Sprouts and Microgreens: Trends, Opportunities, and Horizons for Novel Research

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Abstract: Sprouts and microgreens have attracted tremendous interest across multiple disciplines in recent years. Here, we critically review the most recent advances to underscore research prospects and niches, and related challenges, not yet addressed or fully pursued. In particular, we report a number of themes that merit special attention as a result of their relevance to plant science, nutrition, health, and zootechnics: (1) species not yet or inadequately investigated, such as wild plants, and fruit tree strains; (2) abiotic and biotic factors, and biostimulants, for elicitation strategies and metabolic engineering; (3) sanitization and processing technologies to obtain high-quality products; (4) digestive fate and impact of bioactive elements, antinutrients, and allergens on human nutrition; (5) experimental challenges to researching health benefits; (6) the opportunity to generate natural product libraries for drug discovery; and (7) sprouts in animal feeding to improve both animal health and the nutritional value of animal products for the human diet. The convergence of different themes involving interdisciplinary competencies advocate fascinating research pursuits, for example, the elicitation of metabolic variants to generate natural product collections for identification and selection of bioactive chemicals with a role as nutraceuticals, key constituents of functional foods, or interactive partners of specific drugs.

Keywords: plant; elicitation; post-harvest; food safety; human nutrition; functional foods; nutraceuticals; drug; human health; animal feeding

1. Introduction

In recent years, microscale vegetables have become increasingly popular for homemade food preparations and have been the subject of progressively higher interest by the ready-to-eat market and the dietary supplements industry. While this trend is true for a vast variety of young plants and their constituents [1–3], this review is centered on sprouted seeds and microgreens, namely, shoots with fully expanded cotyledons, including grasses (i.e., shoots of *Poaceae*) [4].

Sprouts and microgreens can be produced quickly, easily, and cost-effectively due to simple requirements for equipment and supplies, and a rapid developmental process varying from a few days (sprouts) to approximately two weeks (microgreens). This, in turn, suggests a unique opportunity for industrial scalability coupled with the prospect for consumers to independently access food with proven or purported nutritional benefits [5,6]. Compared to seeds or mature plants, both sprouts and microgreens are known to contain low amounts of antinutrients, while being rich in amino acids, fatty acids, and simple sugars as a result of enzymatic breakdown of large macromolecules, and bioactive compounds, namely, a cohort of secondary metabolites, also known as phytochemicals [4,7–10].

Most phytochemicals are low-molecular-weight and anti-microbial compounds synthesized de novo (i.e., phytoalexins) or from existing precursors (i.e., phytoanticipins) [11,12]. Among phytoanticipins, glucosinolates are commonly found in *Brassicaceae* sprouts, and among phytoalexins, terpenoids and phenolic compounds are widespread in sprouts of most plant species. Their high concentration in seedlings contributes to increased vigor and tolerance to any stress potentially occurring in early stages of development Table 1.

Table 1. Main functions recognized to the principal phytochemical classes and subclasses.

Class		Function in Plants	References
Polyphenols	Flavonoids	Pigments (i.e., anthocyanins, flavanols, and flavones); UV protection (i.e., flavones and flavonols); scavenger of H_2O_2, superoxide, hydroxyl radical, singlet oxygen, peroxyl radical; feeding and wounding deterrents (i.e., hydrolyzable tannins); insecticidal and antimicrobial action (i.e., isoflavones).	[13–15]
	Phenolic acids	Plant-microbe symbiosis; allelopathic effect (caffeic and ferulic acid); feeding deterrents as astringent compounds (i.e., condensed tannins); resistance to pathogen attack; components of cell-wall; attract pollinators and seed dispersers; signaling molecules (i.e., salicylic acid).	[14,15]
	Stilbenes	Defensive response to pathogens or stress conditions; protective agents from viral and microbial attack, injuries, diseases, UV exposure, aluminum, etc.	[15,16]
	Lignans	Building blocks for the fiber lignin; plant defense diseases and pests.	[15,17]
Terpenoids	Overall	Plant growth regulators; defense against herbivores and pathogens; attracting pollinators influence the growth and the development of neighbor plant.	[13,18]
	Carotenoids	Pigments; protection against photooxidation; light-harvesting pigments.	[13,14]
	Tocotrienols and tocopherols	Singlet oxygen scavengers in photosystem ii; signaling molecules; maintain the integrity of long-chain polyunsaturated fatty acids in the cell membranes.	[19]
N-containing metabolites	Glucosinolates	Herbivore toxins and feeding repellents; insects deterrents and pathogens growth inhibitors (isothiocyanates).	[12–14,20]

Notably, a large array of phytochemicals exhibit antioxidant properties, and, in many instances, the presence of phytochemicals in the diet has been shown to provide beneficial effects to human health; Table 2.

Table 2. Phytochemicals and health benefits.

Chemical Class	Subclass	Health Benefits	References
Polyphenols	Flavonoids	Antioxidants: long-lived radical anions scavengers; anti-lipoperoxidant, -tumoral, -platelet, ischemic, -allergic, -inflammatory activities.	[21]
	Phenolic acids	Radical scavengers; singlet oxygen quenching; block biosynthesis of leukotrienes involved in immunoregulation disease, asthma and allergic reaction (caffeic acid).	[21]
	Stilbenes	Anti-oxidative, -carcinogenic, -tumor properties; retard cardiovascular disease and cancer; prevent lipid oxidation and the formation of toxic oxidation products.	[21]
	Phytoestrogens (i.e., isoflavones, lignans, coumestans)	Prevent menopausal symptoms (hot-flashes, vasomotor symptoms, vaginal atrophy); anti-aging effect on the skin; enhance bone formation and increase bone mineral density; beneficial effects on endothelial cells, vascular smooth muscle; reduce levels of low-density lipoprotein (LDL) cholesterol; anti-mutagenic, -proliferative, -angiogenic, pro-apoptotic and general anti-cancer effects.	[22]
Terpenoids	Carotenoids	Chemical quenchers of $^1O_2$; reduce the risk of prostate cancer and age-related macular degeneration; stimulate the immune system in inflammatory diseases or human immunodeficiency disease.	[23]
	Tocotrienols and tocopherols	Retard cancer lesions and tumors; tackle free radical reactions; scavengers of lipid peroxyl radicals; singlet oxygen quenchers.	[23]
	Phytosterols	Reduce cholesterol levels; attenuate inflammatory processes; induce apoptosis when added to cultured human prostate, breast, and colon cancer cells.	[24]
N-containing metabolites	Glucosinolates	Protect against *Helicobacter pylori* infections, light-induced damage of retina brain ischemia/reperfusion, traumatic brain injury, intracerebral hemorrhage, and contusion spinal cord injury (sulforaphane); decrease infarct size, brain (or spinal cord) edema, and cortical apoptosis (sulforaphane); restoration of skin integrity; inhibit growth of human tumor cells.	[25]

In the last decade, the scientific literature on sprouts has dramatically increased and has been widely reviewed [1–4,23,26]. Nonetheless, there are aspects that have been scarcely or never explored. These gaps in knowledge offer perspectives for multidisciplinary studies involving collaboration across competencies in plant biology, food science and technology, human and animal nutrition, pharmacology, and medicine. This review is aimed at identifying intriguing areas of investigation that merit special attention.

