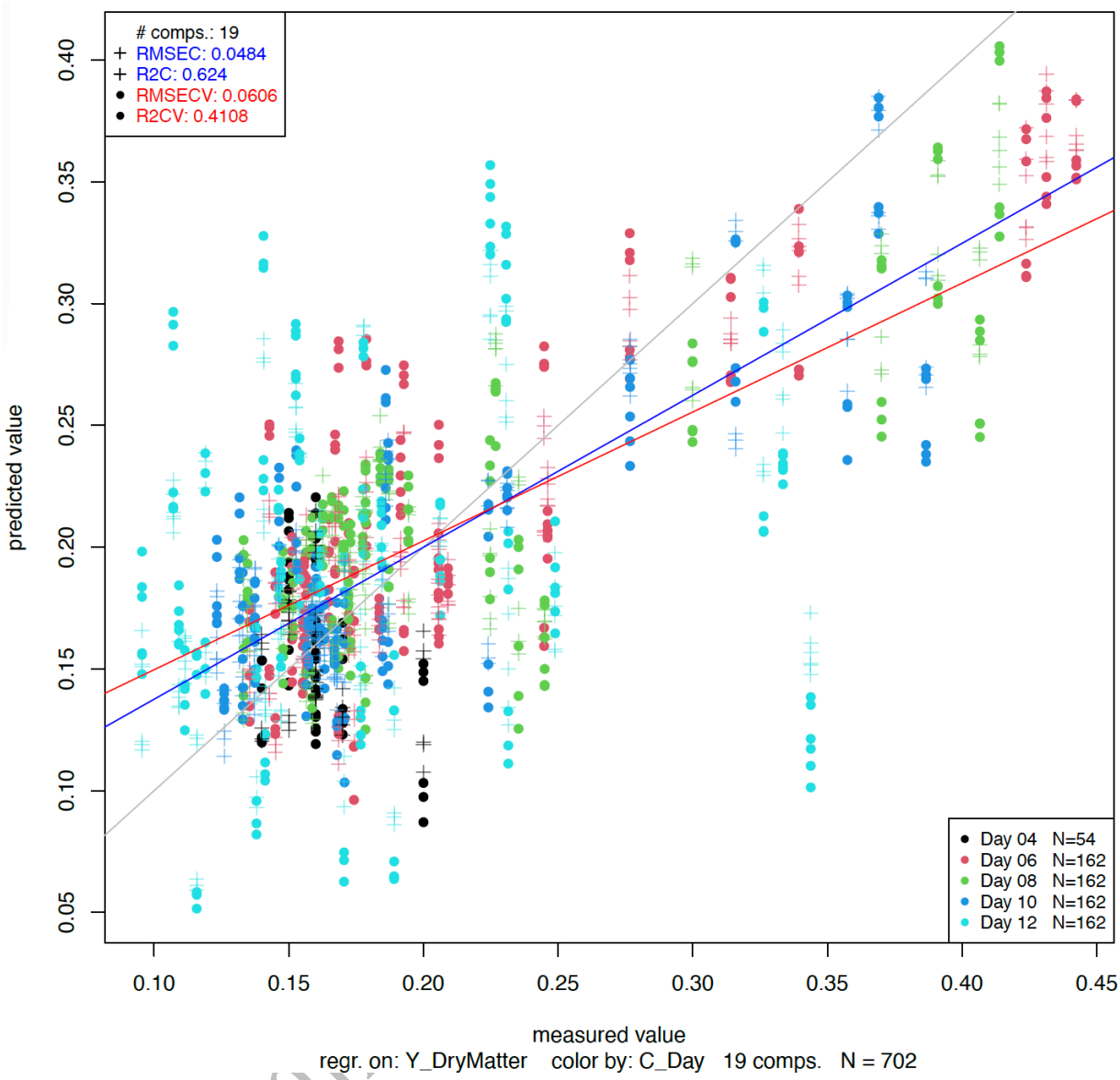

Figure 30. Y-fit graph for the prediction of dry matter content in sunflower sprout samples after the XDS smoothing (Savitzky-Golay filter) and MSC spectra pre-treatments in the 1100-1800nm region.

