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Nutritional and bioactive components of fresh sprouts of selected crop species

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Declaration

I hereby declare that I have solely worked on this thesis entitled "Nutritional and bioactive components of fresh sprouts of selected crop species" and all contextual sources have been duly quoted and acknowledged by means of standard referencing. The thesis does not break the copyright order.

Date

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Abstract

The aim of this study was to investigate the effect of sprouting of selected important grains and seeds of 15 plant species (*Chenopodium quinoa*, *Amaranthus caudatus*, *Medicago sativa*, *Fagopyrum esculentum*, *Glycine max*, *Cicer arietinum*, *Vigna radiata* syn. *Phaseolus aureus*, *Lens culinaris*, *Trigonella foenum graceum*, *Hordeum vulgare*, *Triticum aestivum*, *Eruca sativa*, *Brassica oleraceae* var. *Italica*, *Raphanus sativus*, *Helianthus annus*) on selected nutritional and bioactive components. Extracts from the non-sprouted (dry seeds) samples of the 15 plant species and their sprouts (germinated seeds) were analysed and assessed for the crude protein and protein fractions contents, flavonoid contents, total phenolic content and antioxidant activity. Sprouted legumes and cruciferous vegetables recorded an increase in their crude protein contents (0.03% to 5.86% of dry matter) as compared to their non-sprouted counterparts. Cereals and pseudocereals recorded low total protein contents regardless of their stage (non-sprouted or sprouted form) and subsequently, cereals measured the lowest crude protein contents. Sprouting effected a substantial increase in the types and amounts of flavonoids detected in most samples. A chromatographic separation following the extraction of analytes under investigation resulted in the identification and quantification of eight types of flavonoids: chlorogenic acid, isoorientin, isoquercitrin, kaempferol, quercetin, quercitrin, rutin and vitexin. Rocket, wheat, mungo and bio amaranth recorded the highest increment in total phenolic content (71.0% to 83.8%) after sprouting. There were significant differences in the total phenolic content recorded between the sprouted and non-sprouted samples. With the exception of two samples, all samples exhibited an increase in their antioxidant activity after sprouting (0.06% to 90.7%). From all the analyses performed, it is evident that sprouting has effect on edible seeds and grains. Results from this study have proved that sprouting can be used as an effective and natural technique to enhance the nutritional value and bioactive components of edible seeds and grains.

Key words: Bioactive compounds, cereals, cruciferous vegetables, flavonoids, germination, legumes, sprout.

Table of content

List of figures

List of tables

List of the abbreviations

ANOVA	Analysis of variance
CSN	Czech state norm
DPPH	2, 2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalents
RSC	Radical scavenging capacity
SFP	Sunflower protein
SWF	Sprouted wheat flour
TE	Trolox equivalent
TPC	Total phenolic content
UHPLC	Ultra-high performance liquid chromatography

1. Introduction

The higher cost of nutritious foods, some physiological adaptations to food constraints, increased urbanisation, changes in food systems and lifestyles have contributed to the various forms of malnutrition (FAO 2018). Climate change is also predicted to cause a decrease in nutritional value of many of the food crops for human nutrition when compared with the same plants grown under present climatic conditions (Myers et al. 2014; Ebert et al. 2017). In view of this, improving the nutritive value of our foodstuffs, cereals and legumes is an option that needs to be exploited to alleviate this tragic problem (Finney 1983).

Sprouts, which are fresh, functional and nutraceutical food sources that have become increasingly popular for healthy eating (Ebert 2015; Kyriacou et al. 2016) fall into this domain and are gaining acceptance across the world as they are a good source of nutrients and non-nutrients which include proteins, carbohydrates, minerals, vitamins and antioxidants (Robertson et al. 2002; Gabriel et al. 2007; Peñas et al. 2009). Several epidemiological studies which have been conducted, concluded that consumption of sprouts may help protect against certain chronic diseases and cancers. The high content of phytochemicals present in the sprouts of some edible seeds (mostly cruciferous vegetables) has contributed to this effect hence, the consumption of sprouts can bring about a lot of health benefits (Bellostas et al. 2007; Yang et al. 2013).

Sprouts have the potential to contribute to food and nutrition security in cities as they can be easily grown in urban and peri-urban settings where land is often a limiting factor. This could be done either by specialised producers or the consumers themselves, independent of seasonal growth cycles, inside or around residential areas (Ebert 2015). One of the key aims highlighted by FAO for the "Nutrition Decade" (2016-2025), which is to provide all stakeholders with a unique, time-bound opportunity to strengthen joint efforts and achieve a healthier and more sustainable future can be achieved by the consumption of sprouts (FAO 2018).

This study was therefore carried out to assess selected nutritional and bioactive components of fresh sprouts of selected 15 plant species (*Chenopodium quinoa*, *Amaranthus caudatus*, *Medicago sativa*, *Fagopyrum esculentum*, *Glycine max*, *Cicer arietinum*, *Vigna radiata*, *Lens culinaris*, *Trigonella foenum graceum*, *Hordeum vulgare*,

Triticum aestivum, *Eruca sativa*, *Brassica oleraceae* var. *Italica*, *Raphanus sativus*, *Helianthus annus*).

2. Literature review

2.1. Origin and history of sprouting

Worldwide, cereals and legumes have throughout recorded history served as major dietary components for most humans, animals and as well played important roles in the food and beverage industries. The seeds of these plants have usually been treated by germinating, fermenting or selectively heat treating with the objective of extracting maximum nutrients for minimum costs (Finney 1983).

Germination forms the basis for producing sprouted grains and seeds. The European Sprouted Seeds Association defines sprouts as products obtained through the germination of seeds and their development in water or another medium, harvested before the development of true leaves and intended to be eaten whole with the seed or used for further processes (Lemmens et al. 2019). This is an effective and inexpensive technology for improving certain grain quality (Chavan et al. 1989). Accumulated reports have shown that sprouting helps to improve the nutritional quality of grains and seeds by increasing the content and availability of essential nutrients and lowering the levels of anti-nutrients (Everson et al. 1944; Chavan et al. 1989).

Originally, sprouting of seeds (grains) was used not for direct human consumption but was adopted in addition to other technologies for the production of alcoholic beverages. Adequate scientific and archaeological evidence have shown that beer, the world's most widely consumed alcoholic drink which ranks third overall after water and tea, made by the combined processes of preparing beverages from an infusion of cereal grains (especially, *Hordeum* spp.) that have undergone sprouting and the subsequent fermentation of the sugary solution (wort) has its history embedded in the Neolithic Revolution in the lowlands of the Mesopotamian alluvial plain (Hornsey 2016). This is an indication that sprouting of seeds began with the commencement of agriculture (Hornsey 2003).

Many accumulated naval records have documented the superiority of fresh against dehydrated, sprouted barley and wheat-wort beers in preventing and curing scurvy among sailors. Captain Cook, after being at sea from November 22, 1772 to March 26, 1773 attributed the lack of scurvy aboard ship to the use of fresh wort beer and sweet wort as a prophylaxis and curative agent against scurvy (Smith 1918).

Sprouted seeds used as food originated in the Orient (Far East countries) and has spread and gained popularity in other parts of the world such as Europe and the United States with the passing of years due to their high nutritive value and presumed health benefits (Lorenz 1980; Chavan et al. 1989; Sharma et al. 2002). Though the sprouting of some legumes (such as *Glycine max* and *Vigna radiata*) was developed by the Chinese centuries ago, data published relating to the sprouting of these seeds and their nutritive composition were limited (Fordham et al. 1975). Sprouted mung beans (*Vigna radiata* L.) are the best known sprouts which has been used as food by the Chinese for nearly 5,000 years (Fordham et al. 1975; Larimore 1975). For hundreds of millions of many world citizens, sprouting of legumes and some cereals has been routine in converting feed grains into human foods (Finney 1983).

The consumption of these sprouted grains were used to treat several ailments in the ancient times (Pagand et al. 2017). In a human-based study, 27 severe cases of scurvy were treated with sprouted beans and fresh lemon juice. Sprouted beans outperformed the juice with the result that 70% versus 53% of the cases were cured within 4 weeks (Wiltshire 1918).

2.2. Sprouts in the modern day world

In recent times, consumers are looking for natural, "healthy", inexpensive and convenient food (Robertson et al. 2002; Gabriel et al. 2007; Peñas et al. 2009). Sprouts, which are fresh, functional and nutraceutical food sources that have become increasingly popular for healthy eating (Ebert 2015; Kyriacou et al. 2016) fall into this domain and are gaining acceptance across the world as they are a good source of various nutrients and non-nutrients which include proteins, carbohydrates, minerals, vitamins and antioxidants (Robertson et al. 2002; Gabriel et al. 2007; Peñas et al. 2009). Also, several epidemiological studies which have been conducted, concluded that consumption of sprouts may help protect against certain chronic diseases and cancers. The high content of phytochemicals known as glucosinolates present in the sprouts of some edible seeds (mostly cruciferous vegetables) has contributed to this effect hence, the consumption of sprouts can bring about a lot of health benefits (Bellostas et al. 2007; Yang et al. 2013).

Sprouts have the potential to contribute to food and nutrition security in cities as they can be easily grown in urban and peri-urban settings where land is often a limiting factor. This could be done either by specialized producers or the consumers themselves, independent of seasonal growth cycles, inside or around residential areas (Ebert 2015).

A great variety of sprouts are easily available on the European markets, but the most popular are those from alfalfa (*Medicago sativa*), mung bean (*Vigna radiata* syn. *Phaseolus aureus*) and radish (*Raphanus sativus*). They are consumed often raw or slightly cooked in salads and sandwiches or as decorative appetisers (Weiss & Hammes 2003).

Sprouted seeds have also been used along with some biological agents to produce a synergistic effect. Sadeghi et al. (2017) discovered that broccoli (*Brassica oleraceae* var. *Italica*) sprout extract and probiotics exhibited a synergistic effect on *Helicobacter pylori* growth inhibition. Hence, they concluded that using broccoli sprout extract and probiotic bacteria, a yogurt that is effective on the growth inhibition of *Helicobacter pylori* can be produced. Similarly, Mridula & Sharma (2015) developed a non-dairy probiotic drink using sprouted cereals, legume and soymilk.

The food industry has increasingly launched sprouted products containing grains in recent years and most of these product launches took place in Europe and North America. The products included breakfast cereals, baked goods, pasta, snacks and beverages. However, sprouted grain product launches in the Asia-Pacific region involved ready-to-drink products (Lemmens et al. 2019).

2.3. Uses and applications of sprouted seeds

Sprouting is an inexpensive, effective and simple tool beneficial for enhancing the dietetic and nutraceutical quality of cereals, legumes, pseudocereals and cruciferous vegetables (Cevallos-Casals & Cisneros-Zevallos 2010; Guo et al. 2012; Pajak et al. 2014).

Many studies have recounted higher levels of nutrients and lower contents of antinutrients in sprouted edible seeds compared to the non-sprouted seeds (Pajak et al. 2014). Therefore, sprouting is an outstanding green food engineering strategy to increase the nutritional value of grains (Gan et al. 2019).

Sprouts have long been used in diet as “health food” and food ingredients based on its significant impact on nutritional, flavour and textural benefits over their non-sprouted grain counterparts (Finnie et al. 2019). They have become an extensively accepted food item (Vidal-Valverde & Frias 1992)**.** Consuming sprouts at the beginning of their growing phase ensures very high nutrient concentration (Marton et al. 2010). Sprouts can be used in the preparation of numerous different foods including breakfast items, baked products, salads, snacks, soups, casseroles and pasta (Finnie et al. 2019). Fresh sprouts can be used as substitute for vegetables or they can be dried and milled to flour for consumption in various forms (Chavan et al. 1989).

Several investigations have been carried out on the application of sprouted seeds to different flours. The chief cereal used in the preparation of variable bakery products is wheat. Application of sprouted wheat in bakery products involves both artificially sprouted and field-sprouted wheat (Chavan et al. 1989). Marti et al. (2018) researched on the use of flour from sprouted wheat as a new ingredient in bread-making and reported that high amounts of sprouted wheat flour (SWF) enrichment affected dough rheology. However, SWF improved the dough development and gas production during leavening. At 50% SWF the best bread performance was obtained. 100% SWF increased the slowly digestible starch fraction. Their results showed that controlled (that is in an industrial plant) sprouted wheat flour can be used as new ingredient in bread making. (Marti et al. 2018).

Flour obtained from sprouted cereals can be added with normal flour in the preparation of traditional unleavened pancake like chapaties and bhakari (Chavan et al. 1989).

Soybean and mung bean sprouts have long been a vital, year-round component of Asian and vegetarian diets. They are consumed mainly as porridge, sprouts or noodles (Ebert 2015; Ghani et al. 2016). Mung bean sprouts are now finding their way into supermarkets in America, Europe and East Africa. In the U.S., producers of Chinese foods make various mixtures and preparations of sprouts such as canning, mixing with other vegetables etc. (Ebert et al. 2017).

Sprouted amaranth, buckwheat, millet, oats, sorghum and quinoa are naturally gluten free, hence they can be utilised to improve the nutrition of gluten-free foods. Various grains

and seeds that have been successfully sprouted commercially gives bakers, food scientists and chefs great versatility for innovation (Finnie et al. 2019).

Epidemiological studies have revealed that consumption of sprouts aid in defence against certain chronic diseases and cancers owing to the high amounts of bioactive agents that act as natural antioxidants and help in cancer prevention (Jacobs et al. 1998; Yang et al. 2013). Sprouts can therefore contribute to human nutrition and health by preventing malnutrition and chronic diseases (Gan et al. 2017).

In addition to their culinary use, different Brassicaceae species have been lengthily used in traditional medicine from antique to present day. In the last few decades, epidemiological studies have proved that diets rich in cruciferous vegetables are allied with a lower risk of several types of cancer (Liu et al. 2004). Rich source of health-promoting phytochemicals are more concentrated in cruciferous sprouts such as broccoli and radish than in the adult plant edible organs (Baenas et al. 2017). Similarly, sprouts of mung bean are formulated and offered as dietary supplement in healthcare (Kovacs 1996).

2.4. Sprout production

In 1970, sprouted alfalfa seed consumption in California measured up to 22.7 tonnes of seed and this increased to 659 tonnes of seed a year. This yield of sprouts gave an estimated sprout value of US$8.5 million, surpassing the farm value of Californian lettuce of US$7.3 million (Hesterman & Teuber 1979). Lipton et al. (1981) also indicated that 50,000 tonnes of sprouts were produced in the United States annually. It has been reputed that there are over 450 US-based sprout growers producing over 300,000 tons of sprouts per annum to satisfy the consumer demand (Kurtzweil 1999).

The objective in edible sprout production is typically to harvest germinated seedlings which have not yet formed true leaves with the primary concern being the hypocotyl region. Optimum hypocotyl length and thickness is essential whiles lengthy root systems are completely unwelcome (Price 1988).

2.4.1. Methods and equipment for sprouting

Numerous edible seeds can be sprouted within a short time through simple germination techniques (Gan et al. 2017). Sprout production ranges from home growers by the consumer without sophisticated equipment (Lorenz & D'Appolonia 1980; Price 1988) through to large scale (commercial) producers with equipment for large scale (6 t/day) sprouting systems (Price 1988).

Diverse procedures have been proposed for the sprouting of grains and with each of these methods, whole seeds are mostly soaked in about three times their volume of water until they are saturated before sprouting (Chavan et al. 1989; Yang et al. 2001). The period of saturation varies with the size of the seeds; larger seeds take a longer time than the smaller ones. The residual water after the soaking period is drained and the seeds are placed in a vessel or tied in cloth for germination in a warm place (Lorenz & D'Appolonia 1980). Day-to-day rinsing, aeration and separation of the saturated seeds (sprouts) is critical to evade mould development in each of the techniques (Miller 1978).

2.4.1.1. Home-made production of sprouts

At the domestic level, any appropriate kitchen container such as: plates, bowls, pans, unglazed clay flower pots, trays containing racks covered with damp paper towels or simply tying soaked seeds in a moist cloth has been used to sprout grains; no sophisticated equipment is used (Whyte 1973). Special jars or sprouters and vessels (Figure 1) have been designed to be used for more controlled sprouting of small quantities of grains at home (Hamad & Fields 1979; Lorenz & D'Appolonia 1980).

Figure 1.Vessels for home production of sprouts: Author

2.4.1.2. Commercial production

For commercial production of sprouts, Miller (1978) developed an equipment designed with rotating jars on a device (Figure 2) to gently agitate the seeds every 4 hrs in addition to daily rinsing. This equipment has been successfully used to produce mould-free sprouts of wheat (Miller 1978). In recent studies, sprouting of seeds has been accomplished in dark incubators (Yang et al. 2001) and modern equipment such as the seed germinator (G-120 Snijders, The Netherlands) has been used for sprouting pea (*Pisum sativum*) under controlled conditions (Urbano et al. 2005). Similarly, a climatic test cabinet (CTS) was used in sprouting seeds of alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) (Penas et al. 2008).

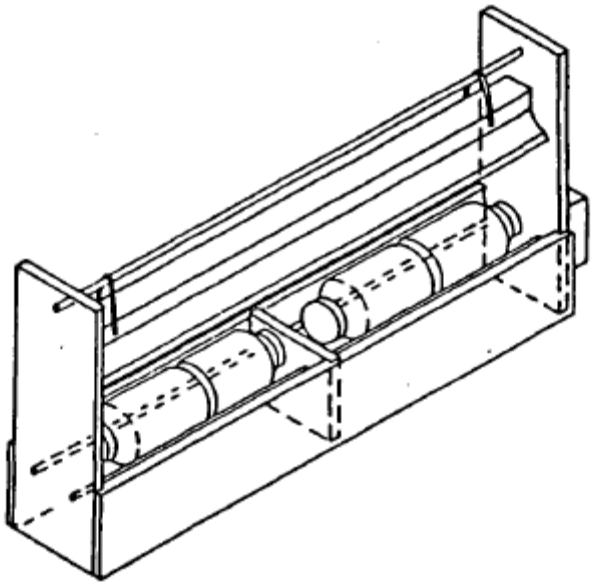

Figure 2. Device for agitating wheat sprouts: Miller (1978)

2.4.1.3. Modifications in methods of sprouting

To curb one of the major challenges in sprout production which is microbiological hazards of sprouts that has led to the outbreaks of infections of bacterial pathogens such as *Salmonella* and *Escherichia coli*, there has been the need for modifications in sprouting some seeds and grains to improve the nutritional safety of sprouts (Taormina et al. 1999; Morabito 2015).