2. Plant Species Scarcely Studied for Sprouting: Limits and Opportunities

Plant species in the *Poaceae*, *Brassicaceae,* and *Fabaceae* families are most exploited for sprouting purposes and therefore most reported by the scientific literature. For these species, research has provided a lot of information on the nutritional traits of sprouts and microgreens [3,4,27].

Other cultivated species are used for sprouting, being appreciated for several peculiar traits: vivid colors (i.e., red for red basil; green for spinach), intense smells (i.e., aromatic herbs), pleasant textures (i.e., regular for *Asteraceae*; juicy for sunflower and beet; crunchy for celery), and variable tastes (i.e., regular for *Asteraceae*; slightly sour for beet; bitter for *Cucurbitaceae*) [3,5]. In this regard, the definition and quantification of organoleptic traits represent a subject still unexplored, even for the species commonly used for sprouting, borrowing the methodology and technology used for vegetables and fruits, such as the gas chromatography-olfactometry. A first preliminary work on this has been carried out by Bianchi et al. [28] to analyze taste quality traits and volatile profile in sprouts and wheatgrass of *Triticum* spp. Another recent study compared 12 microgreens in terms of sensory attributes and visual appearance, by means of a consumer test [29]. Other grains, such as amaranth, quinoa, and buckwheat have been also studied in detail and appreciated as gluten free [30]. Several other cultivated species have been studied occasionally, for example hemp, whose sprouts are destined to become very popular [31].

This section, however, is focused on opportunities and research challenges offered by species underexplored for sprouting like voluntary species, wild relatives, ancestors, and neglected/local accessions of cultivated species, and fruit tree species. All of these would be very interesting for sprouting because they are supposed to have a higher phytochemical content compared to cultivated species [32–34].

Research should identify and quantify phytochemicals of these species but also investigate germination performances (% germination, mean germination time, time to reach a minimum germination threshold) and related methods for improvements. In fact, these species may show dormancy or unpredictable germination, which is a key impediment for sprouting.

2.1. Voluntary Species, Wild Relatives, Ancestors, Neglected/Local Accessions of Cultivated Species

None of these species, unlike cultivated species, were subject to breeding programs aimed at increasing yield and fruit size to the detriment of adaptability. They are characterized by high rusticity and tolerance to extreme environmental conditions that can cope with the negative effects of climate change, thereby representing potential contribution to world food security [35]. Their resilience (adaptability) is often due to biochemical mechanisms involving higher contents of secondary metabolites [36]. This also contributes to the high seedling vigor and fast vegetative growth, which warrants competitive adaptability in early growth stages [37]. For these reasons, sprouts from these species are expected to have a very high nutritional value. Some wild species were proposed and studied for micro-scale vegetables production (Table 3) and many other might be considered for sprouting: *Galium aparine* L. [38], *Convolvulus arvensis* L. [39], *Solanum nigrum* L. [40], and *Papaver rhoeas* L. [41]. An example of sprouts from ancestors of modern species is offered by einkorn, whose sprouts were found to be richer in phenolic substances than those of soft and durum wheat [42].

Table 3. Micro-scale vegetables from voluntary species, wild relatives, and ancient species.

Family	Species	Days after Sowing (DAS)	Secondary Metabolites	References
Amaranthaceae	*Amaranthus caudatus*–amaranth	10	PAs, total PC, FC	[43]
	Amaranthus cruentus–amaranth	4, 6, 7	Total PC, AA	[44]
	Amaranthus hypochondriacus–amaranth	2	AA	[45]
		N.S.	Carotenoids	[46]
	Chenopodium album–pigweed	48 h	AA, total PC	[47]
		48 h	Total PC	[48]
		48 h	Tocopherols	[49]
	Chenopodium quinoa–quinoa	4, 6, 7	Total PC, AA	[44]
		82 h	Total PC, AA, single phenolics	[50]
		4	Total PC, AA	[51]
		5	Total PC	[52]
	Chenopodium berlandieri–huauzontle	12, 24, 36, 48, 72 h	N.R.	[53]
Apiaceae	*Anethum graveolens*–dill	8–12	Total PC, FC, AA	[54]
	Coriandrum sativum L.–coriander	N.S.	Carotenoids, total PC, single phenolics	[55]
		20	AA, carotenoids, total PC, single phenolics	[56]
Asteraceae	*Artemisia dracunculus*–tarragon	1, 3, 5, 7	N/A	[57]
	Taraxacum officinale–common dandelion	16	Anthocyanins and carotenoids	[58]
Boraginaceae	*Phacelia tanacetifolia*–phacelia	7	Total PC, AA, free and bound PAs and flavonoids	[59]
Brassicaceae	*Cichorium intybus*–chicory	12	Total PC, tocopherols, anthocyanins, carotenoids	[60]
	Diplotaxis tenuifolia–wild rocket	4, 7	Total and single GLS	[61]
		7, 21	Anthocyanins, phenolics, AA, resveratrol	[62]
Convolvulaceae	*Ipomea aquatica*–water convolvulus	8–12	Total PC, FC, AA	[54]
Malvaceae	*Corchorus olitorius* L.–jute	22	AA, carotenoids, total PC, single phenolics	[56]

Table 3. *Cont.*

Family	Species	Days After Sowing (DAS)	Secondary Metabolites	References
Poaceae	*Coix lacryma-jobi*–adlay seed	12, 24, 36, 48, 60 h	Free, bound total PC, flavonoid, PAs and AA	[63]
	Phalaris canariensis–canary seed	24, 48, 72, 96, 120 h	Free, bound total PC, PAs and AA	[64]
	Triticum aestivum spp. spelta–spelt	5 and 12	Total PC, free and bound PAs, AA	[42]
	Triticum monococcum ssp. *monococcum*–einkorn	5 and 12	Total PC, free and bound PAs, AA	[42]
		5 and 13	Total PC, FC, AA	[65]
		5 and 12	Total PC, free and bound PAs, AA	[66]
		5 and 9	Total PC, free and bound PAs, AA	[67]
	Triticum turgidum spp. *dicoccum*–emmer	5 and 12	Total PC, free and bound PAs, AA	[42]
		5 and 13	Total PC, FC, AA	[45]
		5 and 12	Total PC, free and bound PAs, AA	[65]
	Zizania latifolia–wild rice		Free, bound and total phenolics	[68]
Lamiaceae	*Salvia hispanica*–chia	7	Total PC, AA, free and bound PAs and FC	[59]
Leguminosae	*Vigna umbellata*–rice bean	6, 12, 18, 24 h	Total PC, single PAs, single flavonoids and AA	[69]
Onagraceae	*Oenothera biennis*–evening primrose	7	Total PC, AA, free and bound PAs and flavonoids	[59]
Portulacaceae	*Portulaca oleracea*–common purslane	1–6	N/A	[70]
		3	Free, bound soluble conjugated total PC, PAs and flavonoids	[71]
	Portulaca grandiflora–moss-rose purslane	1–6	N/A	[70]

AA = antioxidant activity; FC = flavonoid content; GLS = glucosinolates; N/A = not available; N.R. = not reported; N.S. = not specified; PAs = phenolic acids; PC = phenolic content.