Figure 30 shows the Y-fit graph for the prediction of dry matter content in the sunflower sprout. From this figure, it can be seen that the coefficient of determination for calibration (R^2C) was 0.62 with a root mean square error of calibration (RMSEC) of 0.04 m/m%. After the cross-validation, R^2CV and RMSECV were 0.41 and 0.06 m/m% respectively. The number of components (# comps) established to achieve the best model was nineteen with 702 total samples for the five measurement days across the 12-day sprouting period. From the scatter points, it can be seen that

the earlier (Day 4 colored by black, day 6 colored by red, and day 8 colored by green) had lower dry matter content compared to the dry matter values for later days, especially day 10 and day 12.

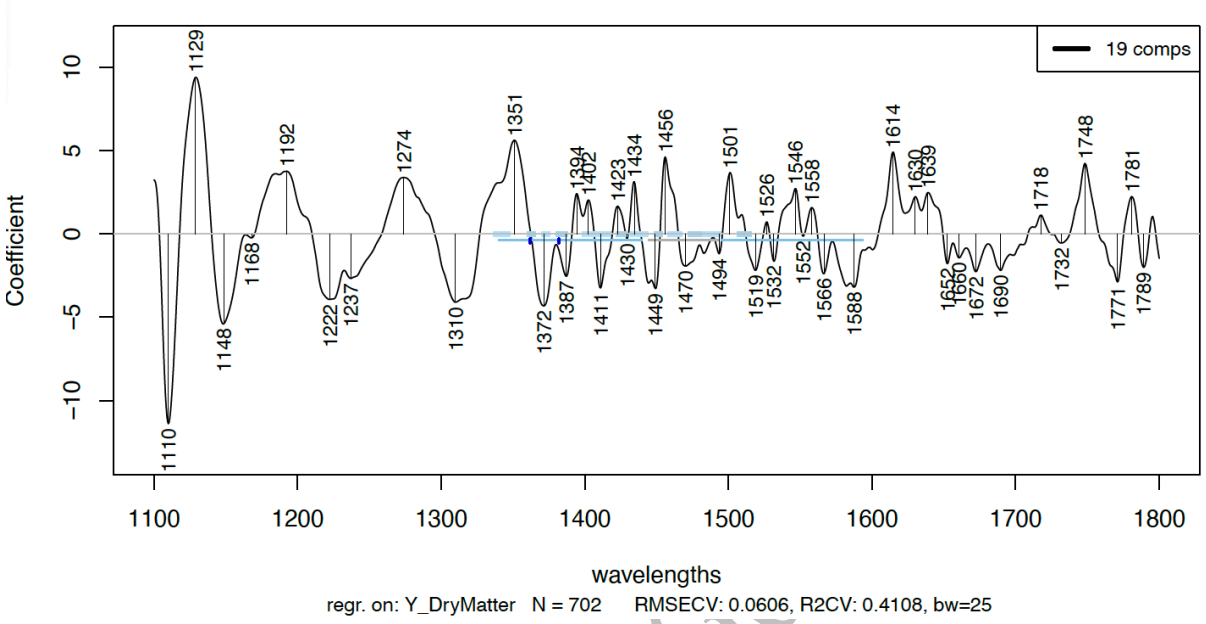

Figure 31. Regression vector of the sunflower sprouts' dry matter content PLSR model, with total sample number (N) = 702 and 19 components.

Figure *31* shows the corresponding regression vector for the PLSR model for sunflower sprouts' dry matter content. Similar to **Figure *27*** and **Figure *29***, it presented a high number of contributing wavelengths with the main contributing wavelengths found at 1129, 1148, 1274, 1310, 1351, 1372, 1456, 1501, 1614, and 1748.