This has been achieved by following the basic techniques of sprouting which have been used of old but with some few reforms. In some investigations, there is pre-treatment of seeds with chemicals such as sodium hypochlorite in the methodologies before soaking in distilled water (Vidal-Valverde et al. 2002; Dueñas et al. 2016). In others, there is combined treatments of seeds with high pressure, temperature and antimicrobial products (Peñas et al. 2009; Peñas et al. 2010). However, in other methodologies, seeds are only soaked directly in water or distilled water before sprouting (Vidal-Valverde & Frias 1992; Paśko et al. 2008; Ebert et al. 2017).

2.4.2. Quality of seeds and conditions of sprouting

Sprouting of seeds neither requires sunlight nor even soil and the period of germination is short with fairly high yields (Lorenz & D'Appolonia 1980; Price 1988).

Though the quality of sprouted seeds may be formed at each stage of its production it is subjected mainly to the seed quality, germination conditions (Chavan et al. 1989) and further storage (Price 1988; Świeca & Gawlik-Dziki 2015).

Chavan et al. (1989) stated that to obtain the highest yield of sprouts and nutritive benefits, seeds should be whole (unbroken and not infested), clean and untreated. An intact embryo on the grain or seed is vital for sprouting to take place Finnie et al. (2019) and the viability of a seed greatly influence its sprouting efficiency (Lorenz & D'Appolonia 1980).

Optimum sprout production is further influenced by an adequate water supply, a desirable temperature and humidity, a certain composition of gases in the atmosphere (O_2, CO_2 and N_2), and absence of germination inhibitors (Lorenz & D'Appolonia 1980; Price 1988). The specific requirements and conditions vary with species and varieties (Mayer & Poljakoff-Mayber 1982).

Inasmuch as the conditions of sprouting directly affect not only the sprout yields, but also the nutrient composition and dry matter losses during germination the period of soaking and the associated treatments (example optimum temperature and humidity, light) need to be standardised (Chavan et al. 1989).

2.5. Biochemical changes associated with sprouting seeds

The process of germination commences with the uptake of water by the dry seed and terminates with radicle penetration through the seed covering layers (Bewley 1997; Weitbrecht et al. 2011). Generally, the complex metabolic events occurring during germination which is mainly linked to water imbibition by the dry seeds exhibits three phases (Figure 3) (Bewley 1997).

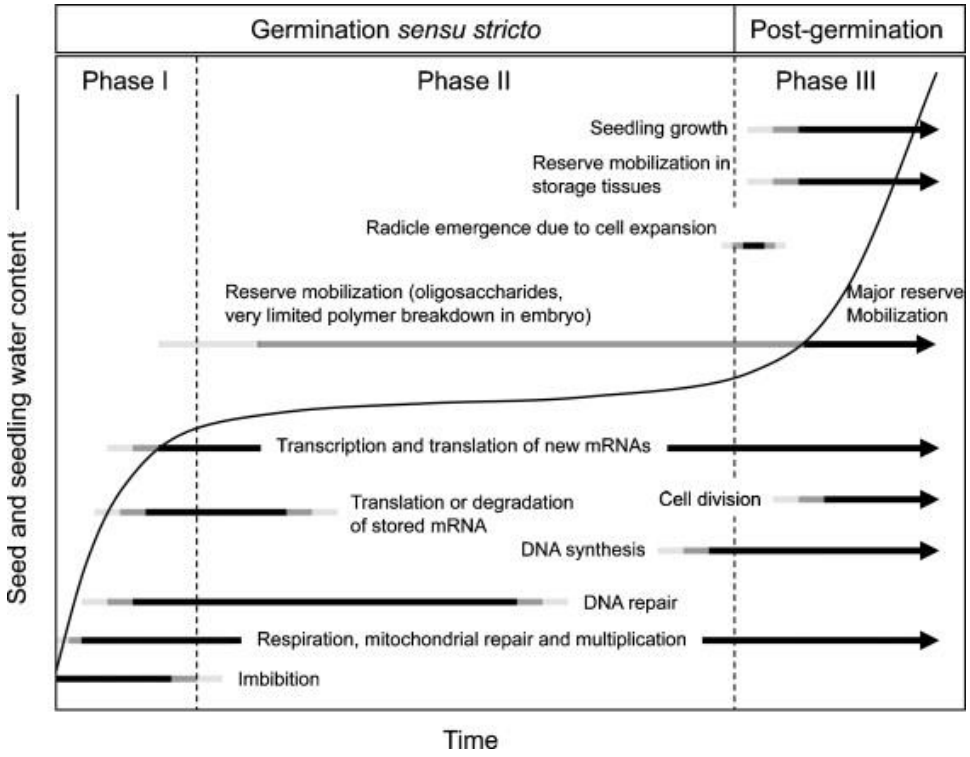

Figure 3. Physical and metabolic events occurring during germination (Phases I and II) and early seedling growth (sprouting) (Phase III): Nonogaki et al. (2010)

Phase I is characterised by the hydration of cell material and matrices after the seed has been steeped in water (Nonogaki et al. 2010). Once the moisture content of the imbibed seed reaches the minimum requirement, there is activation of endogenous metabolism necessary for mobilising reserve material. This is achieved when the seed activates the synthesis and release of plant hormones such as abscisic acid, gibberellic acid and ethylene throughout the seed causing the release of the catabolic enzymes: amylase, proteases and lipases (Nonogaki et al. 2010; Finnie et al. 2019). Phase I is followed by a limited water uptake in phase II and radicle growth at the end of phase II (Nonogaki et al. 2010).

Usually, penetration of the structures surrounding the embryo by the radicle is an evident sign that germination is complete. Successive events, in addition to the mobilisation of the major storage reserves, are associated with growth of the seedling (Bewley 1997). The seed takes up further water in phase III, major mobilisation of reserve material occurs and the seedling starts to grow (Nonogaki et al. 2010). In phase III, nutrients are made

available for biological processes such as respiration and seedling growth (Lemmens et al. 2019).

The reserve biochemical components including protein, starch and lipids which are mainly concentrated in the scutellum of the embryo and endosperm are enzymatically degraded into simple compounds (Lorenz & D'Appolonia 1980; Lemmens et al. 2019). These are synthesised into new compounds or conveyed to other parts of the growing seedling (Lorenz & D'Appolonia 1980; Chavan et al. 1989) and inasmuch as no foreign nutrients are added, only water and oxygen are used up by the sprouting seeds (Mayer & Poljakoff-Mayber 1982).

The catabolism of complex compounds into further simple forms, conversion into vital constituents and breakdown of nutritionally undesirable components brings about the significant nutritional modifications and quality that occur as a result of sprouting (Lorenz & D'Appolonia 1980; Chavan et al. 1989; Finnie et al. 2019).

2.6. Nutritional composition and phytochemistry of sprouts

Edible seeds such as beans are rich in diverse nutrients and phytochemicals and possess various bioactive effects (Hayat et al. 2014). Germination mostly increases the nutritive value of seeds, consequently, the value of the human diet is improved and they compare well with fresh vegetables. (Everson et al. 1944; Chen et al. 1975; Fordham et al. 1975). Current studies confirm that sprouting further improves the dietetic value of grains and seeds by increasing the protein (free amino acids) content, simple sugars, vitamins and bioactive compounds (Kuo et al. 2004; Wang et al. 2005; Gan et al. 2019).

Sprouting also reduces antinutritional and digestion inhibiting factors such as protease inhibitors and lectin (Aguilera et al. 2013).Variations in nutrients and antinutritional factors happening during germination rest on the type of grain (especially, legumes) as well as the sprouting conditions such as time, temperature, light cycle (Frias et al. 1995; Sierra & Vidal-Valverde 1999).

2.6.1. Proteins and protein fractions of sprouts

Legumes are placed second to cereals only in their importance as human food crops (Nair et al. 2013) and grain legumes contribute 33% of the dietetic protein nitrogen (N) needs of humans (Vance et al. 2000). For centuries, these high-protein crops have paid their contribution to human nutrition.

Sprouting of lentils (*Lens culinaris* var. Vulgaris) led to the total elimination of α-galactosides and a considerable increase of thiamin, riboflavin, and niacin (Urbano et al. 1995). Ebert et al. (2017) evaluated the level of protein in mung bean (*Vigna radiata* syn. *Phaseolus aureus*) and soybean (*Glycine max*) sprouts in comparison with mature mung bean grain and vegetable soybean. Mung bean sprouts recorded lower values of protein as compared to the matured mung bean. Soybean sprouts were found superior to mung bean sprouts in terms of the protein content. The vegetable soybean was found superior to germinated soybean in the content of protein by 14% increase. Urbano et al. (2005) found that sprouting of peas significantly increased the palatability and dietetic utilisation of proteins.

Balasaraswathi & Sadasivam (1997) sprouted sunflower (*Helianthus annus*) seeds and analysed their cotyledons for free amino acid, non-protein nitrogen, lysine, tryptophan and methionine contents. The protein content was fractionated. The non-protein nitrogen, total free amino acid, lysine and tryptophan contents increased as a result of soaking and germination. In the sprouted samples of sunflower, a big increment in lysine (2.7 to 11.9 g/16 g N) and tryptophan (0.7 to 7.8 g/16 g N) contents were recorded. The soluble albumin and globulin fractions reduced while the glutelin content increased during sprouting. Subsequently, the sprouted sunflower seeds had improved nutritional quality than the raw seeds.

Limited reports show insignificant differences in the composition of protein as a result of sprouting of cereals (Chavan et al. 1989). Miller (1978) reported that the protein content in wheat after seven days of sprouting increased significantly, whether it was a low protein wheat at 10% or a high protein wheat at 18%.

The storage proteins of cereal seeds are categorised as: albumins (water-soluble), globulins (salt-soluble), prolamins (alcohol-soluble), glutelins (acid- or alkali-soluble) and residue or insoluble proteins (Osborne & Mendel 1914). The major storage protein

constituent of cereals are either prolamins or glutelins hence, the conversion of the prolamin fraction into albumins and globulins during sprouting may improve the quality of cereal proteins (Chavan et al. 1989). Oats are the only exception, with their major storage proteins belonging to the globulin fraction; prolamins form the minor component (Croy et al. 1982; Butt et al. 2008; Klose & Arendt 2012). The total amino acid analysis made during germination of oats revealed an increase in essential amino acids like lysine and tryptophan, which leads to an improved nutritional value of sprouted oats (Klose & Arendt 2012).

In contrast to common grains such as wheat, albumins and globulins constitute the main storage proteins in pseudocereals containing very little or no storage prolamin proteins, which are the main storage proteins in cereals (Mlakar et al. 2009; Alvarez-Jubete et al. 2010). Quinoa and amaranth are well-thought-out to be gluten-free grains for the reason that there is very little or no prolamin component (Valcárcel-Yamani & Caetano da Silva Lannes 2012). Sprouts of buckwheat recorded a significant decrease in the activity of trypsin inhibitor (Ikeda et al. 1984).

2.6.2. Carbohydrates in sprouts

Carbohydrates contribute 50-70% of dietary energy and based on their degree of polymerisation are categorised into three principal groups: sugars (monosaccharides, disaccharides and polyols), oligosaccharides and polysaccharides (starch and nonstarch) (Blazek et al 2009). Starch occurs typically in granular form of various shapes and sizes and it is the most important carbohydrate in all plants (Qian & Kuhn 1999).

In addition to the proteins, legumes are also a source of large amounts of carbohydrates, dietary fibre, water-soluble vitamins and minerals (Vidal-Valverde et al. 2002). Vidal-Valverde & Frias (1992) investigated the effect of germination on soluble carbohydrates, total and digestible starch and components of dietary fibre (neutral detergent fibre, cellulose and hemicellulose) in two varieties of lentils (*Lens culinaris medicus* var. *Vulgaris* and *Variabilis*). The amount of total soluble sugars reduced (from 4.3% to 2.0% and from 5.3% to 2.2%, respectively) in sprouted lentils. Glucose which is absent in raw seeds, was comparatively high (0.6% and 0.7%), fructose increased marginally and sucrose decreased slightly. Oligosaccharides of the raffinose family vanished from the

sprouted seeds. There was a considerable reduction in total starch in the sprouted lentils (from 60.3% to 41.4% and from 57.4% to 36.4%) and the digestibility of the starch was significantly improved. The nutritive value of both varieties of lentils was enhanced with the germination processes.

Oligosaccharides of the raffinose family which comprise α 1, 6-galactose linkages indigestible to mammalian enzymes are significant components of legumes (Vidal-Valverde & Frias 1992) and several findings have attributed flatulence to the activities of anaerobic intestinal microorganisms on the raffinose family of these oligosaccharides. Nevertheless, it has been found that sprouting is an effective tool for eliminating these undesirable carbohydrates of legumes (Frias et al. 1995; Urbano et al. 1995; Sierra & Vidal-Valverde 1999**).**

The total carbohydrates in cereals may account for as much as 68-90% of the seed weight and starch is the major constituent of cereal endosperms, comprising 58-70% of the total kernel weight (Deshpande et al. 1984). An upsurge in the activities of amylases and maltase during germination leads to a gradual reduction in starch with an associated increase in reducing and nonreducing sugars during sprouting of cereal grains (Mayer & Poljakoff-Mayber 1963; Lemar & Swanson 1976). Lemar & Swanson (1976) reported that total carbohydrates reduced somewhat during sprouting of wheat.

2.6.3. Phenols and flavonoids in sprouts

Phytochemicals are non-nutrient bioactive compounds in vegetables, fruits, grains and other plant foods that have the potential of reducing the risk of chronic diseases (Liu 2004). With regards to the major nutrients composition (carbohydrates, proteins and lipids), plant secondary metabolites or phytochemicals (such as phenols and flavonoids) form only a minor component of grains and seeds (Schendel 2019).

Phenolic compounds identified in sprouted grains are mainly present as diverse phenolic acids and flavonoids. The primary phenolic compounds in sprouted grains include: hydroxybenzoic acids, hydroxycinnamic acids, common flavonoids, C-glycosidic flavonoids and isoflavonoids (Gan et al. 2019).

Studies have shown that germination has influence on the total phenolic content in many edible seeds and can progressively accumulate soluble phenolic in sprouts compared with raw seeds. However, some studies have also reported a decline in soluble phenolics in germinated edible seeds and sprouts (Guo et al. 2012).

Flavonoids commonly comprises flavones, flavonols and flavanones and their glycosides (Peterson & Dwyer 1998). Ebert et al. (2017) found that isoflavones reported to have beneficial effects on human health, were at high concentrations in soybean sprouts as compared to their seeds. Similar observation was made by Kim et al. (2007). Hence, Ebert et al. (2017) proposed that soybean sprouts could easily provide the recommended anticarcinogenic dose range from 1.5 to 2.0 mg/Kg of body weight per day.

Paśko et al. (2008) also found that quinoa sprouts were richer in phenolic acids (mainly, gallic acid) and flavonoids (rutin) than the sprouts of amaranth.

2.6.4. Bioactivities of sprouts

Recent studies have discovered that germinated edible seeds and sprouts exhibit a variety of bioactivities, such as antioxidant capacity, anti-inflammatory, antibacterial, antidiabetic and anticancer effects and these bioactivities can be associated with the build-up of diverse bioactive constituents such as polyphenols in these sprouts. These bioactivities of germinated edible seeds and sprouts are suggestive that they possess possible health benefits and can be consumed as portions of our diets for the avoidance of some long-lasting ailments (Gan et al. 2017).

Antioxidant capacity of sprouts

The most widely researched in germinated edible seeds and sprouts are the content of phenolic compounds and antioxidant activity and it has been established that germination can significantly improve the antioxidant capacity of the soluble extracts of sprouts as compared with the raw seeds of numerous edible seeds (Gan et al. 2017). An upsurge of some antioxidant components in germinated seeds and sprouts such as antioxidant vitamins and polyphenols are noted to be contributory factors. Other, studies have also

reported variations in the antioxidant capacity of germinated edible seeds and sprouts (Wu et al. 2013; Guajardo-Flores et al. 2013; Aguilera et al. 2013).

Pająk et al. (2014) reported that sprouts of mung bean, radish, broccoli and sunflower had a significant increase in the levels of phenolic acids, flavonoids and antioxidant activity as compared to their seeds. Hence, sprouts are a very valuable source of natural antioxidants. Furthermore, Dueñas et al. (2009) found that germination significantly increased the bioactive phenolic compounds as well as the antioxidant activity of lupine seeds (*Lupinus angustifolius* L., c.v. Zapatón). This was attributed to the activation of endogenous enzymes and the complex biochemical metabolism of seeds in the course of sprouting resulting in the significant changes in the phenolic composition.

Similarly, Cevallos-Casals & Cisneros-Zevallos (2010) researched on 13 selected seed species and reported that germinated edible seeds are an excellent source of dietary phenolic compounds and antioxidants activity due to the increase in these bioactive compounds upon sprouting.

Paśko et al. (2009) also found that pseudocereals (amaranth and quinoa) sprouts showed relatively high antioxidant activity. The results of their investigation revealed that sprouts have a significantly higher antioxidant activity than seeds, which may be the outcome of difference in the content of polyphenols, anthocyanins and other bioactive compounds. Quinoa was found to be a better alternative for traditional cereals than amaranth. These alternative crops species and sprouts can be used in traditional diet as a beneficial source of food with very high nutritional value since they are good sources of anthocyanins and total phenolic content with high antioxidant activity.