Of course, all of these species, especially the wild ones, will be effectively suitable for sprouting only after solving limitations related to low grain yield and seed dispersal at ripening, and seed dormancy [37]. Since many wild species can be considered weeds in agro-ecosystems, these studies might also provide information for weed science and management.

2.2. Fruit Tree Species

Seeds of fruit tree species often represent an important by-product of juice/jam food industries, as well as a relevant source of phytochemicals [72]. This is the case of limonoids in *Citrus* species [73], and of secoiridoids in *Olea* species [74]. In the last decade, many utilizations were proposed to add value to these agricultural by-products, including the possibility to use them for nutritional purposes [72,75]. The direct consumption of seeds is not possible in many cases, since they are coriaceous, and/or bitter and astringent. Several processing methods have been proposed for extracting phytochemicals from these kinds of seeds, while sprouting is a relatively new option, which would help producing high value food while reducing wastes. To date it has been studied only for grapeseed [76,77], pomegranate [78], olive [79], and *Citrus* species [80]. In most cases, the increase in phytochemical content and antioxidant activity observed in sprouts in relation to seeds were found to be dramatic (e.g., 30-fold for polyphenols and 90-fold for antioxidant activity in pomegranate), and much higher than in herbaceous species, thus advocating further research. However, among these species, only pomegranate showed acceptable germination rate and time, and a suitable consistency and taste to be actually used for the production of edible sprouts. In the other cases, authors realized that sprouts might better be used for extraction of food additives, cosmetics, and pharmaceuticals.

By analogy with fruit tree species, also fruit-shrub species such as blackberries, raspberries, and blueberries, can be postulated as excellent sources of sprouts, but limitations related to seed dormancy and low germination performances appear hard to overcome. However, considering the large amount of seed wastes obtained from processing, even low germination percentages could be suitable for sprouting purposes.

3. Delving into Elicitors

As previously recalled, phytochemicals have several functions in plants, including protection against abiotic and biotic stressing agents, which may threaten seedling health. Thus, any stressing condition during germination can work as an elicitor, i.e., it may stimulate secondary metabolisms and increase the phytochemical content of sprouts.

Elicitation in sprouts has been recently reviewed by Liu et al. [81]. This section is merely intended at mentioning some underexplored elicitation strategies (Table 4) while prospecting new alternatives.

Among abiotic elicitors, salts applied during sprouting represent a generally available and cheap technique to induce plant defense. Recently, a large body of literature has become available about NaCl and Se elicitation in sprouting [81,82], but even for these salts there are still questions that need to be addressed. First, the effect of salts may change with the genotype tolerance; thus, evidences in the literature for any species, often obtained with one genotype or a few, should be validated over a greater number of genotypes. Moreover, when studying the effect of an elicitor for sprouting purposes, a much larger variety of doses should be included. In this regard, while a higher level of stress increases the production of phytochemicals, on the other hand it delays and depresses sprout growth. Thus, the elicitor application should be finely tuned to find out the dose that can maximize the "phytochemical yield" as defined by Falcinelli et al. [65], i.e., the product between the sprout biomass and its phytochemical concentration.

Priming of seeds with salts (halo priming) has been little explored with regard to sprouting. Evidence by Hassini et al. [83,84] on cabbage and broccoli seeds primed with KCl encourage this option. More in general, not only halo priming, but any applicable priming techniques appear to be a compelling area of investigation [85,86].

Se biofortification of sprouts and microgreens represent an intriguing research subject, because it combines the increase of this health promoting micronutrient in sprout tissue with its elicitation effect on secondary metabolites, resulting in an overall improvement of sprout nutritional value. However, this subject is not further discussed here, since it has been thoroughly reviewed in a recent work by D'Amato et al. [82].

Another research subject still unexplored is the effect on the nutritional value of sprouts following the application of elicitation agents to the mother plant. For example, a few studies dealt with the effects of Se in seeds obtained by mother plants subject to Se fertilization [87,88]. In this regard, a challenging matter is represented by possible epigenetic effects that might impact the nutritional value of the corresponding sprouts. Here, a key concept is that different environmental conditions in plants might enhance or suppress the expression of genes associated with phenotypic traits that, eventually, are transferred to the next generations [89]. For example, Marconi et al. [90] found that salinity applied to rapeseed induced genome-wide changes in DNA methylation status, and that these changes accounted for 16.8% of the total site-specific methylation differences in the rapeseed genome, as detected by methylation-sensitive amplified polymorphism (MSAP) analysis. Preliminary results from a follow-up experiment on the rapeseed sprouts obtained from seeds harvested from salt stressed plants have demonstrated that these sprouts have greater phenolic content and antioxidant activity (Benincasa, unpublished results). It should be pointed out that studies on elicitors would most likely be facilitated by a multidisciplinary approach including investigations on the physiology of seed germination and related genetic implications. These studies, altogether, could lead to breeding protocols towards a higher phytochemical content with beneficial implications on plant adaptation and, more downstream, human health.

Elicitation should also be investigated in relation to hormesis [91], i.e., the stimulatory effect caused by a toxic compound when applied at very low doses. The beneficial response to hormetins (i.e., the factors inducing hormesis) likely involves an increase of phytochemical content, which has not yet investigated in detail.

Among physical elicitation factors, several have been recently proposed (Table 4). For example, wounding injuries to cotyledons were applied alone or combined with other treatments in soybean sprouts by Aisyah et al. [92]. Ultrasonication has been applied in the sprouting of legumes [93–95], while audible sounds (in the range 20–20,000 Hz) were used in the sprouting of mung bean [96].

Nanomaterials (1–100 nm) represent another intriguing subject for sprouting. The effect of nano-silver (NAg) was studied in radish sprouts [97], and that of nano-selenium (NSe) in broccoli sprouts [98]. High levels (600 and 800 ppm) of CO_2, previously shown to induce plant defenses [99], was applied in the sprouting of pea with positive effects on nutritional traits [100]. Magnetic fields were applied to mung bean sprouts by Chen et al. [101] and electromagnetic waves, obtained by simply using a microwave oven, were applied to buckwheat seeds by Wang et al. [102]. Besides their application as elicitors, microwaves, as well as gamma and UV radiation, may also be utilized to reduce seed contamination and related sanitary problems [93,103]. The effect of the light spectrum, light intensity, and photoperiod light in the production of micro-scale has been widely studied, as recently reviewed by Alrifai et al. [104]. However, the effect of light spectra on sprouts has been found to be genotype-specific and should be further investigated, considering that the study primarily dealt with red and blue light, whereas the effect of intermediate wavelengths, especially on phytochemical content, has not yet clarified [105,106]. Finally, based on the futuristic view of a human presence in the Space, sprouts and microgreens can be considered as ideal candidates for astronauts' diet [107]. To this purpose, Nakajima et al. [108] explored the effect of microgravity by applying clinorotation to the sprouting of mung bean.