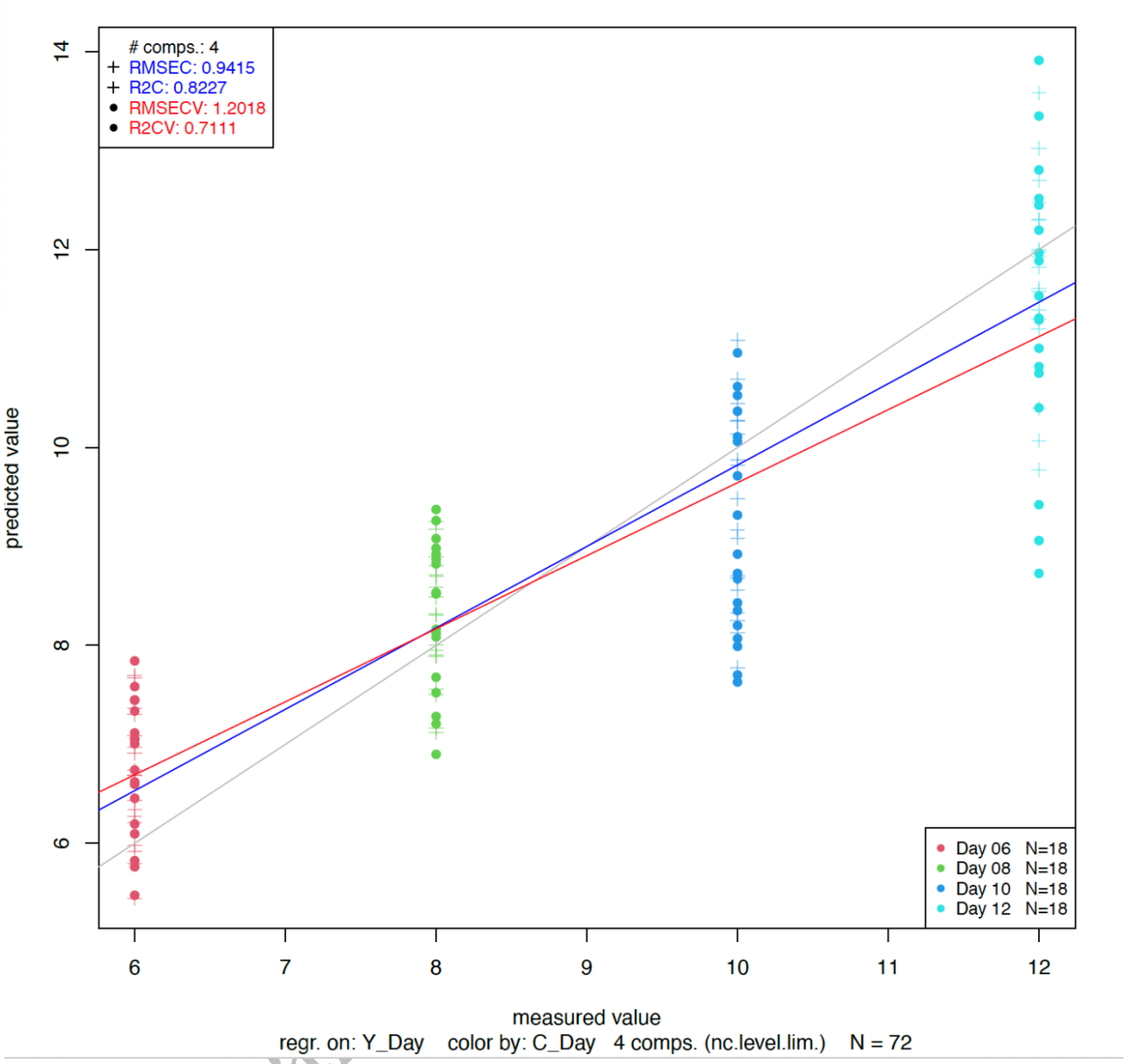

Figure 32. Y-fit graph for the prediction sunflower sprout samples kept at 15°C and 90% of relative humidity during the 12-day sprouting period after the XDS smoothing (Savitzky-Golay filter) and MSC spectra pre-treatments in the 1100-1800nm region.

The PLSR regression model, for samples belonging to 15°C and 90% of relative humidity, according to the days of the experiment, showed a fairly high prediction capacity of R^2 for calibration and cross-validation of 0.82 and 0.71, respectively; and RMSE for calibration and cross-validation of 0.94 days and 1.20 days, respectively. Similar results were presented regarding other temperature and humidity combinations.