3. Aims of the thesis

The main aim of the thesis was to assess selected nutritional and bioactive components of fresh sprouts of selected 15 plant species (*Chenopodium quinoa*, *Amaranthus caudatus*, *Medicago sativa*, *Fagopyrum esculentum*, *Glycine max*, *Cicer arietinum*, *Vigna radiata* syn. *Phaseolus aureus*, *Lens culinaris*, *Trigonella foenum graceum*, *Hordeum vulgare*, *Triticum aestivum*, *Eruca sativa*, *Brassica oleraceae* var. *Italica*, *Raphanus sativus*, *Helianthus annus*).

The sub-objectives were to:

- Evaluate the content of protein and its fractions, flavonoids, phenolics and antioxidant activity of sprouted seeds of selected cereals, pseudocereals, cruciferous vegetables and legumes.
- Assess and evaluate the changes in contents of tested components after the sprouting process.
- Characterise the health benefits of sprouted edible seeds.

The hypotheses tested under the set objectives were:

- Sprouting generally increases the nutritive value of edible seeds adding up some health benefits for humans.
- Comparison between seeds and sprouts from different crop samples yields a variation in their nutritional and polyphenolic composition as well as antioxidant activity.
- Nutritional and bioactive properties of sprouts are species and variety dependent.

4. Material and methods

4.1. Plant material

A total of 15 plant species were involved in the research. Commercially purchased samples included: 6 legumes, 3 cruciferous vegetables, 3 pseudocereals, 2 cereals and 1 oil crop including 4 fresh germs mixtures and 2 sprouted mix seeds. These crops from diverse geographical origins were obtained from various sources. Twenty-eight samples were prepared from the 15 plant species (Table 1). Figures 4 and 5 present samples of non-sprouted and sprouted plant material used for the study respectively.

Table 1. Experimental plant materials

Sample no.	Species name	English/Commercial name	Country of origin	Producer	Sample description	Type of production
	PSEUDOCEREALS					
1	*Chenopodium quinoa*	Bio quinoa black	Bolivia	Country Life	Black seeds	Organic
2	*Chenopodium quinoa*	Bio quinoa red	Peru	Bio nebio	Rusty red seeds	Organic
3	*Amaranthus caudatus*	Bio amaranth	Outside EU	Sunfood	Pale yellow seeds	Organic
4	*Amaranthus caudatus*	Amaranth bio	Czech Republic	Gene bank	Pale yellow seeds	
5	*Fagopyrum esculentum*	Buckwheat	Czech Republic	Pro-Bio	Triangular brown achenes	Organic
6	*Fagopyrum esculentum*	Buckwheat bio	Non EU	DM drogerie markt	Triangular brown achenes	Organic
7	*Fagopyrum esculentum*	Buckwheat groat	China	Country Life	Triangular brown achenes	Organic
	LEGUMES					
8	*Medicago sativa*	Bio alfalfa	Italy	Country Life	Green brown seeds	Organic
9	*Glycine max*	Soya beans	China	Pro-Bio	Rounded pale yellow seeds	Organic
10	*Cicer arietinum*	Chickpea	Italy	Bio nebio	Pale yellow seeds	Organic
11	*Vigna radiata* syn. *Phaseolus aureus*	Mungo	China	Country Life	Green small beans	Organic
12	*Lens culinaris*	Red lens	Turkey	Country Life	Red brown unhulled seeds	Organic

Table 1. Continued

Sample no.	Species name	English/Commercial name	Country of origin	Producer	Sample description	Type of production
	LEGUMES					
13	*Lens culinaris*	Dark green lens	Canada	Country Life	Dark green small seeds	Organic
14	*Lens culinaris*	Green big lens	Turkey	Country Life	Big green brown seeds	Organic
15	*Trigonella foenum graceum*	Fenugreek	Italy	Healthy day	Small brown stony seeds	Organic
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	Mix bio	Italy	Healthy day	Mixture of alfalfa and mungo seeds	Organic
	CEREALS					
17	*Hordeum vulgare*	Barley ´Amistar´	Poland	Flower factory	Hulled brown caryopsis	
18	*Hordeum vulgare*	Barley ´Casino´	Poland	Flower factory	Hulled brown caryopsis	
19	*Triticum aestivum*	Wheat ´Astrid´	Czech Republic	Gene bank	Husked brown caryopsis	
	CRUCIFEROUS VEGETABLES					
20	*Eruca sativa*	Rocket	Poland	Flower factory	Small, round and brown seeds	
21	*Brassica oleraceae* var. *Italica*	Broccoli	Italy	Healthy day	Small black and rusty red seeds	Organic
22	*Raphanus sativus*	Radish	Italy	Healthy day	Ovoid-globose yellowish seeds	Organic

Table 1. Continued

Sample no.	Species name	English/Commercial name	Country of origin	Producer	Sample description	Type of production
	FRESH GERMS/ MIXED SPROUTS					
23	*Medicago sativa, Raphanus sativus, Eruca sativa*	Fresh germs	Czech Republic	Happy eat	Alfalfa, radish, rocket	Organic
24	*Brassica oleraceae* var. *Italica*	Broccoli fresh germs	Czech Republic	Happy eat	Broccoli	Organic
25	*Cicer arietinum, Lens culinaris, Raphanus sativus, Triticum aestivum, Trigonella foenum graceum*	Protein mix fresh germs	Czech Republic	Happy eat	Protein mixture-chickpea, dark green lens, light green lens, wheat, radish, fenugreek	Organic
26	*Vigna radiata* syn. *Phaseolus aureus*	Mungo fresh germs	Czech Republic	Happy eat	Mungo beans	Organic
27	*Helianthus annus*	Sprouted dehulled lyophilised sunflower	Product of EU	Iswari Superfood	Sprouted dehulled sunflower seeds	Organic
28	*Fagopyrum esculentum, Helianthus annus, Chenopodium quinoa*	Mixture of raw sprouted seeds	Product of EU	Iswari Superfood	Sprouted raw seeds of buckwheat, sunflower and quinoa	Organic

Figure 4. Non-sprouted samples (A – *Chenopodium quinoa*, B – *Cicer arietinum*, C – *Hordeum vulgare*, D – *Raphanus sativus*): Author

Figure 5. Sprouted samples (A – *Vigna radiata* syn. *Phaseolus aureus*, B – Fresh germs (*Medicago sativa*, *Raphanus sativus*, *Eruca sativa*), C – *Chenopodium quinoa*, D – *Hordeum vulgare*): Author

4.2. Methods

4.2.1. Sample preparation

With the exception of the 4 fresh germs mixtures (samples 23-26) and the sprouted mix seeds (samples 27 and 28) which were analysed in the sprouted (germinated) forms only, sprouted and non-sprouted (quiescent dry seeds) samples from the remaining 22 samples were analysed.

Seeds were sprouted according to the methodology for home-made sprouting of seeds with modifications. They were germinated between 3 to 5 days at a temperature of 18 to 25 ^{o}C. To obtain the sprouted samples, dry seeds were first washed with distilled water followed by steeping in distilled water for 24 or 48 hours. They were then placed in a single layer on a sheet of filter paper inside perforated plastic trays (size- 4 cm x 17 cm) to ensure drainage of excess water and high moisture content for the sprouting process. The seedlings were carefully watered (sprinkled) twice daily while ensuring maximum aeration. At the end of the designated days of germination, sprouts (germinated seedlings that have not yet formed true leaves) were harvested and then lyophilised.

With the aid of a laboratory grinder, each of the lyophilised samples of all sprouted species were milled to obtain separate powdered specimen of about 10 g. Equivalent amount of milled specimen from each of the 22 quiescent dry seeds samples was also prepared to obtain the non-sprouted plant material for analysis.

The dry matter content of lyophilised sprouted samples was determined by drying samples in an electric hot-air oven at 105 °C for 4 h following the standard method CSN EN ISO 662 (2001).

4.2.2. Crude protein and protein fractions quantification

The crude protein content of each sample (1 g) was determined by the Kjeldahl method using a protein factor of 6.25 (CSN, 2012).

Three protein fractions namely, albumins, globulins and gliadins were extracted using Osborne's method with modifications based on the study conducted by Džunková et al. (2011). Albumins and globulins were isolated first followed by gliadins. For the

determination of protein fractions content, 1 g of each of the milled samples was used. All the analysis were carried out in duplicates.

4.2.3. Flavonoid content determination

0.1 gram of the milled plant material (dry seeds and sprouted seeds) was homogenised and subsequently extracted with a 5 ml extraction agent (methanol: water: formic acid - 80:14:6) on a horizontal shaker (120 min, 200 rpm). After the mixture had been centrifuged (15 min, 10 000 g), the supernatant was filtered (using a microfilter of 0.2 μm) followed by further dilution with 50% MeOH to ensure that the monitored analytes were within the quantification range.

UHPLC (Ultra-High Performance Liquid Chromatography)

A chromatographic system (Dionex UltiMate 3000 UHPLC system, Dionex Softron GmbH, Germany) consisting of a binary pump (HPG-3400RS), autosampler (WPS-3000RS), degasser (SRD-3400) and column heater (TCC-300RS) was used. Chromatographic separation was carried out on an Ascentis Express C18 column (100 mm x 2.1 mm, 2.7 μm, Supelco, Germany). The column was heated to 50 ºC and the injected sample volume was 6 μl. For separation, an elution gradient of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) was applied with a mobile phase flow rate: 350 μl per minute, gradient: 0 min 95% of component A and 5% of component B; 4 min 5% A + 95% B; 8 min 5% A + 95% B; 8.33 min 95% A + 5% B; 10 min 95% A + 5% B.

Mass Spectrometry

A Q Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) with electrospray ionisation (HESI) and Trace Finder 4.1 were used for detection.

Analysis was performed in a negative mode with a heated electrospray injection source (ESI−). High resolution Parallel Reaction Monitoring (PRM) was selected for the targeted

analysis, with a resolution of 17.500 (FWHM) and a following setting: Sheat gas flow rate 50 (unit), Aux gas flow rate 13 (unit), Sweep gas flow rate 3 unit), Spray voltage 2.70 (kV), Capillary temp. 263 (°C), Aux gas heater temp. 425 (°C), S-lens RF level 30, AGC target 2e5. The maximum injection time was 100 ms. Nitrogen was used as Sheat, Aux and Sweep gas. The mass spectrometer was externally calibrated to accurate mass using Positive Ion Calibration Solution and Pierce ESI Negative Ion Calibration Solution (Thermo Scientific). Quantification of the analytes in the extract was achieved via the calibration curve method (Figure 6).

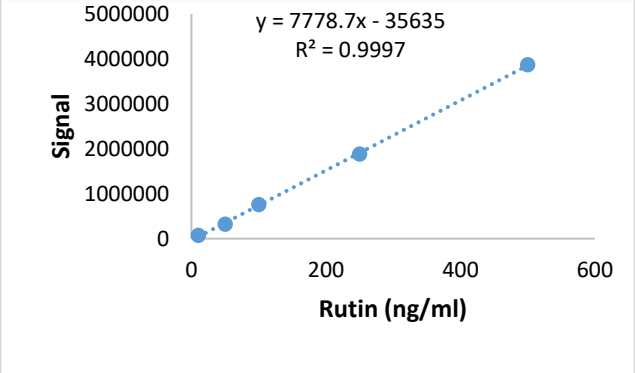

Figure 6. An example of calibration curve (for the flavonoid, rutin)

4.2.4. Folin assay

A spectrophotometric method was used for the determination of total phenolic content (TPC) using Folin-Ciocalteu reagent according to a modified protocol in Holasova et al. (2002). Extraction was done using 2 g of sample with 20 ml of 80% methanol for 60 minutes in centrifugation tubes. The tubes were protected from sunlight. 0.5 ml of the extract was pipetted into 50 ml volumetric flask and diluted with distilled water. This was followed by the addition of 2.5 ml Folin-Ciocalteu reagent (PENTA, Czech Republic) and 7.5 ml 20% sodium carbonate solution after which the mixture was agitated. The mixture was incubated at laboratory temperature for 2 h and the absorbance was measured at wave length (λ), 765 nm on the spectrophotometer Genesys 10UV (Thermo Scientific, USA) against blank samples: 500, 250, 100, 50, 25, 10, 5, 1 µg/ml gallic acid concentration which were used as standards for calibration. The total phenolic content was expressed as gallic acid equivalents (GAE) and the results were quantified using gallic acid (Merck, Germany).

4.2.5. Determination of free radical scavenging activity of sample extracts through DPPH assay

The radical scavenging capacity (RSC) of samples in methanol extract were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl).

To obtain a stock solution, 0.025 g of DPPH was diluted to 100 ml with methanol and it was kept in a cool, dark place. Just before the analysis, a 1:10 dilution of the stock was made with methanol.

20 ml of methanol was added to 1 g of sample and agitated for 90 min while being protected from light followed by filtration of the mixture to obtain extract. 150 µl of DPPH solution with initial absorbance of A=0.6 at 550 nm was added to 20 µl of the extracts on microtiter plates. The mixture was incubated at laboratory temperature for 10 min with the reaction taking place in the dark. Afterwards, the absorbance at 550 nm was read using a spectrophotometer (Sunrise absorbance reader, Tecan, Switzerland). The ability of the sample to scavenge the DPPH radical was determined using the standard curve obtained with Trolox (Sigma Aldrich, Germany) in the range from 0.0 to 0.2 mmol/l. The results were expressed as Trolox equivalent (TE) antioxidant capacity.

4.2.6. Statistical analysis

Data obtained from the experiments were statistically evaluated using analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test. Statistical differences among evaluated species were significant at level of $p \leq 0.05$. Correlation analysis was performed between measured parameters. Statistical analysis was performed by STATISTICA 12.0 CZ software.

5. Results

5.1. Crude protein and protein fractions content of samples

The mean crude (total) protein and protein fractions content of the investigated 15 species in the sprout and non-sprouted forms is shown in Table 2. Generally, the changes in crude protein and protein fractions content after sprouting considerably depended on the species and varieties. Though there were variations following the sprouting of the individual seed samples, overall there was no significant ($p \leq 0.05$) difference in the total protein content, albumin and globulin fractions, gliadin fractions and glutelin fractions between the sprouted and non-sprouted samples (Table 2). Between the repeated analyses (repetition 1 and 2) for each sample of sprout and non-sprout, there was no significant ($p \leq 0.05$) difference in the crude protein content, gliadin fraction and glutelin fraction except, albumin and globulin fraction which recorded some slight difference (Table 2).

5.1.1. Crude protein and protein fractions content of non-sprouted samples

Bio alfalfa, soya bean and rocket recorded the highest total protein content of 39.85%, 38.99% and 35.37% respectively (Table A1) among the non-sprouted samples. Generally, high contents of crude proteins were recorded in legumes of the non-sprouted samples. The values of crude protein content measured in non-sprouted cruciferous vegetables were comparable to those of legumes. The lowest crude protein content among the samples were measured in the three cereals samples: barley ´Casino´, barley ´Amistar´ and wheat ´Astrid´ with respective values of 11.98%, 13.02% and 12.06%. Pseudocereals recorded relatively low amounts of crude proteins (12.61% to 16.99%). Albumin-globulin content was comparably high in legumes (6.16% to 18.78%) and low in cereals (2.56% to 3.17%). The gliadin content measured among the non-sprouted samples were generally low (0.07% to 4.50%). However, cereals recorded comparatively maximum values of gliadins: 4.50%, 3.14% and 2.98% for wheat ´Astrid´, barley ´Casino´ and barley ´Amistar´ respectively. These reported values were significantly ($p \leq 0.05$) different from the rest of the samples. Glutelin contents of non-sprouted samples were generally high

among the legumes with bio alfalfa recording the highest value of 32.71% and the lowest of 4.39% by wheat.

5.1.2. Crude protein and protein fractions content of sprouted samples

Sprouted legumes and cruciferous vegetables recorded high crude protein contents. Bio alfalfa, soya bean and rocket recorded the highest total protein content of 42.71%, 39.67% and 36.13% respectively among the sprouted samples (Table A2). The three samples: wheat ´Astrid´, barley ´Casino´ and barley ´Amistar´ with respective values of 11.75%, 12.79% and 12.84% recorded the lowest total protein contents among the sprouted samples. Generally, pseudocereals also recorded relatively low amounts of crude proteins (12.95% to 16.51%).

The albumin-globulin content measured among the sprouted samples was high in legumes and comparatively low in cereals with red lens measuring the highest content of 21.73%. Generally, low gliadin content was measured in all samples (0.14% to 2.94%). Rocket, wheat ´Astrid´ and bio alfalfa measured the highest gliadin values of 2.94%, 2.29% and 1.52% respectively. Sprouted cereals recorded lower gliadin values with barley ´Amistar´, barley ´Casino´ and wheat ´Astrid´ recording the values of 1.21%, 1.28% and 2.29% respectively.

Fresh germs and mixed sprouts of sample 23 to sample 28 measured high crude protein contents (Table A2). Overall, the fresh germs of sample 23 recorded the highest protein content of 44.65% followed by mungo fresh germs (sample 26), broccoli fresh germs (sample 24), protein mix fresh germs (sample 25) and sprouted dehulled lyophilised sunflower (sample 27) with the respective values 27.84%, 26.93%, 24.52% and 20.57%. Mixed sprouts of sample 28 recorded the minimum crude protein content of 17.39%. There were significant ($p \leq 0.05$) differences in protein content among the fresh germs and mixed sprouts.

5.1.3. Changes in crude protein and protein fractions content of samples after sprouting

Generally, there was an increase in the crude protein content of sprouted legumes and cruciferous vegetables compared to the non-sprouted. Non-sprouted bio alfalfa, soya bean and rocket recorded the highest total protein content of 39.85%, 38.99% and 35.37% respectively whiles in their sprouted counterparts there was a respective increase in total protein content values to 42.71%, 39.67% and 36.13%. Generally, sprouted legumes recorded the highest increase in albumin-globulin content among the sprouted samples and their values were superior as compared to their non-sprouted forms. All sprouted cereals recorded lower gliadin values as compared to their non-sprouted forms.