When dealing with literature about elicitation in sprouts, a recurrent issue is that data from separate studies are often not comparable, due to differences in the process of sprout development. Moreover, growth stage is often described based on the number of days from the start of incubation, but this does not help in case of genotypes or germination conditions implying different germination times and growth rates. For example, in works on rapeseed and wheat sprouted under salinity, the authors compared treatments in sprouts displaying the same growth stage, therefore sampling after different number of days from the start of incubation, given that treatments with high salinity levels had caused delayed germination and growth [65,66,109].

With regard to biotic factors, fungal inoculation represents a new approach in sprouting. There are several work on this topic [92,110–115], showing evidence about the increase of phytochemical content [112,116]; however, little is known about the metabolic pathways impacted by fungal–plant interactions.

The application of yeasts on sprouts is very intriguing, in light of probiotic benefits that may occur together with the elicitation effects [117–119].

The effect of bacteria inclusion in the substrate or the seeds was studied for buckwheat sprouts and microgreens by Briatia et al. [120], while Eissa et al. [100] reported the effect of *Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens,* and their combination on growth performances and nutritional traits of pea sprouts.

Based on the effects shown by fungi and bacteria, one may also wonder whether virus infections play a role in determining the phytochemical content of sprouts. However, to our knowledge, no scientific evidence has ever been shown in this regard.

Another form of biotic elicitation method concerns the application of biostimulants. These include (1) humic and fulvic acids, (2) animal and vegetal protein hydrolysates, (3) macroalgae seaweeds extracts, (4) silicon, (5) microorganisms (arbuscular mycorrhizal fungi; N-fixing bacteria like *Rhizobium, Azotobacter*, and *Azospirillum*) [121]. By definition, biostimulants are mainly employed to enhance plant growth, abiotic stress tolerance and/or quality traits of crops [122], rather than improving the nutritional value of plants for humans. Nonetheless, Witkowicz et al. [123] found that the application of different biostimulants had a significant effect on the nutritional traits of buckwheat sprouts. This influential study, as a result, uncovers new research opportunities, considering the number of key parameters involved (e.g., type, amount, and combination of biostimulants to any plant species) and, within each parameter, the variety of available options.

Elicitation as a consequence of insect attacks is another compelling, and unexplored, area of research. It is well known that plants respond to insects by releasing a wide range of chemical compounds, including volatile entities that convey molecular signals to alert neighboring plants [18,124]. Such volatile chemicals cause phenotypic changes and, therefore, carry a potential role as elicitation factors.

It is worth noting that elicitation can be effectively achieved by implementing combinatorial strategies, as shown by Benincasa et al. [87], when high levels of endogenous Se combined with salinity stress were found to synergistically increase the phytochemical content of maize sprouts. However, to date, elicitors have been generally investigated alone and not in combination, thereby precluding observations about possible additive and synergistic effects that could result from versatile and flexible application of variable conditions and parameters (e.g., type, dose, and application method of abiotic and/or biotic factors).

Table 4. Biotic and abiotic elicitation strategies to raise phytochemical content and corresponding bioactivities in micro-scale vegetables.

Elicitor	Application	Plant	Days after Sowing (DAS)	Phytochemicals/Bioactivities	Reference
Abiotic elicitation–Priming					
	Seeds soaked with 30 mL of 50 mM KCl	*Brassica oleracea* var. *capitata*–cabbage	10	↓total and single GLS	[83]
	Seeds soaked with 50 mM KCl	*Brassica oleracea* var. *italica*–broccoli	10	≈total and single GLS and AA; ↑ flavonoids and TP	[84]
	100% imbibition and aeration of the seeds for 24 h, with MeJA and JA (250 μM) and MET (10 mM)	*Brassica oleracea* var. *italica*–broccoli *Raphanus sativus*–radish	8	↑ total GLS with MeJA and JA priming different effect on single GLS	[85]
Physical factors					
Wounding	Soaked soybeans were wounded prior to germination stage by cutting the cotyledon individually	*Glycine max*–soybean	10	↑glyceollin content (i.e., isoflavones)	[92]
Ultrasound	Seeds treated with ultrasound at 360 and 180 W for 30, 40, and 60 min in the ultrasonic bath	*Phaseolus vulgaris*–common bean	1–4	↑ PAs, flavonoids, anthocyanins, and AA	[93]
	Seeds treated with ultrasound at 100, 200, and 300 W for 30 min in the ultrasonic bath	*Glycine max*–soybean	5	↑daidzein and genistein (i.e., isoflavones); ↓daidzein and genistein (i.e., isoflavones)	[95]
	Seeds subjected to ultrasound at three frequencies (28, 45 and 100 kHz) for 15, 20, and 30 min.	*Arachis hypogaea*–peanut	1, 2, 3, 4, 5	Generally, ↑ resveratrol content (i.e., stilbenes)	[94]
Nanoparticles	Seeds sprinkled every day with NSePs (0, 10, 50 and 100 ppm); NSePs prepared by chemical reduction of NaHSeO$_3$ solution with freshly prepared 0.25% glucose solution (4:1)	*Brassica oleracea*–broccoli	9	≈ carotenes; ≈ phenolics and AA; different effects on single GLS	[98]
Magnetic field	Seeds exposed to 600 mT magnetic field pretreatment (Model: 2G of 755R magnetometer system)	*Vigna radiata*–mung bean	6	↑anthocyanins	[101]
Microwave	Seeds exposed to microwave irradiation at 200, 400, 600, and 800 W for 10 or 30 s	*Fagopyrum esculentum*–tartary buckwheat	3, 5, 7	↑total flavonoids and AA at 600 W	[102]
Clinorotation	Seeds treated with slow-rotating clinostat (CL) with a servo motor and an acrylic chamber. An amplifier controlled the rotation rate, and the chamber was horizontally rotated at 2 rpm	*Vigna radiata*–mung bean	2, 3, 4	≈ carotenoids, except anthocyanins (↓)	[108]
Fungi	*Rhizopus oryzae* spore suspension (0.2 mL/g of beans) added to soybeans during incubation	*Glycine max*–soybean	10	↑ total pterocarpans, coumestans (i.e., isoflavonoids); ≈ isoflavones	[92]
	Rhizopus oryzae, *Fusarium graminearum* CBS 104.09 and *Fusarium oxysporum* CBS 186.53-fungal spore suspension, applied to 2-day-old seedlings	*Sinapis alba*–yellow mustard; *Brassica napus*–rapeseed; *Brassica juncea*–Chinese mustard	7	≈ total GLS except with *Fusarium oxysporum* (↓) in yellow and Chinese mustard	[114]
	Spore suspension of *Rhizopus oryzae* (LU581) and *Aspergillus oryzae* var. effusus (LU009) (0.2 mL/g peanuts) added after soaking or after 2 days of germination	*Arachis hypogaea*–peanut	5 or 7	Effect on stilbenoids depending on the fungus and the time of application	[110]