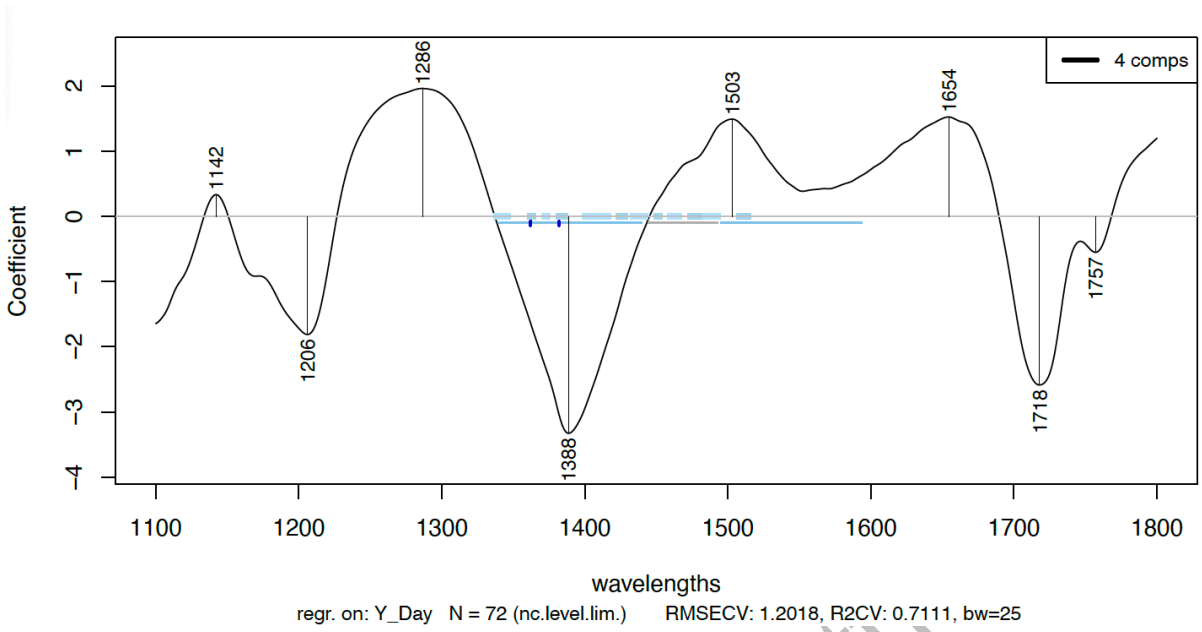

Figure 33. Regression vector of the sunflower sprouts PLSR model, with sprouts kept at 15°C and 90% of relative humidity during the 12-day sprouting period. Total sample number (N) = 72 and 4 components.

As shown in **Figure *33***, the main contributing wavelengths of 1100-1800 nm model for the PLSR model were few and included 1142, 1206, 1286, 1388, 1503, 1654, and 1718.

6. SUMMARY

The main objective of this study was to assess the suitability of NIRS in monitoring the growing process of commercially valuable sprouting plants, namely sunflower under different temperature and humidity levels. Based on the study goal, NIR spectroscopy was applied and the Metrohm XDS RapidContent Analyser enabled the acquisition of the spectra in the select wavelength range of 1100-1800nm.

Advanced statistical techniques like the Principal Component Analysis (PCA) and the Linear Discriminant analysis (LDA) were applied for the accuracy of analysis. The results from all analyses indicate the influence of temperature and humidity on the growing conditions of sunflower sprouts.

Specifically, the PCA results showed some trend of separation based on temperature and humidity conditions/levels. In most cases, the PCA results exhibited separate groupings/clusters that indicate that sunflower sprouts exposed to different combinations of temperature and humidity variables may exhibit distinct patterns during the sprouting process. Notably, from the PCA result of the highest temperature and humidity levels combination, the clear uniquely distinct pattern affirmed previous studies' results that high temperature and humidity levels may induce environmental stress and affect plants' overall growth and development.

These findings were corroborated by the LDA analysis which qualitatively classified the data, with consistently high accuracy and prediction values, hence correctly predicting the growing conditions of sunflower sprouts according to temperature and humidity combinations. While all LDA results had high prediction and recognition values, the results from the analysis of specific conditions of different temperature and humidity levels showed perfect accuracy at 100% for all 9 combinations. However, the full data set analysis for all temperature and humidity levels were misclassified by almost 40%, as it had the lowest prediction and recognition values at 60.595% and 60.295% respectively.

Furthermore, the PLSR regression models showed an average prediction capacity with the regressions (R2) for brix, dry matter, and height above 0.5 in all cases. The PLSR results for the variables were modest considering the full dataset from the temperature and humidity combinations that were used, which the best model was for height.

Overall, the consistently high accuracy of the predictive model indicates its reliability in classifying sprouts at different environmental conditions accordingly and demonstrates the efficiency of NIR for monitoring the growing conditions of sunflower sprouts.