Overall, glutelin content reduced in most sprouted legumes but increased in all three sprouted cereals and cruciferous vegetables as compared to their non-sprouted forms. Non-sprouted bio alfalfa recorded a glutelin content of 32.71% whiles its sprouted form decreased to 25.38%. Glutelin content in non-sprouted radish (9.65%) increased to 15.82% in the sprouted form.

Radish, mix bio and bio alfalfa respectively recorded the highest increase in crude protein content of 5.86%, 4.07% and 2.86% between the sprouted and non-sprouted forms followed by buckwheat and mungo (Table A3).

Generally, cereals and pseudocereals recorded low total protein contents regardless of their stage (non-sprouted or sprouted form).

Table 2. Protein analysis of sprouted and non-sprouted samples

Sample no.	Species name	Total protein content* (%) in d.m.	Change in total protein content (%)	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	PSEUDOCEREALS					
1	*Chenopodium quinoa*	15.57±0.18^{ab}	+0.31	5.97±1.75^{ab}	0.34±0.16^{ab}	9.26±1.75^{bcde}
2	*Chenopodium quinoa*	14.81±2.18^{ab}	-3.71	7.06±0.57^{abc}	0.14±0.08^{a}	7.61±1.61^{abcd}
3	*Amaranthus caudatus*	16.33±1.27^{b}	-1.32	5.58±0.84^{ab}	0.66±0.38^{ab}	10.09±2.06^{cde}
4	*Amaranthus caudatus*	16.28±0.33^{b}	+0.47	6.14±0.34^{ab}	0.43±0.09^{ab}	9.70±0.31^{bcde}
5	*Fagopyrum esculentum*	13.25±0.76^{ab}	+1.28	2.91±1.63^{a}	0.43±0.21^{ab}	9.91±2.07^{bcde}
6	*Fagopyrum esculentum*	14.65±1.64^{ab}	-2.83	6.81±1.61^{abc}	0.36±0.04^{ab}	7.49±1.50^{abcd}
7	*Fagopyrum esculentum*	15.16±0.19^{ab}	+0.10	6.27±0.97^{ab}	0.47±0.07^{ab}	8.43±0.83^{abcd}
	LEGUMES					
8	*Medicago sativa*	41.28±1.71^{g}	+2.86	10.98±5.59^{bcd}	1.26±0.85^{abcd}	29.04±4.27^{h}
9	*Glycine max*	39.33±0.50^{fg}	+0.68	18.86±1.40^{f}	0.64±0.28^{ab}	19.83±1.56^{g}
10	*Cicer arietinum*	21.59±0.22^{c}	+0.03	14.67±1.03^{def}	0.48±0.57^{ab}	6.44±1.24^{ab}
11	*Vigna radiata* syn. *Phaseolus aureus*	25.30±0.64^{cd}	+1.07	18.39±0.44^{f}	0.14±0.10^{a}	6.78±0.54^{abc}
12	*Lens culinaris*	28.14±0.22^{de}	+0.37	19.33±2.85^{f}	0.31±0.11^{ab}	8.50±2.57^{abcd}
13	*Lens culinaris*	27.18±0.31^{de}	+0.32	18.02±2.66^{f}	0.37±0.09^{ab}	8.78±2.42^{abcd}
14	*Lens culinaris*	28.34±0.42^{de}	+0.62	17.40±1.43^{ef}	0.54±0.10^{ab}	10.39±1.13^{de}
15	*Trigonella foenum graceum*	29.12±0.73^{e}	+0.56	6.39±1.40^{ab}	1.08±0.07^{abc}	21.65±0.75^{g}

Table 2. Continued

Sample no.	Species name	Total protein content* (%) in d.m.	Change in total protein content (%)	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	LEGUMES					
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	29.77±2.35^{e}	+4.07	15.44±4.20^{def}	0.25±0.11^{ab}	14.08±2.71^{f}
	CEREALS					
17	*Hordeum vulgare*	12.93±0.20^{ab}	-0.18	3.20±0.33^{a}	2.09±1.03^{cde}	7.65±0.70^{abcd}
18	*Hordeum vulgare*	12.39±0.52^{a}	+0.81	2.38±0.26^{a}	2.21±1.07^{cde}	7.80±1.77^{abcd}
19	*Triticum aestivum*	11.91±.19^{a}	-0.31	3.05±0.24^{a}	3.40±1.27^{e}	5.45±1.26^{a}
	CRUCIFEROUS VEGETABLES					
20	*Eruca sativa*	35.75±0.45^{f}	+0.76	12.29±1.98^{cde}	2.55±0.49^{de}	20.93±2.06^{g}
21	*Brassica oleraceae* var. *Italica*	21.82±0.31^{c}	+0.53	10.00±1.92^{bcd}	1.30±0.58^{abcd}	10.52±2.29^{de}
22	*Raphanus sativus*	29.05±5.16^{de}	+5.86	14.73±3.88^{def}	1.59±0.70^{bcd}	12.73±8.02^{ef}
Repetition	1	22.73±8.88^{a}		9.76±5.57^{a}	1.03±1.20^{a}	11.65±6.77^{a}
	2	22.72±8.88^{a}		10.77±6.62^{b}	0.88±0.73^{a}	11.36±5.91^{a}
Stage	NS	22.43±8.41^{a}		10.64±6.24^{a}	1.01±0.97^{a}	11.09±6.39^{a}
	S	23.02±9.31^{a}		9.90±6.02^{a}	0.90±1.02^{a}	11.91±6.29^{a}

In the table are presented average value±S.D. (Standard Deviation)
*In the same column, values followed by different superscripts (a-h) denote statistically significant differences at $p \leq 0.05$.
(+) indicate an increase in total protein content after sprouting
(-) indicate a decrease in total protein content after sprouting
NS – non-sprouted samples, S – sprouted samples
d.m. – dry matter

5.2. Flavonoid content determination

A chromatographic separation following the extraction of analytes under investigation (sprouted and non-sprouted samples) resulted in the identification and quantification of eight types of flavonoids: chlorogenic acid, isoorientin, isoquercitrin, kaempferol, quercetin, quercitrin, rutin and vitexin (Tables 3 and 4). Generally, the availability and quantity of flavonoids detected in samples depended on the species and varieties. The presence and amounts of each identified bioflavonoid were determined by UHPLC tandem mass spectrometry.

5.2.1. Flavonoid content of non-sprouted samples

Data reported in Table 3 shows the flavonoid concentrations (in μg/g) present in selected non-sprouted seed extracts with varying types and amounts of flavonoids in each of the sample. Among the non-sprouted samples, the three varieties of *Fagopyrum esculentum* and bio alfalfa recorded the maximum types of flavonoids whiles in chickpea, green big lens, wheat ´Astrid´, broccoli and radish, flavonoids (chlorogenic acid, isoorientin, isoquercitrin, kaempferol, quercetin, quercitrin, rutin and vitexin) were either not detected or the amounts discovered were below the limit of quantification (Tables 3).

Buckwheat (sample 5) recorded 7 types of flavonoids whereas in buckwheat bio (sample 6) and buckwheat groat (sample 7) 6 types of flavonoids were identified. The highest content of quercitrin detected among the non-sprouts extracts was in buckwheat (7.58 μg/g). Chlorogenic acid was absent in all pseudocereals with the exception of buckwheat. Buckwheat bio and buckwheat groat recorded the lowest amount of quercetin (0.34 μg/g). Rutin was the only flavonoid detected in the two varieties of amaranth whereas in the two varieties of quinoa, isoquercitrin, quercetin and rutin were the flavonoids identified. The highest of rutin was recorded in bio quinoa red (1,320.15 μg/g) and lowest in amaranth bio (0.62 μg/g) among the non-sprouted samples.

Among the non-sprouted legumes, 6 types of flavonoids were detected in bio alfalfa. Kaempferol was detected in only bio alfalfa among the non-sprouted samples in the quantity 0.4 μg/g. The highest amount of quercetin (12.56 μg/g) among the non-sprouts was recorded in bio alfalfa. Red lens recorded the minimum content of quercitrin (0.4

µg/g) among the non-sprouts samples and this was the only flavonoid detected in red lens. Isoquercitrin was recorded highest in bio alfalfa (3,931.5 µg/g) among the non-sprouted samples with the minimum amount detected in soya beans (0.27 µg/g). Vitexin was present in the highest content in mungo (306.66 µg/g) and lowest in soya beans (0.27 µg/g) of the non-sprouted samples. Fenugreek recorded the highest content (288.82 µg/g) of isoorientin among the non-sprouted samples.

Chlorogenic acid which was detected in a few of the non-sprouted samples and isoorientin were the only two types of flavonoids detected in the cereals (barley). Barley ′Casino′ and barley ′Amistar′ recorded the highest (1.39 µg/g) and the lowest (0.25 µg/g) content of chlorogenic acid respectively. Barley ′Amistar′ recorded the lowest amount of isoorientin (0.41 µg/g) among the non-sprouted samples.

Rocket was the only non-sprouted cruciferous vegetable which recorded the detection and quantification of 3 types of flavonoids (chlorogenic acid, isoquercitrin and quercetin).

5.2.2. Flavonoid content of sprouted samples

Flavonoid contents present in selected sprouted seed extracts are shown in Table 4 with varying types and amounts of flavonoids in each of the sprouted samples. Generally, the three varieties of *Fagopyrum esculentum* recorded the maximum types of flavonoids among the sprouted samples. With the exception of kaempferol the remaining 7 types of flavonoids were detected in buckwheat (sample 5), buckwheat bio (sample 6) and buckwheat groat (sample 7). Mungo and mixture of raw sprouts also recorded a maximum of 6 types of flavonoids. Among the sprouted samples flavonoids were not detected or the amounts determined were below the limit of quantification in chickpea, red lens, barley ′Amistar′ and wheat ′Astrid′.

Mainly among the pseudocereals there was an increase in the types of flavonoids detected. Chlorogenic acid was detected in all varieties of *Fagopyrum esculentum* with buckwheat recording the highest content of 160.19 µg/g among the sprouted samples. Sprouted buckwheat had the highest isoorientin content of 2,209.65 µg/g. Kaempferol was detected highest in bio quinoa red (0.67 µg/g) and bio amaranth recorded the lowest amount (0.31 µ/g) of isoquercitrin.

Quercetin was detected highest in buckwheat (7.25 µg/g) and lowest in buckwheat bio (0.25 µg/g). Buckwheat and buckwheat groat respectively recorded the maximum (1.48 µg/g) and minimum (0.64 µg/g) values of quercitrin. Again, buckwheat recorded the highest content of rutin (1,358.05 µg/g) and vitexin (858.4 µg/g) among the sprouted samples.

Among the sprouted cereals, isoorientin and isoquercitrin were the only flavonoids detected in barley ′Casino′. Barley ′Casino′ recorded the lowest amount of isoorientin among the sprouted samples (0.42 µg/g).

Within the sprouted legumes, mix bio was the sample detected to have the highest amount of isoquercitrin (116.33 µg/g). Vitexin content detected in sprouted soya beans was low (0.3 µg/g). A maximum of 2 flavonoid types was detected in each of the three sprouted cruciferous vegetables. Broccoli recorded the minimum value of kaempferol (0.18 µg/g) among the sprouted samples.

Among the mixtures of fresh germs and sprouts, very high amounts of chlorogenic acid was detected in sprouted dehulled sunflower (10,339.35 µg/g) and mixture of raw sprouted seeds of sample 28 (2,871.75 µg/g). Broccoli fresh germs recorded the maximum value of kaempferol (1.88 µg/g) whiles rutin was detected lowest in fresh germs (2.32 µg/g) among the sprouted samples. Isoquercitrin recorded the minimum amount (0.31 µ/g) also in mungo fresh germs. The lowest recorded vitexin was in the mixture of raw sprouted seeds (0.29 µg/g) among the sprouted samples.

5.2.3. Changes in flavonoid content of samples after sprouting

Generally, additional flavonoids were detected in some sprouted pseudocereals (bio quinoa red, bio amaranth, buckwheat bio and buckwheat groat), legumes (mungo, green big lens, mix bio,) and cruciferous vegetables (broccoli and radish) compared to their non-sprouted forms. In others like sprouted bio alfalfa, soya beans, red lens, barley ′Amistar′ and rocket, there was a reduction in the types of flavonoids detected compared to their non-sprouted forms. Depending on the species and varieties, the amounts of flavonoids determined also changed as a result of sprouting.

Kaempferol was detected in only bio alfalfa among the non-sprouted samples in the quantity 0.4 µg/g whiles in the sprouted samples, kaempferol was detected in bio quinoa red, bio alfalfa, soya beans, mungo, broccoli, fresh germs and broccoli fresh germs in the amounts 0.67 µg/g, 0.42 µg/g, 0.42 µg/g, 0.56 µg/g, 0.18 µg/g, 0.37 µg/g and 1.88 µg/g respectively. Chlorogenic acid was detected in a few of the non-sprouted samples with barley ′Casino′ and barley ′Amistar′ recording both the maximum (1.39 µg/g) and minimum (0.27 µg/g) values respectively. However, chlorogenic acid was absent in the sprouted varieties of barley. On the other hand, it was detected in high amounts in sprouted dehulled sunflower (10,339.35 µg/g), followed by the mixture of raw sprouted seeds (2,871.75 µg/g) and buckwheat (160.19 µg/g).

The highest amount of isoorientin among the non-sprouted samples was detected in fenugreek (288.82 µg/g) with the equivalent sprouted sample recording 769.41 µg/g. Sprouted buckwheat had the highest isoorientin content of 2,209.65 µg/g with its equivalent non-sprouted sample recording a value of 31.02 µg/g. Isoquercitrin was recorded in the highest amount in bio alfalfa (3,931.5 µg/g) among the non-sprouted samples with the lowest value detected in soya beans (0.27 µg/g). Nevertheless, sprouted bio alfalfa recorded a relatively low amount of isoquercitrin (35.48 µ/g) and mix bio was the sample detected to have the highest amount (116.33 µg/g) of isoquercitrin among the sprouted extracts with mungo fresh germs and bio amaranth having the minimum values (0.31 µ/g).

Quercetin was detected in highest amount in bio alfalfa (12.56 µg/g) among the non-sprouted samples whiles in the sprouted forms, buckwheat recorded the maximum content (7.25 µg/g) of quercetin. Quercitrin was the only flavonoid detected in non-sprouted red lens however, it was not detected in the sprouted form. Sprouted buckwheat recorded the highest content of rutin (1,358.05 µg/g) whiles among the non-sprouted samples bio quinoa red contained the highest amount of rutin (1,320.15 µg/g). There was an increase in the rutin content of sprouted bio quinoa black, sprouted bio amaranth and sprouted amaranth bio compared to their non-sprouted forms. Though there was an increase in the content of vitexin in sprouted mungo to 457.3 µg/g compared to its non-sprouted counterpart, buckwheat which detected minimal amount of vitexin in its non-sprouted form (27.3 µg/g) recorded the maximum content of vitexin (858.4 µg/g) among the

sprouted samples. Generally, sprouting resulted in an increase of the bioflavonoid contents of the selected seeds under investigation.

Table 3. Bioflavonoid analysis of non-sprouted samples

Sample no.	Species name	Chlorogenic acid (µg/g in d.m.)	Isoorientin (µg/g in d.m.)	Isoquercitrin (µg/g in d.m.)	Kaempferol (µg/g in d.m.)	Quercetin (µg/g in d.m.)	Quercitrin (µg/g in d.m.)	Rutin (µg/g in d.m.)	Vitexin (µg/g in d.m.)
	PSEUDOCEREALS								
1	*Chenopodium quinoa*	< LOQ	N/F	0.67	<LOQ	0.41	N/F	9.58	N/F
2	*Chenopodium quinoa*	N/F	N/F	1.54	<LOQ	0.73	N/F	1,320.15	N/F
3	*Amaranthus caudatus*	N/F	N/F	<LOQ	N/F	<LOQ	N/F	1.93	N/F
4	*Amaranthus caudatus*	N/F	N/F	N/F	N/F	<LOQ	N/F	0.62	N/F
5	*Fagopyrum esculentum*	0.98	31.02	93.45	<LOQ	3.1	7.58	292.09	27.3
6	*Fagopyrum esculentum*	<LOQ	1.64	3.05	<LOQ	0.34	0.69	133.88	0.72
7	*Fagopyrum esculentum*	< LOQ	1.26	2.87	<LOQ	0.34	0.77	152.23	0.76
	LEGUMES								
8	*Medicago sativa*	<LOQ	3.41	3,931.5	0.4	12.56	N/F	5.78	0.31
9	*Glycine max*	N/F	2.4	0.27	N/F	<LOQ	N/F	N/F	0.27
10	*Cicer arietinum*	N/F	N/F	<LOQ	N/F	<LOQ	N/F	N/F	<LOQ
11	*Vigna radiata* syn. *Phaseolus aureus*	N/F	10.17	0.43	N/F	<LOQ	N/F	1.03	306.66
12	*Lens culinaris*	N/F	N/F	N/F	N/F	<LOQ	0.4	N/F	N/F
13	*Lens culinaris*	N/F	N/F	4.94	N/F	N/F	0.79	N/F	N/F
14	*Lens culinaris*	N/F	N/F	<LOQ	N/F	<LOQ	<LOQ	<LOQ	N/F
15	*Trigonella foenum graceum*	N/F	288.82	N/F	N/F	<LOQ	N/F	N/F	161.2
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	<LOQ	1.26	1,109.32	<LOQ	8.1	N/F	N/F	63.61