Table 4. *Cont.*

Elicitor	Application	Plant	Days after Sowing (DAS)	Phytochemicals/Bioactivities	Reference
Biotic elicitation					
	Rhizopus oryzae (LU 581) and *Rhizopus oligosporus* (approximately 1.5×10^7 CFU/g seed) added to 2-day-old seedlings	*Vigna radiata*–mung bean; *Phaseolus vulgaris*–common bean; *Glycine max*–soybean; *Lupinus albus*–white lupine; *Lupinus angustifolius*–blue lupine; *Lupinus luteus*–yellow lupine; *Arachis hypogaea*–peanut;	8	↑ TP except in mung bean and blue lupin; different effects on phenolic subclasses (i.e., flavonoids, phenolic acids, stilbenoids, etc.)	[113]
	Spore suspension of *Rhizopus oryzae* (0.2 mL/g beans) added to 2-day-old seedlings	*Phaseolus vulgaris*–common bean; *Phaseolus coccineus*–runner bean; *Lablab purpureus*–Lablab bean; *Vigna angularis*–adzuki bean; *Vigna unguiculata*–cowpea; *Vigna radiata*–mung bean; *Psophocarpus tetragonolobus*–Winged bean	7	↑or ≈all isoflavonoids (i.e., coumestans, isoflavans, pterocarpans, isoflavones, isoflavanones)	[111]
	Spore suspension of *Rhizopus oryzae* (0.2 mL/g beans) added to 2-day-old seedlings	*Lupinus albus*–white lupine; *Lupinus angustifolius*–blue lupine; *Lupinus luteus*–yellow lupine	7	≈ or ↑genistein derivatives	[112]
	4-day-old seedlings inoculated by soaking in spore solution of *Rhizopus oryzae* for 50 min	*Triticum aestivum*–soft wheat	7	↑ variety of secondary metabolites mainly constituted by benzoxazin-3-one glycosides and benzoxazolinones	[115]
Yeasts	Seeds soaked in distilled water or *Lactobacillus plantarum* 299v water suspension (1×10^8 CFU per 1 g of seeds (8.00 log10 CFU/g) for 4, 6, and 8 h	*Lens culinaris*–lentil; *Glycine max*–soybean; *Vigna angularis*–adzuki bean; *Vigna radiata*–mung bean	4	≈ or ↓ phenolics and condensed tannins	[117]
	Seeds soaked in water or *Lactobacillus plantarum* 299v water suspension (8.00 log10 CFU/g of seeds)	*Lens culinaris*–lentil; *Glycine max*–soybean; *Vigna angularis*–adzuki bean; *Vigna radiata*–mung bean	4	≈ ability to quench ABTS$^{•+}$ and $^{•}$OH; different effects on the ability to quench $O_2^{•-}$;different effects on total and single phenolics	[119]
	Seeds soaked in distilled water or probiotic water suspension (1×10^6 CFU per 1 g of seeds) of *Saccharomyces cerevisiae* var. *boulardii* seedlings sprayed daily with 5 mL of Milli-Q water or 5 mL of probiotic water suspension on 1st day of cultivation (1×10^6 CFU per 1 g of seeds)	*Lens culinaris*–lentil; *Vigna angularis*–adzuki bean	4	≈ phenolics and antioxidant activities	[118]
	Seeds soaked in distilled water or a *Lactobacillus plantarum* 299 V water suspension (1×10^8 CFU per 1 g of seeds) for 4 h	*Glycine max*–soybean	4	↓ total isoflavones in fresh sprouts; different effects on single isoflavones; ↑ antioxidant activity	[125]
	Sprouts treated with 50, 100, 200, 400 and 800 mg/L of YPS (polysaccharide fraction of YE, Y4250) on days 0, 3, 6, and 9	*Fagopyrum esculentum*–tartary buckwheat	0, 3, 6, 9	↑ flavonoids in 3-,6-, and 9-day -old sprouts	[126]

Table 4. *Cont.*

Elicitor	Application	Plant	Days after Sowing (DAS)	Phytochemicals/Bioactivities	Reference
Biotic elicitation					
Bacteria	Seeds were surface-sterilized with sodium hypochlorite and rinsed with distilled water. Then, seeds were soaked in liquid suspension of endophytic bacterium, (ST-B2 strain, isolated from stem of buckwheat) at 0, 10% and 20% concentrations for 4 h at room temperature	*Fagopyrum esculentum*–buckwheat	10	Not analyzed	[120]
	Bacillus megaterium 10^8 CFU/mL added 10 days before the cultivation of seeds, while *Azotobacter chroococcum* and *Pseudomonas fluorescens* 10^8–10^9 CFU/mL were added after pea sprouts emergence and after the 1st cut. Eight sub-treatments from different combinations of microbial inoculants were carried out	*Pisum sativum*–pea	14 (1st cut) and 12 (2nd cut)	Not analyzed	[100]
	Seeds soaked for 30 min in 1:40 solutions of PGPs named Serenade ASO, containing *Bacillus subtilis* QST 713 at a concentration of 13.96 g/L (1.34%)	*Fagopyrum esculentum*–buckwheat	14	Not analyzed	[123]
Algae	Seeds soaked for 30 min in PGPs: 1:100 solution of Kelpak SL (*Ecklonia maxima* extract), and 2 g in 500 mL of Polyversum WP (10^6 *Pythium oligandrum* oospores per 1 g)	*Fagopyrum esculentum*–buckwheat	14	Not analyzed	[123]
Other biostimulants	Seeds soaked for 30 min in 1:500 solution of PGPs named Asahi SL (mixture of sodium p-nitrophenolate, sodium o-nitrophenolate, sodium 5-nitroguaiacolate)	*Fagopyrum esculentum*–buckwheat	14	Not analyzed	[123]

AA = antioxidant activity; CBS = CentraalBureau voor Schimmelcultures (Fungal Biodiversity Centre); CFU = colony-forming unit; GLS = glucosinolates; JA = jasmonic acid; MeJA = ethyl jasmonate; MET = DL-methionine; PAs = phenolic acids; PGPs = plant growth promoters; TP = total phenolics; YE = yeast extract; YPS = Yu-Ping-Feng polysaccharides.