Although the major sprout of interest used for this study was the sunflower sprout, complementary studies were done on other plants like pea plants during my MSc program which supports the findings of this research, where NIRS were used to assess the temperature and humidity stress factors.

Conclusively, this study addresses a critical knowledge gap in the existing literature regarding the growing conditions of sunflower sprouts under temperature and humidity factors/different stress factors/varying environmental conditions. While the study has demonstrated that different growing conditions result in varying compositions of the plant, which may be useful knowledge for fulfilling the dietary needs of people, however, as it is not conclusive research, the potential for improvements exists, including developing rapid and nondestructive techniques to monitor these changes to help in precision food production and precision diet. Furthermore, the potential for improvements may include expanding the scope of environmental variables (e.g light and water) beyond just temperature and humidity, long-duration different sprouts large sample size analysis, nutritional profiling of the monitored sprouts, and more robust regression models for prediction.

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APPENDICES

Appendix 1. Applications of sprouts for food product formulation and improvement in the food industry (Miyahira et al., 2021)

Sprouts	Food applications	Positive aspects	Negative aspects	References
Amaranth	Sprouted amaranth flour as an ingredient in food formulations	Increase in the concentrations of soluble protein, total phenolic content, total flavonoid content, total anthocyanin content, and antioxidant activity	Not reported	Sandoval-Sicairs et al., 2020
		Increase in antioxidant activity, total phenolic and flavonoid contents, protein, and dietary fiber contents of amaranth seeds. Decrease in total lipid content.	Not reported	Perales-Sánchez et al., 2014
Amaranth and chia	Beverages with sprouted amaranth and chia flours	Increase in protein and dietary fiber contents, and high sensory acceptability.	Not reported	Argüelles-López et al., 2018
Blue maize	Sprouted blue maize flour as an ingredient in food formulations	Increase in protein content, antioxidant activity, and total phenolic, dietary fiber, and anthocyanin contents.	Not reported	Chavarín-Martínez et al., 2019
Brown Rice	Bread made with sprouted brown rice flour	No significant difference in the acceptability scores for aroma, flavor, and taste between the formulated bread and the control bread.	Bread formulations had lower loaf volume and greater hardness than wheat bread due to the absence of gluten in rice.	Charoenthaikij et al., 2010
Buckwheat	Bread made with sprouted buckwheat flour	Breads made using buckwheat flour still contained flavonoids in significant amounts.	The negative impact of baking on the polyphenol content suggests that some degradation may have occurred.	Alvarez-Jubete et al., 2010
Chia	Sprouted chia flour as an ingredient in food formulations	Higher protein and total phenolic contents, antioxidant activity, γ-aminobutyric acid, essential amino acids, and total dietary fiber contents than non-sprouted grain chia flour.	Not reported	Gómez-Favela et al., 2017
Lentil	Bread made with sprouted lentil flour	Increase in the content of phenols and flavonoids in bread plus 10% of sprouted grain lentil flour; Sensory acceptance.	Higher hardness and less cohesiveness than wheat bread possibly due to the greater resistance of the swollen starch during the cooking process.	Hernandez-Aguilar et al., 2020
Moth bean	Sprouted moth bean flour as an ingredient in food formulations	Higher gelation and thermal stability, and lower viscosity degradation than non-sprouted beans. Higher gelation and thermal stability, and lower viscosity degradation than non-sprouted beans.	Decrease in ash content due to the draining out of macro and microelements from the flour through soaking and cooking.	Medhe et al., 2019