Table 3. Continued

Sample no.	Species name	Chlorogenic acid (µg/g in d.m.)	Isoorientin (µg/g in d.m.)	Isoquercitrin (µg/g in d.m.)	Kaempferol (µg/g in d.m.)	Quercetin (µg/g in d.m.)	Quercitrin (µg/g in d.m.)	Rutin (µg/g in d.m.)	Vitexin (µg/g in d.m.)
	CEREALS								
17	*Hordeum vulgare*	0.25	0.41	N/F	N/F	<LOQ	N/F	N/F	N/F
18	*Hordeum vulgare*	1.39	0.58	N/F	N/F	<LOQ	N/F	N/F	N/F
19	*Triticum aestivum*	<LOQ	N/F	N/F	N/F	<LOQ	N/F	<LOQ	N/F
	CRUCIFEROUS VEGETABLES								
20	*Eruca sativa*	0.44	N/F	4.3	<LOQ	0.4	N/F	<LOQ	<LOQ
21	*Brassica oleraceae* var. *Italica*	N/F	N/F	<LOQ	<LOQ	<LOQ	N/F	N/F	<LOQ
22	*Raphanus sativus*	N/F	N/F	N/F	N/F	<LOQ	<LOQ	<LOQ	<LOQ

LOQ = 0.25 µg/g
N/F – Not Found
d.m. – dry matter

Table 4. Bioflavonoid analysis of sprouted samples

Sample no.	Species name	Chlorogenic acid (µg/g in d.m.)	Isoorientin (µg/g in d.m.)	Isoquercitrin (µg/g in d.m.)	Kaempferol (µg/g in d.m.)	Quercetin (µg/g in d.m.)	Quercitrin (µg/g in d.m.)	Rutin (µg/g in d.m.)	Vitexin (µg/g in d.m.)
	PSEUDOCEREALS								
1	*Chenopodium quinoa*	N/F	N/F	0.9	<LOQ	0.52	N/F	12.15	N/F
2	*Chenopodium quinoa*	N/F	N/F	2.1	0.67	5.78	N/F	18.1	N/F
3	*Amaranthus caudatus*	N/F	N/F	0.31	N/F	<LOQ	N/F	7.17	N/F
4	*Amaranthus caudatus*	N/F	N/F	N/F	N/F	N/F	<LOQ	26.3	N/F
5	*Fagopyrum esculentum*	160.19	2,209.65	29.91	<LOQ	7.25	1.48	1,358.05	858.4
6	*Fagopyrum esculentum*	0.31	1.29	3.8	<LOQ	0.25	0.73	150.4	1.64
7	*Fagopyrum esculentum*	0.34	1.42	3.9	<LOQ	0.32	0.64	133.01	1.84
	LEGUMES								
8	*Medicago sativa*	N/F	N/F	35.48	0.42	5.03	N/F	10.09	N/F
9	*Glycine max*	N/F	N/F	N/F	0.42	<LOQ	N/F	N/F	0.3
10	*Cicer arietinum*	N/F	N/F	<LOQ	N/F	N/F	N/F	N/F	N/F
11	*Vigna radiata* syn. *Phaseolus aureus*	N/F	51.95	8	0.56	6.01	N/F	7.68	457.3
12	*Lens culinaris*	N/F	N/F	N/F	N/F	<LOQ	<LOQ	N/F	N/F
13	*Lens culinaris*	3.56	N/F	N/F	N/F	N/F	0.79	N/F	N/F
14	*Lens culinaris*	0.27	N/F	N/F	N/F	<LOQ	<LOQ	N/F	N/F
15	*Trigonella foenum graceum*	N/F	769.41	N/F	N/F	N/F	N/F	N/F	769.18

Table 4. Continued

Sample no.	Species name	Chlorogenic acid (µg/g in d.m.)	Isoorientin (µg/g in d.m.)	Isoquercitrin (µg/g in d.m.)	Kaempferol (µg/g in d.m.)	Quercetin (µg/g in d.m.)	Quercitrin (µg/g in d.m.)	Rutin (µg/g in d.m.)	Vitexin (µg/g in d.m.)
	LEGUMES								
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	N/F	4.11	116.33	N/F	7.19	<LOQ	6.72	160.26
	CEREALS								
17	*Hordeum vulgare*	<LOQ	<LOQ	N/F	N/F	<LOQ	N/F	N/F	N/F
18	*Hordeum vulgare*	<LOQ	0.42	0.61	N/F	<LOQ	N/F	<LOQ	N/F
19	*Triticum aestivum*	N/F	<LOQ	N/F	N/F	N/F	N/F	<LOQ	N/F
	CRUCIFEROUS VEGETABLES								
20	*Eruca sativa*	<LOQ	N/F	4.84	<LOQ	0.54	N/F	<LOQ	N/F
21	*Brassica oleraceae* var. *Italica*	N/F	N/F	N/F	0.18	N/F	N/F	N/F	10.09
22	*Raphanus sativus*	N/F	N/F	N/F	N/F	N/F	N/F	N/F	30.41

Table 4. Continued

Sample no.	Species name	Chlorogenic acid (µg/g in d.m.)	Isoorientin (µg/g in d.m.)	Isoquercitrin (µg/g in d.m.)	Kaempferol (µg/g in d.m.)	Quercetin (µg/g in d.m.)	Quercitrin (µg/g in d.m.)	Rutin (µg/g in d.m.)	Vitexin (µg/g in d.m.)
	FRESH GERMS/ MIXED SPROUTS								
23	*Medicago sativa, Raphanus sativus, Eruca sativa*	3.75	N/F	1.86	0.37	0.95	<LOQ	2.32	N/F
24	*Brassica oleraceae* var. *Italica*	N/F	N/F	N/F	1.88	<LOQ	N/F	N/F	N/F
25	*Cicer arietinum, Lens culinaris, Raphanus sativus, Triticum aestivum, Trigonella foenum graceum*	N/F	1.2	0.56	N/F	<LOQ	<LOQ	N/F	4.32
26	*Vigna radiata* syn. *Phaseolus aureus*	N/F	1.22	0.31	N/F	<LOQ	N/F	1.2	24.04
27	*Helianthus annus*	10,339.35	N/F	0.73	N/F	<LOQ	N/F	N/F	N/F
28	*Fagopyrum esculentum, Helianthus annus, Chenopodium quinoa*	2,871.75	0.68	1.14	<LOQ	0.37	<LOQ	65.44	0.29

LOQ = 0.25 µg/g
N/F – Not Found
d.m. – dry matter

5.3. Determination of total phenolic content

The TPC of extracts of samples under study (sprouted and non-sprouted forms) was determined using Folin-Ciocalteu reagent and their mean values are shown in Table 5. Overall, the changes in TPC after sprouting was species and varieties dependent. Rocket, wheat ′Astrid′, mungo and bio amaranth recorded the highest increase in TPC with the values: 83.8%, 73.9%, 71.0% and 71.0% respectively between the sprouted and non-sprouted samples. There was a decrease in the TPC of barley ′Casino′, bio quinoa red and green big lens with respective values -69.8%, -38.2% and -21.5%. Statistically, there were significant ($p \leq 0.05$) differences in the total phenolic content recorded between the sprouted and non-sprouted samples (Table 5).

Table 5. Determination of total phenolic content in extracts of sprouted and non-sprouted samples

Sample no.	Species name	TPC* (mg/g) in d.m.	Change in TPC (%)
	PSEUDOCEREALS		
1	*Chenopodium quinoa*	1.91±1.34^{ab}	+66.1
2	*Chenopodium quinoa*	0.90±0.20^{a}	-38.2
3	*Amaranthus caudatus*	1.87±1.46^{ab}	+71.0
4	*Amaranthus caudatus*	1.87±1.29^{ab}	+65.5
5	*Fagopyrum esculentum*	1.89±0.64^{ab}	+38.5
6	*Fagopyrum esculentum*	3.67±2.41^{b}	+63.5
7	*Fagopyrum esculentum*	1.89±1.27^{ab}	+64.4
	LEGUMES		
8	*Medicago sativa*	2.25±0.80^{ab}	+40.1
9	*Glycine max*	2.52±1.69^{ab}	+64.2
10	*Cicer arietinum*	1.29±0.92^{ab}	+67.0
11	*Vigna radiata* syn. *Phaseolus aureus*	1.55±1.21^{ab}	+71.0
12	*Lens culinaris*	1.63±1.10^{ab}	+64.7
13	*Lens culinaris*	0.68±0.07^{a}	+13.7
14	*Lens culinaris*	0.72±0.10^{a}	-21.5
15	*Trigonella foenum graceum*	2.35±0.60^{ab}	+30.7
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	2.08±1.15^{ab}	+56.1
	CEREALS		
17	*Hordeum vulgare*	1.95±1.25^{ab}	+62.5
18	*Hordeum vulgare*	0.85±0.31^{a}	-69.8
19	*Triticum aestivum*	1.81±1.50^{ab}	+73.9

Table 5. Continued

Sample no.	Species name	TPC* (mg/g) in d.m.	Change in TPC (%)
	CRUCIFEROUS VEGETABLES		
20	*Eruca sativa*	1.51±1.54^{ab}	+83.8
21	*Brassica oleraceae* var. *Italica*	2.01±1.01^{ab}	+52.4
22	*Raphanus sativus*	2.36±0.70^{ab}	+34.7
Stage	NS	1.11±0.44^{a}	
	S	2.48±1.08^{b}	

In the table are presented average value±S.D.
*In the same column, values followed by different superscripts (a-b) denote statistically significant differences at $p \leq 0.05$.
(+) indicate an increase in TPC after sprouting
(-) indicate a decrease in TPC after sprouting
NS – non-sprouted samples, S – sprouted samples
d.m. – dry matter

5.4. Determination of free radical scavenging activity (antioxidant activity) of sample extracts

Extracts of sprouted and non-sprouted samples were assessed for their capacity to scavenge DPPH radicals and their antioxidant activity data in terms of free radical inhibition are presented in Table 6. Generally, all the samples exhibited an increase in their antioxidant activity after sprouting with the exception of wheat ´Astrid´ and barley ´Casino´ which recorded a decline in their scavenging capacities. The free-radical scavenging capacities of samples was species and variety dependent. Among the quadruplicate analyses (repetitions) performed for each sample of sprout and non-sprout, there was no significant ($p \leq 0.05$) difference in the scavenging capacities (Table 6). However, there was a significant ($p \leq 0.05$) difference in the antioxidant activity between sprouted and non-sprouted extracts.

5.4.1. Antioxidant activity of non-sprouted samples

Generally, most of the non-sprouted samples did not exhibit strong scavenging capacities of the DPPH radicals (Table A4). However, buckwheat groat recorded a significantly ($p \leq 0.05$) higher antioxidant activity of 219.04 mg TE/100 g compared to the rest of non-sprouted samples. Buckwheat bio, mungo, rocket, buckwheat and wheat ´Astrid´ followed with the values: 92.64 mg TE/100 g, 88.73 mg TE/100 g, 73.76 mg TE/100 g, 72.26 mg TE/100 g and 64.17 mg TE/100 g respectively (Table A4). The lowest scavenging capacities were recorded by bio quinoa black (14.82 mg TE/100 g), red lens (15.54 mg TE/100 g) and mix bio (16.19 mg TE/100 g). There was no significant ($p \leq 0.05$) difference in the scavenging capacities of the quadruplicate analyses performed for each of the non-sprouted samples (Table A4).

5.4.2. Antioxidant activity of sprouted samples

Comparable to the non-sprouted samples, high antioxidant activities through to moderate and weak antioxidant activities were measured among extracts of the sprouted samples (Table A5). Among the sprouted samples, all three cruciferous vegetables: rocket, broccoli and radish exhibited a strong capacity in scavenging DPPH radicals with the respective values: 222.68 mg TE/100 g, 228.83 mg TE/100 g and 229.22 mg TE/100 g. The highest antioxidant activity were exhibited by: mungo (233.40 mg TE/100 g), buckwheat (232.87 mg TE/100 g) and buckwheat groat (232.01 mg TE/100 g). Wheat ´Astrid´ and barley ´Casino´ recorded the lowest scavenging capacities of the DPPH radicals (16.90 mg TE/100 g and 34.63 mg TE/100 g respectively) among the sprouted samples.

Among the pseudocereals, all three varieties of sprouted *Fagopyrum esculentum* exhibited a comparably higher scavenging capacities (208.91 mg TE/100 g to 232.87 mg TE/100 g) than the varieties of *Chenopodium quinoa* (128.22 mg TE/100 g and 159.80 mg TE/100 g) and *Amaranthus caudatus* (77.35 mg TE/100 g and 107.72 mg TE/100 g).

Sprouted bio alfalfa recorded the second highest antioxidant activity (157.22 mg TE/100 g) after mungo among the legumes and among the cereals barley ´Amistar´ recorded the highest scavenging capacity of 86.11 mg TE/100 g.

With the exception of fresh germs and mixed sprouts of sample 27 and sample 28 which exhibited a moderate antioxidant activity of 188.03 mg TE/100 g and 113.94 mg TE/100 g, the remaining freshly bought germs and sprouts of sample 23, 24, 25 and 26 measured a higher scavenging capacity of the DPPH radicals values: 217.36 mg TE/100 g, 234.62 mg TE/100 g, 234.79 mg TE/100 g and 233.40 mg TE/100 g respectively.

Among the quadruplicate analyses performed for each of the sprouted sample, there was no significant ($p \leq 0.05$) difference in the scavenging capacities (Table A5).

5.4.3. Changes in antioxidant activity of samples after sprouting

Changes in antioxidant activity of samples after sprouting was dependent on species and varieties (Table 6). With the exception of extracts of wheat ´Astrid´ and barley ´Casino´ which recorded a reduction in their scavenging capacities of the DPPH radicals (-279.7% and -22.5% respectively), the remaining sprouted samples recorded an increase in their antioxidant capacities compared to their non-sprouted counterparts (Table 6).

The highest increase in antioxidant activity were recorded by bio quinoa black (90.7%), radish (89.72%), broccoli (88.5%), red lens (82.9%) and bio alfalfa (81.4%).

Buckwheat groat which recorded the highest antioxidant activity among the non-sprouted samples however measured a marginal increase of 0.06% in its scavenging capacity of the DPPH radical after sprouting. Among the cereals, only barley ´Amistar´ recorded an increase in antioxidant activity after sprouting. Overall, sprouting increased the free radical scavenging capacities of the samples.

Table 6. Free radical scavenging capacity (antioxidant activity) of extracts of sprouted and non-sprouted samples against DPPH

Sample no.	Species name	mg TE/100 g* in d.m.	Change in antioxidant activity (%)
	PSEUDOCEREALS		
1	*Chenopodium quinoa*	87.31±78.44^{abc}	+90.7
2	*Chenopodium quinoa*	89.07±44.28^{abcd}	+61.1
3	*Amaranthus caudatus*	65.12±46.39^{ab}	+79.1
4	*Amaranthus caudatus*	60.28±18.54^{ab}	+44.1
5	*Fagopyrum esculentum*	152.57±85.97^{cd}	+69.0
6	*Fagopyrum esculentum*	150.77±62.37^{cd}	+55.7
7	*Fagopyrum esculentum*	225.53±7.11^{e}	+0.06
	LEGUMES		
8	*Medicago sativa*	93.25±68.72^{abcd}	+81.4
9	*Glycine max*	62.79±33.61^{ab}	+51.3
10	*Cicer arietinum*	43.77±25.06^{a}	+67.8
11	*Vigna radiata* syn. *Phaseolus aureus*	161.06±77.45^{de}	+62.0
12	*Lens culinaris*	53.30±40.86^{a}	+82.9
13	*Lens culinaris*	87.55±50.95^{abc}	+70.2
14	*Lens culinaris*	55.85±36.97^{ab}	+76.0
15	*Trigonella foenum graceum*	43.65±19.09^{a}	+77.4
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	31.09±17.56^{a}	+64.8

Table 6. Continued

Sample no.	Species name	mg TE/100 g* in d.m.	Change in antioxidant activity (%)
	CEREALS		
17	*Hordeum vulgare*	56.91±61.52^{ab}	+67.8
18	*Hordeum vulgare*	38.52±10.99^{a}	-22.5
19	*Triticum aestivum*	40.54±69.57^{a}	-279.7
	CRUCIFEROUS VEGETABLES		
20	*Eruca sativa*	148.22±80.02^{cd}	+66.9
21	*Brassica oleraceae* var. *Italica*	127.60±108.27^{bcd}	+88.5
22	*Raphanus sativus*	126.41±110.17^{bcd}	+89.72
	1	87.08±74.06^{a}	
Repetition	2	96.95±79.47^{a}	
	3	87.78±76.82^{a}	
	4	92.04±74.87^{a}	
Stage	NS	49.04±48.66^{a}	
	S	132.89±75.02^{b}	

In the table are presented average value±S.D.
*In the same column, values followed by different superscripts (a-e) denote statistically significant differences at $p \leq 0.05$.
(+) indicate an increase in antioxidant activity after sprouting
(-) indicate a decrease in antioxidant activity after sprouting
NS – non-sprouted samples, S – sprouted samples
d.m. – dry matter

6. Discussion

This study provides a comprehensive overview of the effects of sprouting on selected nutritional and bioactive components of an appreciably wide range of different plant species (edible seeds and grains) as compared to several studies. Different species and varieties yielded different responses to the sprouting treatment.

6.1. Crude protein and protein fractions content of analysed samples

6.1.1. Crude protein content of analysed samples

High contents of crude proteins were recorded in legumes of the non-sprouted samples, followed by cruciferous vegetables and pseudocereals. Cereals recorded the lowest crude protein content among the non-sprouted samples. The crude protein content recorded in this research are in agreement with reports from Shewry et al. (1995) who stated that the amount of protein present in edible seeds ranges from approximately 10% in cereals to approximately 40% in certain legumes of the dry weight, forming a major source of dietary protein.

Pseudocereals like amaranth, buckwheat and quinoa are noted to be high quality grain crops with their protein content usually higher than most cereals, but lower than legumes protein content (Abugoch et al. 2008; Koziol 1992). This has been confirmed in this study by the values recorded for pseudocereals (12.61% to 16.99% of dry matter) and cereals (11.98% to 13.02% of dry matter). Valcárcel-Yamani & Caetano da Silva Lannes (2012) also reported that the protein content for amaranth and quinoa are 14.0-16.5% and 12.9-16.5 % respectively.