4. Sanitization and Processing

Sprouts and microgreens are mainly used for homemade preparations and fresh food markets. Suitable handling practices, techniques for domestic and industrial food storage, and safety procedures are therefore required to ensure shelf life and food quality, and prevent microbiological contaminations that could pose risks to human health. In this regard, research must consider as well optimized germination protocols while preserving the nutritional value of sprouts and microgreens. This may include, for example, breeding programs aimed at genetic improvement and valorization of autochthonous cultivars and/or wild species to select genotypes associated with improved post-harvest storage.

Sprouts and microgreens are subject to different processing avenues, such as freezing, drying, waving, microwaving, frying, toasting, boiling, or centrifugation (for sprout juice production), to arrive at final applications where, ideally, phytochemical content, and overall nutritional value are fully retained. This is particularly important when sprouts represent the source of supplements to improve food quality, as in sprouted bread [127], other bakery products, and juices.

Research in this field would therefore benefit most from interdisciplinary collaborations between geneticists, microbiologists, and food technologists.

4.1. Microbiological Safety of Sprouts and Microgreens

When, in several instances, food-borne illness outbreaks have been linked to sprouts' consumption, at the same time no reported outbreaks or illnesses were associated to microgreens. This is not very surprising if we consider that, from a microecological standpoint, production of sprouts, and microgreens differ in a number of operating steps: microgreens undergo a brief seed-soaking period, are generally grown on substrate, and are consumed, after cutting, without the root system. On the other hand, sprouts are eaten raw together with their roots. Thus, many food agencies classify sprouts as "high-risk" products, while microgreens are considered much safer, although some (low) risks can arise from fresh herbs (basil, thyme) or baby-leaf vegetables (baby spinach) [128–131].

The most common agents causing outbreaks in relation to sprouts consumption include *Salmonella*, *Escherichia coli* O157:H7, and Shiga-toxin-producing *E. coli* [128,129,131,132]. Instead, other bacterial pathogens (e.g., *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Yersinia enterocolitica*) have rarely been reported [131,133]. Indeed, *Salmonella* and *E. coli* O157:H7, unlike *Listeria monocytogenes*, can internalize into seedlings, thus, making ineffective simple washing treatments or topical disinfectants [129,134]. Consequently, pre-harvest interventions could effectively enhance the microbiological safety of sprouts [129], even in the absence of a validated Critical Control Point (CCP) intended to at least reduce levels of the fecal pathogen to acceptable standards [131,133,135].

Typically, contaminations occur first in the seeds, and then spread to sprouts. Microbiological threats can be minimized through Good Agricultural Practices (GAP) as well as Good Handling Practices (GHP) along seed production stages [136]. In particular, soil amendments (i.e., compost or manure) and irrigation water require adequate controls, especially for species not usually employed in the whole sprouts/microgreens production chain (e.g., alfalfa and clover seeds not specifically produced for sprouts cultivation) and, more particularly, those newly introduced or potentially considered for consumers and the fresh market. For example, Liu et al. [137] recently showed that *S. enterica* and *E. coli* O157:H7 strains artificially inoculated into alfalfa, fenugreek, lettuce, and tomato flowers, had subsequently moved into the seeds (produced by contaminated flowers), and then to sprouts and seedlings, thus, showing the ability of *Salmonella* to survive and spread.

Efforts regarding sprout safety have mainly been focused on pathogen detection, as well as seed testing (including seed traceability) and disinfection. In this regard, a large body of literature has been produced to show effective seed disinfection procedures and, at the same time, specific steps aimed at ensuring high germination percentage and rate together with optimal yields and quality of sprouts. Disinfection methods fall into three main categories, i.e., chemical, physical, and biological, which have extensively been reviewed by Ding et al. [128], and by Warriner and Smal [129].

The application of 20,000-ppm calcium hypochlorite is likely the most commonly used chemical treatment. However, although recommended by the Food and Drug Administration (FDA), this method carries distinct drawbacks concerning worker safety and environmental impact, difficulty to make organic food claims, disinfection efficacy that may vary in relation to the physical properties of the seed surface (e.g., smooth vs scarified) [128,130], and impact on product quality [138]. Consequently, there is a need for alternative methods that optimally combine disinfection efficacy with safety and environment-friendly features. Table 5 summarizes the studies that have been performed in this field over the last 5 years (2016 onward) for applications in sprouts production. Notably, it should be clarified that, to date, seed disinfection is primarily based on physical methods, and that combination of physical, chemical, and biological treatments have also been shown to be particularly valuable in light of observed synergistic effects [128].

However, microbial control studies have generally been centered on seeds of a relatively small number of species, thereby leaving ample room for additional research on those of less common varieties. In this context, another research gap to fill is about the development of methods that can routinely be performed in household preparations, when seed disinfection is often neglected [139].

GAP should be applied during sprouting (e.g., germination and irrigation water tests [140]) whereas GHP should be implemented during harvest and post-harvest procedures [141]. Moreover, post-harvest microbiological control by washing treatments could damage vegetable tissues, thereby decreasing sprouts' shelf life. Physical disinfection methods, including irradiation, have proven to be effective post-harvest practices in the preparation of microbiologically safe sprouts [129]. Gamma irradiation (1–2.5 kGy) in combination with ultrasonication (4–10 min; 40–50 °C), blanching (50–70 °C for 4–10 min using potable water) and ascorbate dip (0.25%, 5% and 1% up to 10 min at 4 ± 1 °C) ensured microbial safety in mung bean, chickpea, and alfalfa sprouts together with an extended shelf life of the final products [103].

Similar concepts, rationales, and methods apply to microgreen productions although an additional CCP, as for raw products in general, should be indicated (see also Turner et al. [142]). Indeed, harvesting by cutting may increase susceptibility to contamination, since the cut site may be a breach from where pathogens can enter the system [130], as already reported in lettuce [143]. Screening for pathogens in sprouting seedbeds should also be performed [129], since soil and soil substitutes could provide a direct nutrient source as well as a protective surface from mechanical damage, thus promoting their proliferation or survival [144–146]. In this regard, Işık et al. [144] have studied the possible transfer of non-pathogenic and pathogenic *E. coli* from contaminated growth media to the edible part of radish and lettuce microgreens. Moreover, hydroponic systems, which are preferably chosen by modern commercial facilities, are not safer than other growing systems [142]. Unlike sprouts, microgreens sanitization processes before and after harvest require additional research, particularly to address the correlation between morphological characteristics and efficacy of sanitizers.

Lastly, in terms of pathogen detection, efforts are necessary to develop rapid and cost-effective screening tests that, ideally, are applicable to homemade productions (e.g., biosensors capable of detecting toxic molecules or pathogens in fresh products).