Mung bean	Noodle with sprouted mung bean flour	Improvement in protein content and functional properties such as water absorption, water solubility, oil absorption ability, and water retention.	Reduction of fat content due to the consumption of fat in the germination process. Reduction of pasting viscosity with the increase of germination time due to the starch degradation.	Liu et al., 2018
	Bread made with composite flour	Increase in phenolic and protein contents.	Decrease in loaf height and volume due to the decrease in the swelling index. Increase in loaf weight due to increased water retention. Lower acceptance score.	Menon et al., 2015
Quinoa	Sprouted quinoa flour as an ingredient in food formulations	Increase in copper and zinc availability improved the stability of the foam, increase in amylolytic enzyme levels.	Decreased the ability to foam due to proteolytic modification.	Suárez-Estrella et al., 2020
Sorghum	Sprouted sorghum flour as an ingredient in food formulations	Reduction of antinutritional factors such as phytate, tannin, oxalate, and improved functional properties	Reduction of bulk density and viscosity due to the action of amylase.	Ojha et al., 2018
Wheat	Sprouted wheat flour as an ingredient in food formulations	Increased the levels of tocopherols, niacin, riboflavin, as well as free and bound phenolic compounds.	Not reported	Zilic et al., 2014
		Gluten degradation promoted by germination.	Impairment of the functional properties of germinated wheat flour due to higher solvent retention.	Boukid et al., 2018
	Bread made with sprouted wheat flour	Increased phenolics and protein contents.	Decrease in starch digestibility due to the increased content of resistant starch.	Świeca et al., 2017
		Increase in antiradical and chelating compounds as well as phytochemicals. The bioactive compounds were potentially bioaccessible. The replacement of wheat flour by SF in up to 10% had little influence on the total acceptability.	Bread with less elastic, little sprung back after compression and characterized by sticky, wet crumbs when 15 and 20% of the wheat flour was replaced by germinated flour.	Gawlik-Dziki et al., 2017
	Wheat bread enriched with sprouted wheat flour rich in phenolic compounds	Baking properties comparable to those of control flour.	Decrease in total phenolic content, total flavonoid content, and antioxidant activity.	Tian et al., 2019
Wheat, barley, lentil, nozzle grain, and quinoa	Bread made with sprouted wheat flour	Flour: Increase in peptides, free amino acids, and γ-aminobutyric acid contents. Decreased concentrations of phytic acid, condensed tannins, raffinose, and trypsin inhibitors. Bread: high digestibility protein content; No significant differences in the specific volume.	Flour: increased microbiological contamination. Bread: higher value of hardness and fracturability.	Montemurro et al., 2019

Appendix 2. Pharmacological properties of sprouts, health benefits and their food applications (Aloo et al., 2021; Waliat et al., 2023).

Sprouts	Pharmacological properties/ Phytochemicals	Health benefits	Food Applications	References
Pea sprouts	Salicylic derivatives	Antimicrobial, Anti-inflammatory, analgesic, antipyretic effects, cardioprotective, and neuroprotective activities.	Used in cooking	Ho et al., 2006
Ramson sprouts	Alliins, flavonoids, polyphenols, and thiosulfinates	Anti-inflammatory, antioxidant, antidiabetic activities	Used as healthy herbs and food spices	Sobolewska et al., 2015; Silva et al., 2013.
Lentil sprouts	Phytic acid, phytosterols, and saponins	Antioxidant, cholesterol-reducing, cardioprotective, anticarcinogenic, immunomodulation properties	Sprouted lentil flour used in breadmaking	Hernandez-Aguilar et al., 2020
Fenugreek sprouts	Sapogenins, fenugreekine, saponins, coumarin, and nicotinic acid	Antioxidant, blood sugar regulating, cholesterol-reducing, anti-inflammatory, anticoagulant properties	Used as food additives: colour and seasoning enhancer	El-GebalY et al., 2022
Ginger and turmeric	Gingerols, paradols, phenolics terpenoids, shogaols, and curcuminoids	Anti-inflammatory, antioxidant, antibacterial, antioxidants, and anticarcinogenic properties	Used as preservatives, spices, flavour and colour enhancer	Retana-Cordero et al., 2021
Amaranth and chia	Polyphenols, and proteins	Antioxidant, anti-inflammatory, blood sugar-regulating properties	Sprouted amaranth and chia flours used in making functional beverages	Argüelles-López et al., 2018
Wheat sprouts	Phenolic acids, tocopherols, and carotenoids, quercetin, lectins	Antioxidant, anti-inflammatory, and cardioprotective properties	Sprouted wheat flour used in bakery products	Ojha et al., 2018
Mung bean sprouts	Flavonoids, isoflavonoids, flavone and isoflavone	Antioxidant, anti-inflammatory, phytoestrogenic, neuroprotective, anticarcinogenic activities	Sprouted mung bean flour used in making noodle	Diego et al., 2020
Buckwheat sprouts	Quercetin, lectins, anthocyanins and flavonoids	Anti-inflammatory, hypocholesterolemic, antioxidant, antidiabetic, and anticancer activities.	Sprouted buckwheat flour used in breadmaking	Alvarez-Jubete et al., 2010; Bastida et al., 2015; Watanabe and Ayugase et al., 2008
Quinoa sprouts	Total phenolics and anthocyanins	Anticancer, antioxidant, Anti-inflammatory, antidiabetic activities	Sprouted quinoa flour used as an ingredient in food formulations	Liu et al., 2018; Guo et al., 2011, Charron et al., 2007.