Non-sprouted legumes and cruciferous vegetables recorded a comparably higher total protein contents (of 21.58% to 39.85% and 21.55% to 35.37% of dry matter respectively) than the non-sprouted cereals and pseudocereals. Values obtained in this study for the protein content of non-sprouted buckwheat and wheat ´Astrid´ are comparable to those reported in the investigation carried out by Donkor et al. (2012). Similar to results from the research of Donkor et al. (2012), cereals and pseudocereals recorded low total protein contents regardless of their stage (non-sprouted or sprouted form).

Sprouting effected an increase in the total protein contents of the sampled legumes (21.61% to 42.71% d.m.) and cruciferous vegetables (22.08% to 36.13% d.m.). Additionally, sprouts of radish, mix bio and bio alfalfa respectively recorded the highest increase in crude protein content of 5.86%, 4.07% and 2.86% followed by buckwheat and mungo. In this study, soya bean at the sprouted stage recorded an increase in the total protein content which is in agreement with findings by Fordham et al. (1975) and Everson et al. (1945). This study also recorded an increase in the protein content of mung bean after sprouting. However, Ebert et al. (2017) reported that sprouts of mung bean and soybean recorded lower values of proteins as compared to the mature dry seeds of mung bean and soybean. The differences in the results reported could result from multiple factors such as the methodology (sprouting conditions, procedure of extraction), varieties of plant species used (Pajak et al. 2014) as well as the country of origin and producer of seeds.

6.1.2. Protein fractions content of analysed samples

Generally, sprouted cereals had reduced quantities of gliadins, albumin-globulins as compared to their non-sprouted forms but increased in the glutelin fraction content. The breakdown of compounds and synthesis of new ones during sprouting may have contributed to these variations- undesirable ones are reduced or eliminated and desired ones are synthesised in the course of sprouting. A reduction in the gliadin content of sprouted cereals (especially, wheat) infers that it can be applied in the diet of individuals with the celiac disease. A disease caused by a reaction to gliadin found in wheat and other similar proteins found in cereal grains (Mir et al. 2018). Change in nutrients occurring during germination rest on the type of grain as well as the sprouting conditions (Frias et al. 1995; Sierra & Vidal-Valverde 1999).

Not only does sprouting improve the nutritional content but also, the nutritional quality of seeds like beans have frequently been improved with sprouting (Hamad & Fields 1979).

6.2. Flavonoid content of sample extracts

The presence and amounts of the eight types of flavonoids identified revealed that the three varieties of *Fagopyrum esculentum* and bio alfalfa possessed the maximum types of flavonoids (6 to 7) among the non-sprouted samples whiles amongst the sprouted samples, the three varieties of *Fagopyrum esculentum* detected the maximum of 7 types of flavonoids. In a study on buckwheat by Zielinska et al. (2007), rutin was found to be the only flavonoid in the unsprouted grain. Sprouting of these buckwheat grains resulted in the presence of high levels of isoorientin, vitexin and rutin. Similarly, Watanabe et al. (1997) also identified rutin, quercetin and vitexin in buckwheat hulls. *Fagopyrum esculentum* is considered health food, fairly due to their flavonoid content (Nemzer 2019). These reports are in agreement to the findings of this study. Similarly, the high amounts

In this study, rutin was the main flavonoid detected in the varieties of amaranth and quinoa in both sprouted and non-sprouted forms. Similarly, Pasko et al. (2008) identified rutin as the main flavonoid present in sprouts of quinoa and amaranth seeds.

Kaempferol was detected in only bio alfalfa among the non-sprouted samples. However, broccoli fresh germs of sample 24 recorded the maximum value of kaempferol among the sprouted samples. According to Shields (2017), kaempferol is a natural flavonol found in vegetables and herbs.

Among the mixtures of fresh germs and sprouts, very high amounts of chlorogenic acid was detected in sprouted dehulled sunflower and mixture of raw sprouted seeds of sample 28. Among the 13 plant species (mung bean, alfalfa, fava, fenugreek, mustard, wheat, broccoli, sunflower, soybean, radish, kale, lentil and onion) investigated by Cevallos-Casals & Cisneros-Zevallos (2010) sprouts of sunflower recorded the highest amount of chlorogenic acid which was confirmed in this study. Similarly, Sastry & Rao (1990) reported chlorogenic acid as one of the major flavonoids in sunflower seed.

6.3. Total phenolic content of sample extracts

Overall, there was a significant difference in the total phenolic content recorded between the sprouted and non-sprouted extracts. Rocket, wheat ´Astrid´, mungo and bio amaranth in respective order recorded the highest increase in TPC after sprouting. In a study carried

out on four selected grains (amaranth, quinoa, buckwheat and wheat) by Alvarez-Jubete et al. (2010), buckwheat was found to possess a significantly higher total phenolic content in the seed extracts followed by quinoa, wheat and amaranth. Sprouting, however increased the TPC of the samples. These findings are comparable to records from this study where buckwheat was found to be superior in TPC when compared to quinoa, wheat and amaranth both in the sprouted and non-sprouted forms. Similarly, sprouted mung bean, wheat, fenugreek, alfalfa, soybean, broccoli showed high phenolic contents in the investigation carried out by Cevallos-Casals & Cisneros-Zevallos (2010).

According to the report by Donkor et al. (2012), the sprouted forms of the 7 grains investigated on contained substantial amounts of total phenolics as compared with the non- sprouted grains. The effect of sprouting on the total phenolic contents of many edible bean seeds and cereal grains has been investigated and it has been found that mostly, germination accumulates phenolic compounds in sprouted edible seeds as compared with raw seeds (López-Martínez et al. 2017).

6.4. Antioxidant activity of sample extracts

All the samples under investigation in this study exhibited an increase in their antioxidant activity after sprouting with the exception of wheat ´Astrid´ and barley ´Casino´ which recorded a decline in their scavenging capacities. Subsequently, there was a significant difference in the recorded antioxidant activity between sprouted and non-sprouted seed extracts. Wu et al. (2011) and Oracz et al. (2016) reported that in the course of seed sprouting, complex biochemical transformations occur and since sprouting is an aerobic process, it consequently augment the activities of reactive oxygen species (ROS) which leads to changes in the quantities of prevailing antioxidant compounds as well as the formation of new ones. Hence, the records obtained in this study which indicate various increment in antioxidant activity of samples as a result of sprouting are in agreement with these reports. The free-radical scavenging capacities of samples was species and variety dependent.

Generally, moderate to weak scavenging capacities of DPPH radicals were detected among the non-sprouted samples as compared to the sprouted samples. However, non-sprouted buckwheat groat recorded a significantly higher antioxidant activity of 219.04

mg TE/100 g compared to the rest of non-sprouted samples. Buckwheat bio, mungo, rocket, buckwheat and wheat ´Astrid´ followed with values ranging from 92.64 mg TE/100 g to 64.17 mg TE/100 g. The lowest scavenging capacities were recorded by bio quinoa black, red lens and mix bio. It is evident that, varieties of buckwheat recorded a higher antioxidant activity at the non-sprouted stage. These findings are in agreement with studies carried out by Donkor et al. (2012), where buckwheat in the non-sprouted form also showed the highest inhibition against the DPPH free radicals among some selected grains. Similarly, in the investigation carried out by Alvarez-Jubete et al. (2010), buckwheat seed extract was found to possess the highest radical scavenging capacity against the DPPH radical among the four selected grains.

Comparatively, high antioxidant activities were measured among extracts of the sprouted samples. All three sprouted cruciferous vegetables: rocket, broccoli and radish exhibited a strong capacity in inhibiting DPPH radicals (222.68 mg TE/100 g to 229.22 mg TE/100 g). Cruciferous vegetables are noted to possess high antioxidant activity due to the presence of the major bioactive compound glucosinolates (Manchali et al. 2012). However, the highest antioxidant activity were exhibited by: mungo, buckwheat and buckwheat groat.

Among the legumes, sprouted bio alfalfa recorded the second highest antioxidant activity after mungo. Similarly, in a study carried out on 13 selected plant species, mung bean exhibited the highest antioxidant activity followed by bio alfalfa (Cevallos-Casals & Cisneros-Zevallos 2010).

Among the pseudocereals, all three varieties of sprouted *Fagopyrum esculentum* exhibited a comparably higher scavenging capacities than the varieties of *Chenopodium quinoa* and *Amaranthus caudatus*. These findings are in agreement with the studies made by Donkor et al. (2012) and Alvarez-Jubete et al. (2010) where sprouted buckwheat also showed the highest inhibition (against the DPPH radical) among the selected grains. Paśko et al. (2009) also found that pseudocereals (amaranth and quinoa) sprouts showed relatively high antioxidant activity. However, quinoa exhibited higher scavenging capacities than amaranth and these are consistent with records from this study. Paśko et al. (2009) also found quinoa to be a better alternative for traditional cereals than amaranth.

6.4.1. Changes in antioxidant activity of samples after sprouting

Samples which recorded the highest increase in antioxidant activity after sprouting in their respective order were: bio quinoa black, radish, broccoli, red lens and bio alfalfa. Similarly, Pająk et al. (2014) reported that sprouts of mung bean, radish and broccoli had a significant increase in their antioxidant activity as compared to their seeds. Cevallos-Casals & Cisneros-Zevallos (2010) also reported sprouts of radish and broccoli to possess high antioxidant activities.

It is inferred from the consistent values recorded by all varieties of *Fagopyrum esculentum* after sprouting that *Fagopyrum esculentum* is a potential natural rich source of antioxidant compounds. This is in agreement with the report that sprouting has a profound influence on the antioxidant activity of buckwheat (Nemzer & Huang 2019).

Overall, sprouting increased the free radical scavenging capacities of samples.

6.5. Nutraceutical benefits of sprouted seeds of selected plant species

Plants are used traditionally not only for nutrition, but also for therapy of diseases, since they contain pharmacological active substances (Herr & Büchler 2010). According to this study, bio quinoa black, radish, broccoli, red lens and bio alfalfa have shown to be substantially effected by the sprouting treatment due to the significant increase in their antioxidant activity. The varieties of buckwheat and most legumes investigated on also recorded high amounts of antioxidant activity after sprouting. As a result of their beneficial effects contributing to human health, antioxidants have received much attention in modern society (Sanna et al. 2011). It is well established that these antioxidant compounds can prevent cardiovascular and neurodegenerative diseases by scavenging free radicals, mainly reactive oxygen species, produced during cell metabolism (Zafra-Stone et al. 2007). Hence, these edible seeds which recorded substantial amounts of antioxidant activity can be consumed as functional foods to meet the demands of humanity to fight against these non-communicable diseases. Antioxidants, are therefore important in the overall nutritional profiles of sprouted seeds (Nemzer & Huang 2019).

The group of cruciferous vegetables (rocket, broccoli and radish) investigated on in this study were substantially influenced by the sprouting process. This was realised in the high

antioxidant activity recorded after sprouting. Epidemiological studies have recommended the intake of cruciferous vegetables such as broccoli which lowers risks for the induction of certain forms of cancer (Latté et al. 2011). This family of cruciferous vegetables have attracted a great deal of attention in modern times due to the rich source of glucosinolates whose degradation products, the isothiocyanates may possess cancer preventive and therapeutic activity (Manchali et al. 2012; Herr & Büchler 2010; Cohen et al. 2010).

High amounts and types of flavonoids detected in sprouted buckwheat in this study, suggest that this crop can be consumed as a nutraceutical food. The intake of flavonols is found to be associated with a wide range of health benefits which includes antioxidant potential and reduced risk of vascular diseases (Panche et al. 2016). Sprouted quinoa, amaranth, legumes and cruciferous vegetables which also recorded increases in their flavonoid content in this study could be consumed as nutraceutical foods. The cardio-protective properties of flavonoids is due to their ability to control oxidative stress and act as anti-inflammatory agents (Kaleem & Ahmad 2018). Flavonoids have been identified to be suitable candidates for promotion of brain health as a result of their multitarget nature and relative safety in the central nervous system (Akinmoladun et al. 2018). Consequently, consuming sprouted grains and seeds will serve a lot of health benefits. Also, the total amount of grains that are consumed worldwide as staple food far exceeds that of fruits and vegetables hence the intake of antioxidants as part of grain products (example, sprouted buckwheat foods) can ensure overall health benefits. Sprouted seeds may therefore have better health promotion properties in terms of dietary antioxidant sources, compared to their respective non-sprouted seeds (Nemzer & Huang 2019).

7. Conclusion

This study provides a comprehensive overview of the effects of sprouting on edible seeds and grains of an appreciable range of different plant species including some varieties. Based on the results of this study, it can be concluded that sprouting effected a change in the selected nutritional and bioactive components of the sprouted samples (germinated seeds and grains) as compared to the non-sprouted (dry seeds) forms. Overall, this study has shown that sprouting increases the nutritional and bioactive components of edible seeds and grains. Consequently, changes recorded were species and variety dependent.

The crude protein content generally increased in sprouted legumes and cruciferous vegetables as compared to cereals and pseudocereals which recorded low protein contents regardless of their stage. Decrease in gliadin contents of all cereal after sprouting and increase in albumin-globulin content of most legumes was also recorded. TPC generally increased in samples with sprouting treatment. Furthermore, sprouting effected a substantial increase in the types and amounts of flavonoids detected in most samples. With the exception of two samples, all samples exhibited an increase in their antioxidant activity after sprouting.

Plant species such as buckwheat, amaranth, quinoa, mungo, bio alfalfa and the cruciferous vegetables have been identified to be potential rich sources of natural bioactive components that increases with sprouting. Buckwheat has proven to be a rich source of flavonoid and antioxidant activities based on the findings of this study. Sprouting had a great impact on cruciferous vegetables by causing a substantial increase in their antioxidant activity. Hence, sprouting can be used as an effective and natural technique to enhance the nutritional value and bioactive components of edible seeds and grains which will alleviate the nutritional deficiencies posed to the world and serve as nutraceutical foods.

Further research is recommended to be carried out on the potential active components (compounds) of buckwheat and mungo responsible for their antioxidant activities.

8. References

Abugoch LE, Romero N, Tapia CA, Silva J, Rivera M. 2008. Study of some physicochemical and functional properties of quinoa (*Chenopodium quinoa* Willd) protein isolates. Journal of Agricultural and Food Chemistry **56**: 4745–4750.

Aguilera Y, Liébana R, Herrera T, Rebollo-Hernanz M, Sanchez-Puelles C, Benítez V, Martín-Cabrejas MA. 2013. Effect of illumination on the content of melatonin, phenolic compounds and antioxidant activity during germination of lentils (*Lens culinaris* L.) and Kidney Beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry **62**: 10736–10743.

Akinmoladun AC, Farombi TH, Farombi EO. 2019. Food for brain health: flavonoids. 370–386 in Melton L, Shahidi F, Varelis P, editors. Encyclopedia of Food Chemistry. Elsevier, Amsterdam.

Alvarez-Jubete L, Wijngaard H, Arendt EK, Gallagher E. 2010. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry **119**: 770–778.

Baenas N, Gómez-Jodar I, Moreno DA, García-Viguera C, Periago PM. 2017. Broccoli and radish sprouts are safe and rich in bioactive phytochemicals. Postharvest Biology and Technology **127**: 60–67.

Balasaraswathi R, Sadasivam S. 1997. Changes in oil, sugars and nitrogenous components during germination of sunflower seeds, *Helianthus annus*. Plant Foods for Human Nutrition **51**: 71–77.

Bellostas N, Kachlicki P, Sørensen JC, Sørensen H. 2007. Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food. Scientia Horticulturae **114**: 234–242.

Bewley JD. 1997. Seed germination and dormancy. The Plant Cell **9**:1055–1066.

Blazek J, Salman H, Rubio AL, Gilbert E, Hanley T, Copeland L. 2009. Structural characterization of wheat starch granules differing in amylose content and functional characteristics. Carbohydrate Polymers **75**: 705–711.

Butt MS, Tahir-Nadeem M, Khan MKI, Shabir R, Butt MS. 2008. Oat: unique among the cereals. European Journal of Nutrition **47**: 68–79.

Cevallos-Casals B.A, Cisneros-Zevallos L. 2010. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chemistry **119**: 1485–1490.

Chavan JK, Kadam SS, Beuchat LR. 1989. Nutritional improvement of cereals by sprouting. Critical Reviews in Food Science and Nutrition **28**: 401–437.

Chen LH, Wells CE, Fordham JR. 1975. Germinated seeds for human consumption. Journal of Food Science **40**: 1290–1294.

Cohen JH, Kristal AR, Stanford JL. 2000. Fruit and vegetable intakes and prostate cancer risk. Journal of the National Cancer Institute **92**: 61–68.

Croy RRD, Lycett GW, Gatehouse JA, Yarwood JN, Boulter D. 1982. Cloning and analysis of cDNAs encoding plant storage protein precursors. Nature, International Journal of Science **295**: 76–79.

Deshpande SS, Sathe SK, Salunkhe DK, Rackis JJ. 1984. Dry beans of Phaseolus: A review. Part 3. C R C Critical Reviews in Food Science and Nutrition **21**: 137–195.

Ding J, Feng H. 2019. Controlled germination for enhancing the nutritional value of sprouted grains. 91–112 in Feng H, Nemzer B, DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Donkor ON, Stojanovska L, Ginn P, Ashton J, Vasiljevic T. 2012. Germinated grains—Sources of bioactive compounds. Food Chemistry **135**: 950–959.

Dueñas M, Sarmento T, Aguilera Y, Benitez V, Mollá E, Esteban RM, Martín-Cabrejas MA. 2016. Impact of cooking and germination on phenolic composition and dietary fibre fractions in dark beans (*Phaseolus vulgaris* L.) and lentils (*Lens culinaris* L.). LWT - Food Science and Technology **66**: 72–78.

Dueñas M, Hernández T, Estrella I, Fernández D. 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chemistry **117**: 599–607.