4.2. Post-Harvest Shelf Life and Processing

Sprouts and microgreens consist of young tissues characterized by higher respiration rates than their mature counterparts, leading to a more rapid post-harvest deterioration of quality, even at relatively low temperatures. Several practices can contribute to the extension of sprouts and microgreens shelf life, including pre-harvest and pre-storage calcium applications [147,148] to pre-packaging washing and dewatering processes [2]. Microgreens could also be commercialized directly in containers and harvested by the end user, bypassing post-harvest handling, and ensuring greater freshness quality [2,6]. Genotype, growing period, cultivation systems and time for harvesting could affect post-harvest quality of microgreens, since these factors significantly influence the accumulation of non-enzymatic antioxidant compounds (i.e., carotenoids, phenols and ascorbic acid), as already reported

for other ready-to-eat vegetables [149]. In sprouts, however, knowledge about polyphenol oxidases (PPOs) remain elusive except for some data concerning PPO inhibition mechanisms. In this regard, Sikora et al. [150] found two PPO isoenzymes in lentil sprouts, and over 40% total PPO activity inhibited by 2 mM ascorbic acid. Notably, ascorbic acid resulted effective also in terms of overall acceptability characteristics (i.e., color and appearance, taste, aroma, texture) in 7-day old sunflower microgreens [151].

In any case, temperature (firstly), atmospheric composition, packaging technologies, initial microbial load, and light exposure during post-harvest are among the most critical factors underlying shelf life.

A 0 °C temperature can be considered the optimal storage temperature for most of leafy and fresh-cut vegetables, in that it causes a reduction of both plant and microorganisms' metabolic activities [7]. However, information on specie-specific chilling sensitivity and respiration rates, together with the effects of cold storage on nutrient digestibility, bio accessibility of pro-health constituents and organoleptic quality of sprouts and microgreens, await further investigations in order to improve post-harvest methodologies. In 4-day-old soybean sprouts, the contents of isoflavones after a 7-day cold storage (4 °C) were generally equal or higher than in fresh sprouts [125], while 4 °C storage for more than 7 days revealed a significant decrease of glucosinolates in *Brassica oleracea* sprouts [152]. Plant species seem to play a pivotal role also in terms of dynamics of sprouts' microbiome during cold storage [153], but further investigations are still needed. Recently, high hydrostatic pressure (HHP) was effectively tested under cold storage (4 °C) on mung bean sprouts, showing positive effects on amino acid concentrations [154]. Moreover, Westphal et al. [155] have demonstrated that high-pressure processing (HPP) had no negative effects on the glucosinolate-myrosinase system in broccoli sprouts.

Packaging proved to be significant for the shelf life of sprouts and microgreens [156]. In general, higher products' post-harvest performance is supported by a relatively high O_2 atmosphere equilibrated under modified atmosphere packaging (MAP) with high oxygen transmission rate films [2,157]. To this purpose, Wilson et al. [158] reviewed innovative MAP technologies in fresh and freshly-cut vegetables including differentially permeable films with antimicrobial properties, elevated levels of non-traditional gases (i.e., argon, xenon), and smart packaging coupled to sensor technologies. Further efforts should be made to test all these packaging possibilities in sprouts and microgreens production chains. Studies should also address the effects of light exposure during storage on post-harvest decay and product's quality, considering that, especially for microgreens, available data appear somewhat inconclusive [142].

Beside fresh consumption, sprouts and microgreens are suitable for creating novel food products, such as juices [159], fermented drinks (i.e., juices [160]), or foods such as breakfast or snack foods, seasonings (e.g., vinegar or sauce), and weaning foods [161], probiotic beverage or foods [162], yogurt [163], powder enriched flours [164,165], and bakery products [127,166–168], and, more recently, tea [169], as well as food matrices [170]. Furthermore, the use in flour mixture of powder from sprouted wheat pomace, as by-product from juice production, was recently evaluated and found to increase of nutritional value in relation to wheat flour [171]. Processing treatments play a determinant role in maintaining biochemical quality of raw material, depending on the specific kind of processing as well as its application conditions, plant genotype, ontogenetic stage, overall plant composition, and chemical structure of specific metabolites. Conditions and methods of sprouts drying should be chosen based on whether quality can be preserved or enhanced. For example, in broccoli sprouts the highest efficiency of grinding as well as the higher total phenolic content and antioxidant activity were obtained from freeze-dried (frozen and lyophilized at 40 °C; 52 Pa) materials, although air-dried at 40 °C (air flowing, 0.5 ms^{-1}) could be also recommended [172]. In alfalfa and flax sprouts, freeze-drying minimized the chemical alteration of the product, resulting in a better preservation of antioxidants and an increase in phytosterol availability [173]. Conversely, air drying at 70 and 80 °C for 24 h, as opposed to freeze drying (below −50 °C and 0.3 mbar vacuum pressure, 36 h), increased total phenolic content and antioxidant capacity of mung bean sprouts [174]. Pan-roasting of cereal (barley, wheat) sprouts was

shown a promising heat processing for tea production, based on higher total phenolics and flavonoid contents and antioxidative effects in-vitro compared to a steaming process [169]. With respect to plant varieties, the phenolic content measured in 4-days-old sprouts of *Chenopodium quinoa* appear to be thermostable in the range 30–60 °C in the white variety (from Chile), whereas it significantly decreased after drying at 60 °C in the red variety (from Bolivia) [175]. The impact of bread-making on secondary plant metabolites was evaluated in dough enriched with 7-days-old pak choi and kale microgreens [165]. Authors confirmed the effects of thermal processing in reducing the concentrations of secondary plant metabolites; however, after bread-making it was observed the formation of structurally different secondary plant metabolites (e.g., pheophytin) and the increase in hydroxycinnamic acid derivatives (in kale), which may also confer health-promoting effects. In the case of legume-enriched breads, the bread-making process led to a slight reduction in kaempferol glycosides and chrysoeriol glycosides in bread supplemented with pea and lupin microgreens, respectively [176].

For juice production, thermal processing continues to be the principal pasteurization method given its historical use, cost, and predictability [177]. However, recent nonthermal pasteurization methods such as HPP and ultraviolet-C (UV-C) have been applied for juice pasteurization [178,179]. For wheatgrass juice, Ali et al. [180] indicated HHP as the preferred alternative pasteurization process due to its negligible impact on antioxidants and the increase in chlorophyll content.

Table 5. Overview of recent studies (dated 2016 onward) about disinfection methods applied to seeds employed for sprout production.