Appendix 3. Confusion table of the PCA-LDA models for the sunflower sprouts subjected to three different humidity levels (70%, 80%, and 90%) according to the measurement days.

Humidity	70%RH					80%RH						90%RH					
Average Recognition (91.498%)						Average Recognition (87.958%)						Average Recognition (86.318%)					
Days	D4	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12
D4	94.57	1.86	0	0	0	D4	100	5.56	0	0	0	D4	94.57	0.92	1.86	0	0
D6	5.43	83.31	10.19	0.92	0	D6	0	72.22	5.56	0	0	D6	0	95.36	2.78	6.47	0
D8	0	4.64	89.81	5.56	0	D8	0	5.56	94.44	4.64	0	D8	0	0	88.89	0	0
D10	0	10.19	0	91.67	1.86	D10	0	15.75	0	85.17	12.03	D10	5.43	1.86	5.56	76.86	24.08
D12	0	0	0	1.86	98.14	D12	0	0.92	0	10.19	87.97	D12	0	1.86	0.92	16.67	75.92
Humidity	70%RH					80%RH						90%RH					
Average Prediction (89.482%)						Average Prediction (85.557%)						Average Prediction (85.891%)					
Days	D4	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12
D4	88.18	3.72	0	0	0	D4	100	5.56	0	0	0	D4	94.17	0	1.83	0	0
D6	11.82	83.29	9.28	1.83	0	D6	0	72.22	5.56	0	0	D6	0	94.5	3.72	5.56	0
D8	0	3.72	90.72	5.56	0	D8	0	5.56	90.77	3.72	3.72	D8	0	1.83	88.89	1.83	0
D10	0	9.27	0	87.06	1.83	D10	0	14.83	1.83	81.45	12.94	D10	5.83	1.83	3.72	75.94	24.06
D12	0	0	0	5.56	98.17	D12	0	1.83	1.83	14.83	83.33	D12	0	1.83	1.83	16.67	75.94

Appendix 3

Appendix 4. Confusion table of the PCA-LDA models for the sunflower sprouts subjected to three different temperatures levels (15˚C, 19˚C, 23˚C) according to the measurement days.

Temperature	T15˚C				T19˚C					T23˚C					
Average Recognition (92.817%)					Average Recognition (92.144%)					Average Recognition (71.942%)					
Days	D6	D8	D10	D12	Days	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12
D6	100	2.78	0	0	D6	94.49	0	0	0	D4	82.58	2.47	0	0	1.55
D8	0	89.81	2.78	0	D8	3.66	87.03	3.7	0	D6	11.92	70.98	9.88	10.19	0
D10	0	7.42	84.25	2.78	D10	0	12.97	89.83	2.78	D8	0	4.01	71.6	5.56	0.93
D12	0	0	12.97	97.22	D12	1.84	0	6.47	97.22	D10	4.6	19.14	14.51	59.88	22.84
										D12	0.91	3.4	4.01	24.38	74.69
Temperature	T15˚C				T19˚C					T23˚C					
Average Prediction (92.595%)					Average Prediction (90.732%)					Average Prediction (70.415%)					
Days	D6	D8	D10	D12	Days	D6	D8	D10	D12	Days	D4	D6	D8	D10	D12
D6	100	1.83	0	0	D6	96.26	0	1.83	0	D4	77.36	4.31	0.61	1.24	1.85
D8	0	92.61	5.56	0	D8	1.87	88.89	3.72	0	D6	16.98	68.52	9.87	8.65	0
D10	0	5.56	83.33	5.56	D10	0	9.28	83.33	5.56	D8	0	4.94	70.38	4.94	2.46
D12	0	0	11.11	94.44	D12	1.87	1.83	11.11	94.44	D10	3.79	18.52	13.58	60.49	20.37
										Days	D4	D6	D8	D10	D12

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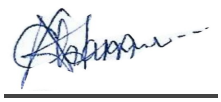

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