Džunková M, Janovská D, Hlásná Čepková P, Prohasková A, Kolář M. 2011. Glutelin protein fraction as a tool for clear identification of Amaranth accessions. Journal of Cereal Science **53**: 198–205.

Ebert AW, Chang CH, Yan MR, Yang RY. 2017. Nutritional composition of mung bean and soybean sprouts compared to their adult growth stage. Food Chemistry **237**: 15–22.

Ebert A. 2015. High value specialty vegetable produce. Handbook of vegetables **2**: 119–143.

Everson GJ, Steenbock H, Cederquist DC, Parsons HT. 1944. The effect of germination, the stage of maturity and variety upon the nutritive value of soybean proteins. The Journal of Nutrition **27**: 225–229.

FAO. 2018. The state of food security in the world. FAO, Rome. http://www.fao.org/state-of-food-security-nutrition/en/

Finney PL. 1983. Effect of Germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. Mobilization of reserves in germination 229–305.

Finnie S, Brovelli V, Nelson D. 2019. Sprouted grains as a food ingredient. 113–142 in Feng H, Nemzer B, DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Fordham JR, Wells CE, Chen LH. 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. Journal of food science Volume **40**: 552–556.

Frias J, Diaz-Pollan C, Hedley CL, Vidal-Valverde C. 1995. Evolution of Trypsin Inhibitor Activity during Germination of Lentils. Journal of Agricultural and Food Chemistry **43**: 2231–2234.

Gabriel AA, Berja MC, Estrada AMP, Lopez MGAA, Nery JGB, Villaflor EJB. 2007. Microbiology of retail mung bean sprouts vended in public markets of National Capital Region, Philippines. Food Control **18**: 1307–1313.

Gan RY, Chan CL, Yang QQ, Li HB, Zhang D, Ge YY, Corke H. 2019. Bioactive compounds and beneficial functions of sprouted grains. 191–246 in Feng H, Nemzer B,

DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Gan RY, Lui WY, Wu K, Chan C L, Dai SH, Sui ZQ. 2017. Bioactive compounds and bioactivities of germinated edible seeds and sprouts: an updated review. Trends in Food Science & Technology **59**: 1–14.

Ghani M, Kulkarni KP, Song JT, Shannon JG, Lee JD. 2016. Soybean sprouts: A review of nutrient composition, health benefits and genetic variation. Plant Breeding and Biotechnology **4**: 398–412.

Guajardo-Flores D, Serna-Saldívar SO, Gutiérrez-Uribe JA. 2013. Evaluation of the antioxidant and antiproliferative activities of extracted saponins and flavonols from germinated black beans (*Phaseolus vulgaris* L.). Food Chemistry **141**: 1497–1503.

Hamad AM, Fields ML. 1979. Evaluation of the protein quality and available lysine of germinated and fermented cereals. Journal of Food Science **44**: 456–459.

Hayat I, Ahmad A, Masud T, Ahmed A, Bashir S. 2014. Nutritional and Health Perspectives of Beans (*Phaseolus vulgaris* L.): An overview. Critical Reviews in Food Science and Nutrition **54**: 580–592.

Herr I, Büchler MW. 2010. Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. Cancer Treatment Reviews **36**: 377–383.

Hesterman OB, Teuber LR. 1979. Alfalfa sprouts: methods of production, current research and economic importance. 24–27. Proceedings Ninth California Alfalfa Symposium, California.

Hornsey IS. 2016. Beer: History and Types. Encyclopaedia of food and health 345–354.

Hornsey IS. 2003. A history of beer and brewing. Cambridge: Royal Society of Chemistry 742.

Ikeda K, Arioka K, Fujii S, Kusano T, Oku M. 1984. Effect of buckwheat protein quality of seed germination and changes in trypsin inhibitor content. Cereal Chemistry **61**: 236.

Jacobs DR, Marquart L, Slavin J, Kushi LH. 1998. Whole-grain intake and cancer: An expanded review and meta-analysis. Nutrition and Cancer **30**: 85–96.

Kaleem M, Ahmad A. 2018. Flavonoids as Nutraceuticals. 137–155 in Grumezescu AM, Holban AM, editors. Therapeutic, probiotic and unconventional foods. Elsevier, Amsterdam.

Kim SJ, Maeda T, Sarker MZI, Takigawa S, Matsuura-Endo C, Yamauchi H, Mukasa Y, Saito K, Hashimoto N, Noda T, Saito T, Suzuki T. 2007. Identification of anthocyanins in the sprouts of buckwheat. Journal of Agricultural and Food Chemistry **55**: 6314–6318.

Kitamura S. 2006. Transport of flavonoids: from cytosolic synthesis to vacuolar accumulation. In Grotewold E, editor. The Science of Flavonoids. Springer, New York, NY.

Klose C, Arendt EK. 2012. Proteins in oats; their synthesis and changes during germination: A review. Critical Reviews in Food Science and Nutrition **52**: 629–639.

Kovacs J. (1996) Composition of dehydrated powdered mung bean sprout and plant fiber for use as dietary supplement in healthcare. U.S.A.: Washington, DC: The United States Patent and Trademark Office. Patent No. 5,487,894.

Koziol MJ. 1992. Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa*). Journal of Food Composition and Analysis **5**: 35–68.

Kuo YH, Rozan P, Lambein F, Frias J, Vidal-Valverde C. 2004. Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. Food Chemistry **86**: 537–545.

Kurtzweil P. 1999. Questions keep sprouting about sprouts. Food and Drugs Administration Consumer Magazine **33**: 18–22.

Kyriacou MC, Rouphael Y, Di Gioia F, Kyratzis A, Serio F, Renna M, De Pascale S, Santamaria P. 2016. Micro-scale vegetable production and the rise of microgreens. Trends in Food Science Technology **57**: 103–115.

Laila O, Murtaza I. 2014. Seed sprouting: A way to health promoting treasure. International Journal of Current Research and Review **6**: 70–74.

Larimore B B. 1975. Sprouting For All Seasons: How and what to sprout, including delicious, easy-to-prepare recipes. Horizon Publishers, Bountiful, UT.

Latté KP, Appel K-E, Lampen A. 2011. Health benefits and possible risks of broccoli – An overview. Food and Chemical Toxicology **49**: 3287–3309.

Lemar LE, Swanson BG. 1976. Nutritive value of sprouted wheat flour. Journal of Food Science **41**: 719-720.

Lemmens E, Moroni AV, Pagand J, Heirbaut P, Ritala A, Karlen Y, Le KA, Van den Broeck HC, Brouns FJPH, De Brier N, Delcour JA. 2019. Impact of cereal seed sprouting on its nutritional and technological properties: A critical review. Comprehensive Reviews in Food Science and Food Safety **18**: 305–328.

Lipton WJ, Asai WK, Fouse DC. 1981. Deterioration and CO_2 and ethylene production of stored mung bean sprouts. Journal of the American Society for Horticultural. Science. **106**: 817–820.

Liu RH. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of Nutrition **134**: 3479–3485.

López-Martínez LX, Leyva-López N, Gutiérrez-Grijalva EP, Heredia JB. 2017. Effect of cooking and germination on bioactive compounds in pulses and their health benefits. Journal of Functional Foods **38**: 624–634.

Lorenz K, D'Appolonia B. 1980. Cereal sprouts: Composition, nutritive value, food applications. C R C Critical Reviews in Food Science and Nutrition **13**: 353–385.

Manchali S, Chidambara Murthy KN, Patil BS. 2012. Crucial facts about health benefits of popular cruciferous vegetables. Journal of Functional Foods **4**:94–106.

Marti A, Cardone G, Pagani MA, Casiraghi MC. 2018. Flour from sprouted wheat as a new ingredient in bread-making. LWT-Food Science and Technology **89**: 237–243.

Marton M, Mandoki Z, Csapo-Kiss Z, Csapo J. 2010. The role of sprouts in human nutrition. Acta Univsitatis Sapientiae **3**: 81–117.

Mayer AM, Poljakoff-Mayber A. 1982. The germination of seeds. New York: Robert Maxwell, M.C. 22–39.

Miller BF. 1978. Effects of sprouting on nutritional value of wheat. 144 in Agricultural Reviews and Manual Western series. Proceedings of the 10th National Conference on Wheat Utilization Research, Tucson. US Department of Agriculture, California.

Mlakar SG, Turinek M, Jakop M, Bavec M, Bavec F. 2009. Nutrition value and use of grain amaranth: potential future application in bread making. Agricultura **6**: 43–53.

Morabito S. 2015. Developments in improving the safety of sprouts. Advances in Microbial Food Safety **2**: 351–378.

Mridula D, Sharma M. 2015. Development of non-dairy probiotic drink utilizing sprouted cereals, legume and soymilk. LWT - Food Science and Technology **62**: 482–487.

Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey ADB, Bloom AJ, … Usui Y. 2014. Increasing CO_2 threatens human nutrition. Nature, International Journal of Science **510**: 139–142.

Nair RM, Yang R-Y, Easdown WJ, Thavarajah D, Thavarajah P, Hughes Jd'A, Keatinge JD. 2013. Biofortification of mung bean (*Vigna radiata*) as a whole food to enhance human health. Journal of the Science of Food and Agriculture **93**: 1805–1813.

Nemzer B, Lin Y, Huang D. 2019. Antioxidants in sprouts of grains. 55–68 in Feng H, Nemzer B, DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Nonogaki H, Bassel GW, Bewley JD. 2010. Germination—Still a mystery. Plant Science **179**: 574–581.

Oracz K, Karpiński S. 2016. Phytohormones signalling pathways and ROS involvement in seed germination. Frontiers in Plant Science 7.

Osborne TB, Mendel LB. 1914. Nutritive properties of proteins of the maize kernel. The Journal of Biological Chemistry **18**: 1–16.

Pagand J, Heirbaut P, Pierre A, Pareyt B. 2017. The magic and challenges of sprouted grains. Cereal Foods World **62**: 221–226.

Pająk P, Socha R, Gałkowska D, Rożnowski J, Fortuna T. 2014. Phenolic profile and antioxidant activity in selected seeds and sprouts. Food Chemistry **143**: 300–306.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: an overview. Journal of Nutritional Science **5**: 1–15.

Paśko P, Bartoń H, Zagrodzki P, Gorinstein S, Fołta M, Zachwieja Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chemistry **115**: 994–998.

Paśko P, Sajewicz M, Gorinstein S, Zachwieja Z. 2008. Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. Acta Chromatographica **20**: 661–672.

Peñas E, Gómez R, Frías J, Vidal-Valverde C. 2010. Effects of combined treatments of high pressure, temperature and antimicrobial products on germination of mung bean seeds and microbial quality of sprouts. Food Control **21**: 82–88.

Peñas E, Gómez R, Frías J, Vidal-Valverde C. 2009. Efficacy of combinations of high pressure treatment, temperature and antimicrobial compounds to improve the microbiological quality of alfalfa seeds for sprout production. Food Control **20**: 31–39.

Peñas E, Gómez R, Frías J, Vidal-Valverde C. 2008. Application of high-pressure treatment on alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) seeds to enhance the microbiological safety of their sprouts. Food Control **19**: 698–705.

Peterson J, Dwyer J. 1998. Flavonoids: Dietary occurrence and biochemical activity. Nutrition Research **18**:1995–2018.

Price TV. 1988. Seed sprout production for human consumption- A Review. Canadian Institute of Food Science and Technology Journal **21**: 57–65.

Qian J, Kuhn M. 1999. Characterization of *Amaranthus cruentus* and *Chenopodium quinoa* Starch. Starch/Starke **51**: 116–120.

Robertson LJ, Johannessen GS, Gjerde BK, Loncarevic S. 2002. Microbiological analysis of seed sprouts in Norway. International Journal of Food Microbiology **75**: 119–126.

Sadeghi AR, Pourahmad R, Mokhtare M. 2017. Enrichment of probiotic yogurt with broccoli sprout extract and its effect on *Helicobacter pylori*. Applied Food Biotechnology **4**:55–59.

Šamec D, Salopek-Sondi B. 2019. Cruciferous (Brassicaceae) Vegetables. 195–202 in Nabavi SM, Silva AS, editors. Nonvitamin and Nonmineral Nutritional Supplements. Elsevier, Amsterdam.

Sanna D, Delogu G, Mulas M, Schirra M, Fadda A. 2011. Determination of free radical scavenging activity of plant extracts through DPPH assay: An EPR and UV–Vis study. Food Analytical Methods **5**: 759–766.

Sastry MCS, Rao MSN. 1990. Binding of chlorogenic acid by the isolated polyphenol-free 11 S protein of sunflower seed (*Helianthus annus*). Journal of Agricultural and Food Chemistry **38**: 2103–2110.

Schendel RR. 2019. Phenol content in sprouted grains. 247–315 in Feng H, Nemzer B, DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Sharma RR, Demirci A, Beuchat LR, Fett WF. 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. Journal of Food Protection **65**: 447–451.

Shewry PR, Napier JA, Tatham AS.1995. Seed storage proteins: structures and biosynthesis. The Plant Cell **7**: 945–956.

Shields M. 2017. Chemotherapeutics. Pharmacognosy 295–313.

Sierra I, Vidal-Valverde C. 1999. Kinetics of free and glycosylated B6 vitamers, thiamin and riboflavin during germination of pea seeds. Journal of the Science of Food and Agriculture **79**: 307–310.

Smith AH. 1918. Beer and scurvy. Some notes from history. The Lancet **2**:813–815.

Świeca M, Gawlik-Dziki U. 2015. Effects of sprouting and postharvest storage under cool temperature conditions on starch content and antioxidant capacity of green pea, lentil and young mung bean sprouts. Food Chemistry **185**: 99–105.

Taormina PJ, Beuchat LR, Slutsker L. 1999. Infections associated with eating seed sprouts: An International Concern. Emerging Infectious Diseases **5**: 626–634.

Urbano G, López -Jurado M, Hernandez J, Fernandez M, Moreu MC, Frias J, Vidal-Valverde C. 1995. Nutritional assessment of raw, heated, and germinated lentils. Journal of Agricultural and Food Chemistry **43**: 1871–1877.

Urbano LG, Aranda P, Vilchez A, Aranda C, Cabrera L, Porres J M, López -Jurado M. 2005. Effects of germination on the composition and nutritive value of proteins in *Pisum sativum*. Food Chemistry **93**: 671–679.

Valcárcel-Yamani B, Caetano da Silva Lannes S. 2012. Applications of quinoa (*Chenopodium quinoa* Willd.) and Amaranth (*Amaranthus* spp.) and their influence in the nutritional value of cereal based food. Food and Public Health **2**: 265–275.

Vance CP, Graham PH, Allan DL. 2000. Biological Nitrogen Fixation: Phosphorus - A Critical Future Need? 509–514 in: Pedrosa FO, Hungria M, Yates G, Newton WE, editors. Nitrogen fixation: From molecules to crop productivity. Current Plant Science and Biotechnology in Agriculture. Springer, Dordrecht.

Vidal-Valverde C, Frias J. 1992. Changes in carbohydrates during germination of lentils. Zeitschrift für Lebensmittel-Untersuchung und-Forschung **194**: 461–464.

Vidal-Valverde C, Frias J, Sierra I, Blazquez I, Lambein F, Kuo YH. 2002. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. European Food Research and Technology **215**: 472–477.

Wang K-H, Lai Y-H, Chang J-C, Ko T-F, Shyu S-L, Chiou RY-Y. 2005. Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. Journal of Agricultural and Food Chemistry **53**: 242–246.

Watanabe M, Ohshita Y, Tsushida T. 1997. Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Möench) hulls. Journal of Agricultural and Food Chemistry **45**: 1039–1044.

Weiss WP, Hammes. 2003. Thermal seed treatment to improve the food safety status of sprouts. Journal of Applied Botany **77**: 152–155.

Weitbrecht K, Müller K, Leubner-Metzger G. 2011. First off the mark: early seed germination. Journal of Experimental Botany **62**: 3289–3309.

Whyte KC. 1973. The complete sprouting cookbook. Troubador Press, San Francisco.

Wiltshire HW. 1918. A note on the value of germinated beans in the treatment of scurvy. The Lancet **2**:811–13.

Wu F, Yang N, Toure´ A, Jin Z, Xu X. 2013. Germinated brown rice and its role in human health. Critical Reviews in Food Science and Nutrition **53**: 451–463.

Wu Z, Song L, Huang D. 2011. Food grade fungal stress on germinating peanut seeds induced phytoalexins and enhanced polyphenolic antioxidants. Journal of Agricultural and Food Chemistry **59**: 5993–6003.

Wu F, Xu X. 2019. Sprouted grains-based fermented products. 143–173 in Feng H, Nemzer B, DeVries JW, editors. Sprouted grains nutritional value, production and applications. Elsevier, Amsterdam.

Yang Y, Meier F, Ann Lo J, Yuan W, Lee Pei Sze V, Chung H-J, Yuk H-G. 2013. Overview of recent events in the microbiological safety of sprouts and new intervention technologies. Comprehensive Reviews in Food Science and Food Safety **12**: 265–280.

Yang F, Basu TK, Ooraikul B. 2001. Studies on germination conditions and antioxidant contents of wheat grain. International Journal of Food Sciences and Nutrition **52**: 319–330.

Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D. 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. Molecular Nutrition & Food Research **51**: 675–683.

Zielinska D, Szawara-Nowak D, Ornatowska A, Wiczkowski W. 2007. Use of cyclic voltammetry, photochemiluminescence, and spectrophotometric methods for the measurement of the antioxidant capacity of buckwheat sprouts. Journal of Agricultural and Food Chemistry **55**: 9891–989

9. Appendix

Table A1. Protein analysis of non-sprouted samples

Table A2. Protein analysis of sprouted samples

Table A3. Changes in total protein and protein fractions content between sprouted and non-sprouted samples

Table A4. Radical scavenging capacity (antioxidant activity) of extracts of non-sprouted samples against DPPH

Table A5. Radical scavenging capacity (antioxidant activity) of extracts of sprouted samples against DPPH

Table A1. Protein analysis of non-sprouted samples

Sample no.	Species name	Total protein content* (%) in d.m.	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	PSEUDOCEREALS				
1	*Chenopodium quinoa*	15.42±0.05^{ab}	6.41±1.47^{abc}	0.24±0.17^{a}	8.76±1.35^{ab}
2	*Chenopodium quinoa*	16.66±0.68^{ab}	7.54±0.25^{abcd}	0.14±0.12^{a}	8.99±0.31^{ab}
3	*Amaranthus caudatus*	16.99±1.76^{ab}	5.19±0.85^{ab}	0.38±0.34^{a}	11.42±2.27^{abc}
4	*Amaranthus caudatus*	16.04±0.31^{ab}	5.93±0.32^{abc}	0.42±0.09^{ab}	9.69±0.10^{ab}
5	*Fagopyrum esculentum*	12.61±0.32^{a}	4.13±1.31^{a}	0.30±0.22^{a}	8.18±0.77^{ab}
6	*Fagopyrum esculentum*	16.07±0.06^{ab}	7.63±0.14^{abcd}	0.36±0.06^{a}	8.09±0.14^{ab}
7	*Fagopyrum esculentum*	15.11±0.23^{a}	5.77±1.35^{ab}	0.48±0.00^{ab}	8.86±1.12^{ab}
	LEGUMES				
8	*Medicago sativa*	39.85±0.80^{e}	6.16±0.39^{abc}	0.99±1.28^{abc}	32.71±0.87^{d}
9	*Glycine max*	38.99±0.53^{e}	18.78±2.31^{f}	0.43±0.26^{ab}	19.78±2.59^{bc}
10	*Cicer arietinum*	21.58±0.34^{bc}	14.62±1.69^{def}	0.09±0.04^{a}	6.87±1.39^{a}
11	*Vigna radiata* syn. *Phaseolus aureus*	24.77±0.27^{cd}	18.31±0.73^{f}	0.07±0.07^{a}	6.39±0.54^{a}
12	*Lens culinaris*	27.95±0.01^{cd}	16.93±1.13^{ef}	0.39±0.09^{a}	10.63±1.23^{abc}
13	*Lens culinaris*	27.02±0.39^{cd}	15.73±0.32^{ef}	0.42±0.12^{ab}	10.88±0.05^{abc}
14	*Lens culinaris*	28.03±0.28^{d}	16.24±0.82^{ef}	0.47±0.11^{ab}	11.32±0.65^{abc}
15	*Trigonella foenum graceum*	28.84±1.13^{d}	6.32±2.36^{abc}	1.03±0.08^{abc}	21.48±1.16^{cd}

Table A1. Continued

Sample no.	Species name	Total protein content* (%) in d.m.	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	LEGUMES				
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	27.74±0.17^{cd}	12.65±4.66^{bcdef}	0.35±0.00^{a}	14.74±4.50^{abc}
	CEREALS				
17	*Hordeum vulgare*	13.02±0.30^{a}	2.92±0.13^{a}	2.98±0.19^{de}	7.12±0.62^{a}
18	*Hordeum vulgare*	11.98±0.39^{a}	2.56±0.11^{a}	3.14±0.12^{ef}	6.28±0.40^{a}
19	*Triticum aestivum*	12.06±0.08^{a}	3.17±0.20^{a}	4.50±0.01^{f}	4.39±0.27^{a}
	CRUCIFEROUS VEGETABLES				
20	*Eruca sativa*	35.37±0.06^{e}	13.65±2.11^{cdef}	2.15±0.30^{cde}	19.58±2.35^{bc}
21	*Brassica oleraceae* var. *Italica*	21.55±0.02^{bc}	9.62±2.48^{abcde}	1.54±0.17^{abcd}	10.38±2.66^{abc}
22	*Raphanus sativus*	26.12±6.74^{cd}	14.59±5.66^{def}	1.89±0.89^{bcde}	9.65±11.51^{ab}
Repetition	1	22.44±8.68^{a}	10.24±5.84^{a}	1.1±1.16^{a}	11.11±6.98^{a}
Repetition	2	22.45±8.73^{a}	9.29±5.39^{a}	0.97±1.27^{a}	12.18±6.66^{a}

In the table are presented average value±S.D. (Standard Deviation)
*In the same column, values followed by different superscripts (a-f) denote statistically significant differences at $p \leq 0.05$.
d.m. – dry matter

Table A2. Protein analysis of sprouted samples

Sample no.	Species name	Total protein content* (%) in d.m.	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	PSEUDOCEREALS				
1	*Chenopodium quinoa*	15.73 ± 0.06^{d}	5.54 ± 2.51^{abcd}	0.44 ± 0.08^{abc}	9.75 ± 2.53^{abcd}
2	*Chenopodium quinoa*	12.95 ± 0.26^{b}	6.58 ± 0.02^{abcd}	0.15 ± 0.06^{a}	6.23 ± 0.18^{a}
3	*Amaranthus caudatus*	15.67 ± 0.11^{d}	5.98 ± 0.88^{abcd}	0.93 ± 0.11^{abc}	8.76 ± 0.66^{abc}
4	*Amaranthus caudatus*	16.51 ± 0.14^{e}	6.35 ± 0.26^{abcd}	0.44 ± 0.12^{abc}	9.72 ± 0.53^{abcd}
5	*Fagopyrum esculentum*	13.89 ± 0.06^{c}	1.70 ± 0.63^{a}	0.56 ± 0.13^{abc}	11.63 ± 0.57^{abcd}
6	*Fagopyrum esculentum*	13.24 ± 0.06^{bc}	5.99 ± 2.25^{abcd}	0.36 ± 0.00^{ab}	6.90 ± 2.30^{ab}
7	*Fagopyrum esculentum*	15.21 ± 0.21^{d}	6.76 ± 0.18^{abcd}	0.46 ± 0.13^{abc}	7.99 ± 0.26^{abc}
	LEGUMES				
8	*Medicago sativa*	42.71 ± 0.23^{s}	15.80 ± 0.65^{fgh}	1.52 ± 0.48^{cd}	25.38 ± 0.41^{f}
9	*Glycine max*	39.67 ± 0.01^{r}	18.95 ± 0.71^{cgh}	0.84 ± 0.08^{abc}	19.88 ± 0.78^{ef}
10	*Cicer arietinum*	21.61 ± 0.19^{h}	14.73 ± 0.60^{efg}	0.86 ± 0.63^{abc}	6.02 ± 1.41^{a}
11	*Vigna radiata* syn. *Phaseolus aureus*	25.84 ± 0.13^{i}	18.47 ± 0.16^{cgh}	0.21 ± 0.05^{ab}	7.16 ± 0.02^{ab}
12	*Lens culinaris*	28.32 ± 0.11^{lm}	21.73 ± 0.02^{ch}	0.23 ± 0.04^{ab}	6.36 ± 0.05^{ab}
13	*Lens culinaris*	27.34 ± 0.19^{jk}	20.32 ± 0.04^{ch}	0.33 ± 0.01^{ab}	6.69 ± 0.22^{ab}
14	*Lens culinaris*	28.65 ± 0.25^{m}	18.57 ± 0.18^{cgh}	0.61 ± 0.02^{abc}	9.47 ± 0.10^{abc}

Table A2. Continued

Sample no.	Species name	Total protein content* (%) in d.m.	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	LEGUMES				
15	*Trigonella foenum graceum*	29.40±0.02^{n}	6.45±0.50^{abcd}	1.12±0.03^{abc}	21.82±0.49^{ef}
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	31.81±0.12^{o}	18.23±0.20^{cgh}	0.16±0.02^{a}	13.42±0.07^{cd}
	CEREALS				
17	*Hordeum vulgare*	12.84±0.05^{b}	3.47±0.03^{abc}	1.21±0.04^{abcd}	8.17±0.04^{abc}
18	*Hordeum vulgare*	12.79±0.02^{b}	2.19±0.25^{ab}	1.28±0.02^{bcd}	9.31±0.22^{abc}
19	*Triticum aestivum*	11.75±0.03^{a}	2.94±0.27^{abc}	2.29±0.08^{de}	6.52±0.38^{ab}
	CRUCIFEROUS VEGETABLES				
20	*Eruca sativa*	36.13±0.14^{p}	10.92±0.00^{def}	2.94±0.00^{e}	22.27±0.14^{f}
21	*Brassica oleraceae* var. *Italica*	22.08±0.04^{h}	10.37±2.10^{de}	1.06±0.86^{abc}	10.65±2.93^{abcd}
22	*Raphanus sativus*	31.98±0.51^{o}	14.87±3.62^{efg}	1.29±0.56^{bcd}	15.82±4.69^{de}
	FRESH GERMS/ MIXED SPROUTS				
23	*Medicago sativa, Raphanus sativus, Eruca sativa*	44.65±0.07^{t}	10.78±3.71^{def}	0.90±0.07^{abc}	32.98±3.71^{g}
24	*Brassica oleraceae* var. *Italica*	26.93±0.01^{j}	6.07±1.55^{abcd}	0.68±0.02^{abc}	20.18±1.58^{ef}

Table A2. Continued

Sample no.	Species name	Total protein content* (%) in d.m.	Albumin-globulin* (%) in d.m.	Gliadin* (%) in d.m.	Glutelin* (%) in d.m.
	FRESH GERMS/ MIXED SPROUTS				
25	*Cicer arietinum, Lens culinaris, Raphanus sativus, Triticum aestivum, Trigonella foenum graceum*	24.52±0.08^{ch}	17.37±2.44^{cgh}	0.14±0.04^{a}	7.02±2.32^{ab}
26	*Vigna radiata* syn. *Phaseolus aureus*	27.84±0.05^{kl}	7.29±1.52^{bcd}	0.79±0.06^{abc}	19.77±1.51^{ef}
27	*Helianthus annus*	20.57±0.17^{g}	7.88±1.81^{cd}	0.58±0.49^{abc}	12.12±1.15^{abcd}
28	*Fagopyrum esculentum, Helianthus annus, Chenopodium quinoa*	17.39±0.38^{f}	4.03±0.11^{abc}	0.90±0.01^{abc}	12.45±0.49^{bcd}
Repetition	1	23.90±9.58^{a}	10.86±6.37^{b}	0.85±0.70^{a}	12.19±6.74^{a}
Repetition	2	23.82±9.58^{a}	9.88±6.26^{a}	0.81±0.64^{a}	13.12±7.37^{b}

In the table are presented average value±S.D
*In the same column, values followed by different superscripts (a-t) denote statistically significant differences at $p \leq 0.05$.
d.m. – dry matter

Table A3. Changes in total protein and protein fractions content between sprouted and non-sprouted samples

Sample no.	Species name	Total protein content (%)	Albumin-globulin (%)	Gliadin (%)	Glutelin (%)
	PSEUDOCEREALS				
1	*Chenopodium quinoa*	+0.31	-0.87	+0.2	+0.99
2	*Chenopodium quinoa*	-3.71	-0.96	+0.01	-2.76
3	*Amaranthus caudatus*	-1.32	+0.79	+0.55	-2.66
4	*Amaranthus caudatus*	+0.47	+0.42	+0.02	+0.03
5	*Fagopyrum esculentum*	+1.28	-2.43	+0.26	+3.45
6	*Fagopyrum esculentum*	-2.83	-1.64	0	-1.19
7	*Fagopyrum esculentum*	+0.1	+0.99	-0.02	-0.87
	LEGUMES				
8	*Medicago sativa*	+2.86	+9.64	+0.53	-7.33
9	*Glycine max*	+0.68	+0.17	+0.41	+0.1
10	*Cicer arietinum*	+0.03	+0.11	+0.77	-0.85
11	*Vigna radiata* syn. *Phaseolus aureus*	+1.07	+0.16	+0.14	+0.77
12	*Lens culinaris*	+0.37	+4.8	-0.16	-4.27
13	*Lens culinaris*	+0.32	+4.59	-0.09	-4.19
14	*Lens culinaris*	+0.62	+2.33	+0.14	-1.85
15	*Trigonella foenum graceum*	+0.56	+0.13	+0.09	+0.34

Table A3. Continued

Sample no.	Species name	Total protein content (%)	Albumin-globulin (%)	Gliadin (%)	Glutelin (%)
	LEGUMES				
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	+4.07	+5.58	-0.19	-1.32
	CEREALS				
17	*Hordeum vulgare*	-0.18	+0.55	-1.77	+1.05
18	*Hordeum vulgare*	+0.81	-0.37	-1.86	+3.03
19	*Triticum aestivum*	-0.31	-0.23	-2.21	+2.13
	CRUCIFEROUS VEGETABLES				
20	*Eruca sativa*	+0.76	-2.73	+0.79	+2.69
21	*Brassica oleraceae* var. *Italica*	+0.53	+0.75	-0.48	+0.27
22	*Raphanus sativus*	+5.86	+0.28	-0.6	+6.17

(+) indicate increase in protein component after sprouting
(-) indicate decrease in protein component after sprouting
d.m. – dry matter

Table A4. Free radical scavenging capacity (antioxidant activity) of extracts of non-sprouted samples against DPPH

Sample no.	Species name	mg TE/100g* in d.m.
	PSEUDOCEREALS	
1	*Chenopodium quinoa*	14.82±15.40[a]
2	*Chenopodium quinoa*	49.91±21.22[abc]
3	*Amaranthus caudatus*	22.53±12.80[a]
4	*Amaranthus caudatus*	43.22±1.32[abc]
5	*Fagopyrum esculentum*	72.26±6.91[abc]
6	*Fagopyrum esculentum*	92.64±6.49[c]
7	*Fagopyrum esculentum*	219.04±1.23[d]
	LEGUMES	
8	*Medicago sativa*	29.29±5.39[abc]
9	*Glycine max*	41.13±32.70[abc]
10	*Cicer arietinum*	21.32±7.10[a]
11	*Vigna radiata* syn. *Phaseolus aureus*	88.73±6.15[bc]
12	*Lens culinaris*	15.54±4.83[a]
13	*Lens culinaris*	40.16±7.24[abc]
14	*Lens culinaris*	21.62±5.17[a]
15	*Trigonella foenum graceum*	32.40±22.26[abc]
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	16.19±8.89[a]
	CEREALS	
17	*Hordeum vulgare*	27.70±3.71[ab]
18	*Hordeum vulgare*	42.41±5.56[abc]
19	*Triticum aestivum*	64.17±98.52[abc]

Table A4. Continued

Sample no.	Species name	mg TE/100g* in d.m.
	CRUCIFEROUS VEGETABLES	
20	*Eruca sativa*	73.76±12.11^{abc}
21	*Brassica oleraceae* var. *Italica*	26.38±5.02^{ab}
22	*Raphanus sativus*	23.60±11.47^{a}
	1	45.37±44.93^{a}
Repetition	2	55.34±59.13^{a}
	3	43.74±45.98^{a}
	4	51.70±45.69^{a}

In the table are presented average value±S.D.
*In the same column, values followed by different superscripts (a-d) denote statistically significant differences at $p \leq 0.05$.
d.m. – dry matter

Table A5. Free radical scavenging capacity (antioxidant activity) of extracts of sprouted samples against DPPH

Sample no.	Species name	mg TE/100g* in d.m.
	PSEUDOCEREALS	
1	*Chenopodium quinoa*	159.80 ± 10.29^{hij}
2	*Chenopodium quinoa*	128.22 ± 5.97^{efgh}
3	*Amaranthus caudatus*	107.72 ± 4.56^{defg}
4	*Amaranthus caudatus*	77.35 ± 4.90^{bcde}
5	*Fagopyrum esculentum*	232.87 ± 0.73^{k}
6	*Fagopyrum esculentum*	208.91 ± 4.62^{jk}
7	*Fagopyrum esculentum*	232.01 ± 2.03^{k}
	LEGUMES	
8	*Medicago sativa*	157.22 ± 8.91^{ghi}
9	*Glycine max*	84.45 ± 17.80^{bcdef}
10	*Cicer arietinum*	66.23 ± 8.36^{abcd}
11	*Vigna radiata* syn. *Phaseolus aureus*	233.40 ± 2.51^{k}
12	*Lens culinaris*	91.07 ± 8.27^{cdef}
13	*Lens culinaris*	134.94 ± 3.96^{fgh}
14	*Lens culinaris*	90.08 ± 6.11^{cdef}
15	*Trigonella foenum graceum*	54.90 ± 4.23^{abc}
16	*Medicago sativa, Vigna radiata* syn. *Phaseolus aureus*	45.99 ± 6.93^{abc}
	CEREALS	
17	*Hordeum vulgare*	86.11 ± 80.88^{cdef}
18	*Hordeum vulgare*	34.63 ± 14.51^{ab}
19	*Triticum aestivum*	16.90 ± 9.80^{a}

Table A5. Continued

Sample no.	Species name	mg TE/100g* in d.m.
	CRUCIFEROUS VEGETABLES	
20	*Eruca sativa*	222.68±2.91^{k}
21	*Brassica oleraceae* var. *Italica*	228.83±1.85^{k}
22	*Raphanus sativus*	229.22±1.27^{k}
	FRESH GERMS/ MIXED SPROUTS	
23	*Medicago sativa, Raphanus sativus, Eruca sativa*	217.36±5.89^{k}
24	*Brassica oleraceae* var. *Italica*	234.62±0.56^{k}
25	*Cicer arietinum, Lens culinaris, Raphanus sativus, Triticum aestivum, Trigonella foenum graceum*	234.79±0.86^{k}
26	*Vigna radiata* syn. *Phaseolus aureus*	233.40±2.51^{k}
27	*Helianthus annus*	188.03±44.85^{ijk}
28	*Fagopyrum esculentum, Helianthus annus, Chenopodium quinoa*	113.94±17.47^{defgh}
Repetition	1	142.23±74.94^{a}
	2	153.88±75.29^{a}
	3	149.29±78.95^{a}
	4	146.84±77.33^{a}

In the table are presented average value±S.D.
*In the same column, values followed by different superscripts (a-k) denote statistically significant differences at $p \leq 0.05$.
d.m. – dry matter