Disinfection Method	Disinfection Conditions	Plant Species	Pathogen/s	Treatment Effectiveness	Effects on Seed Germination	Effects on Sprouts Yield/Quality	References
Chemical							
Bleach	1 mL NaClO (4% *v/v*), 15 min	Fenugreek	Shiga toxin-producing *Escherichia coli* O104:H4	On seeds: no detectable colonies	Not affected	Not affected	[181]
Acidified (organic acids) essential oil nanoemulsions	50 mM acetic acid 50 mM levulinic acid carvacrol nanoemulsion, 4000/8000 ppm, 30 min	Mung bean, broccoli	*Salmonella enterica* Enteritidis cocktail	On seeds: 2–4 log CFU/g reduction–specie dependent; on sprouts: no detectable pathogens (<1.5 log CFU/g)	Not affected	Not affected	[182]
Chlorinated water	Treatment 1: seeds soaking, 100 ppm, 60 min; treatment 2: sprouts washing, 4 times/5 min each, 100 ppm	Mung bean	Shiga toxin-producing *Escherichia coli* *Salmonella* spp. *Listeria monocytogenes*	On seeds: 3.0 log CFU/g reduction; on sprouts: 7.0 log CFU/g reduction	–	–	[183]
Chlorine dioxide (ClO$_2$)	3 ppm ClO$_2$, 10 min	Alfalfa	*Listeria monocytogenes*	On seeds: not effective; on sprouts: not effective	–	–	[184]
Chlorophyllin (Chl)	1.5×10^{-5} M Chl, incubation time 1 h, 405 nm, radiant exposure: 18 J cm^{-2}, 1 h	Wheat	*Escherichia coli*	On seeds: ~1.5 log CFU/g.	–	–	[185]
Peracetic acid (PAA)-based solution	PAA solution (5% *v/v*) diluted in EtOH (20% *v/v*) 1:9	Flax, chia	*Salmonella* spp. *Enterococcus faecium*	On seeds: 4.0–5.0 log CFU/g reduction–specie dependent	Not affected	–	[186]
Saline organic acid solutions	Tempering solutions: lactic acid (5% *v/v*) and NaCl (~26% w/v)	Soft wheat, durum wheat	*Salmonella enterica* *Escherichia coli* O157:H7 Shiga toxin-producing *Escherichia coli*	On seeds: 1.6–2.6 log CFU/g – specie and pathogen dependent	–	–	[187]
Slightly acidic/acidic electrolyzed water (SAEW/AEW)	Available chorine concentrations (ACC) 150 mg/L, 24 h, 1.5 *w/v*	Brown rice	*Bacillus cereus*	On sprouts: 2.3 log CFU/g reduction	↑↓ pH dependent	↑↓ growth–pH dependent	[188]
SAEW	ACC 10.3-91.7 mg/L sprouts washing (1:3 *w/v*), 5 min	Buckwheat	*Escherichia coli* O78 *Listeria monocytogenes*	1.1–2.7 log CFU/g reduction–pathogen dependent	Not affected	↓ sprout length–ACC dependent	[189]
SAEW	ACC 70.0 mg/L seeds washing (1:5 *w/v*), 3 times	Pea	Natural population of total bacteria	~ 2.0 log CFU/g reduction.	–	Yield not affected; ↑ soluble sugars; ↓ nitrite	[190]
SAEW	ACC 25.0-45.0 mg/L seeds soaking (1:5 *w/v*), 0.5 h	Alfalfa	Natural *Enterobacteriaceae*	On seeds: ~ 2.5 log CFU/g reduction; on sprouts: 0.7–1.8 log CFU/g reduction	↑ or not affected – pH dependent	Not affected	[191]
Zinc diacetate	Concentration 400 mg/L seeds soaking 0.66 h, 18 °C	Durum wheat	Natural bacteria population	On sprouts: 2.0 log CFU/g reduction	Slightly decreased	↑ ash, lipids, proteins, zinc, vitamin C, total phenol content	[192]
Physical							
Atmospheric pressure plasma discharge	Atmospheric pressure volumetric dielectric barrier discharge (DBD), 10 kHz, 8 KV, 500 ns pulses, 5/10 min	Alfalfa, onion, radish, cress	*Escherichia coli*	On seeds: 1.4–3.4 log CFU/g reduction–specie dependent	↑↓ depending on temperature, time, pulse frequency or voltage	–	[193]

Table 5. *Cont.*

Disinfection Method	Disinfection Conditions	Plant Species	Pathogen/s	Treatment Effectiveness	Effects on Seed Germination	Effects on Sprouts Yield/Quality	References
Gamma ray irradiation	7 kGy, 0.073 kGy/min, 4–5 °C	Mung bean, clover, fenugreek	*Escherichia coli*	On seeds: 5.0 log CFU/g reduction–specie dependent	↓ species dependent	↓ yield	[194]
Electron-beam irradiation	12 kGy, 140 kV, 87 mm/s (12 kGy) and 250 mm/s (4 kGy)	Mung bean, clover, fenugreek	*Escherichia coli*	On seeds: not completely inactivated–specie dependent	Not affected	Not affected	[193]
Gamma ray irradiation	7 kGy, 0.073 kGy/min, 4–5 °C	Mung bean, clover, fenugreek	*Escherichia coli*	On seeds: 5.0 log CFU/g reduction–specie dependent	↓ species dependent	↓ yield	[194]
Chlorine + drying + dry heat	ClO_2-200 mg/mL, 5 min; 45 °C, 23% RH, 24 h; 80 °C, 23% RH, 48 h	Pack choi	*Salmonella enterica Escherichia coli* O157:H7	On seeds: >3.8 log CFU/g reduction–pathogen dependent	Not affected	–	[195]
Cold atmospheric pressure plasma	400 W, 5 min	Lentil	*Escherichia coli Salmonella* spp. *Listeria monocytogenes*	On seeds: ~5.0 log CFU/g reduction–pathogen dependent	↓ after 180 s	–	[196]
High-intensity light pulses	19.35 J/cm^2, 15s	Chia	*Salmonella* Typhimurium	On seeds: 4.0 log CFU/g reduction.	–	–	[197]
Intense pulsed light	24.50 and 20.81 J/cm^2 – depending on plant species	Radish, pak choi	Natural total aerobic mesophilic bacteria	On seeds: 1.4–1.8 log CFU/g reduction–specie dependent	Not affected or slightly decreased	Sprout length not affected	[198]
Nonthermal plasma	Corona discharge plasma jet, 3 min	Radish	Aerobic bacteria *Bacillus cereus Escherichia coli Salmonella* spp.	On seeds: 1.2–2.1 log CFU/g reduction–pathogen dependent; on sprouts: >2.0 log CFU/g reduction–pathogen dependent	↑↓ treatment time dependent	↓↑ yield–treatment time (min)dependent; ↓ slightly brightness and redness; not affected reducing sugars, total phenolics, radical scavenging activity	[199]
Nonthermal plasma	Corona discharge plasma jet, 3 min	Rapeseed	Aerobic bacteria *Bacillus cereus Escherichia coli Salmonella* spp.	On seeds: 1.2–2.2 log CFU/g reduction–pathogen dependent; on sprouts: ~2.0 log CFU/g reduction–pathogen dependent	↑↓ treatment time dependent	↓↑ yield – treatment time dependent; not affected reducing sugars, total phenolics, radical scavenging activity; ↓ slightly "appearance", "flavor", "taste", "overall acceptance"–treatment time dependent	[200]
Nonthermal plasma	Corona discharge plasma jet, 3 min	Broccoli	Aerobic bacteria *Bacillus cereus Escherichia coli Salmonella* spp.	On seeds: 1.2–2.3 log CFU/g reduction–pathogen dependent; on sprouts: ~2.0 log CFU/g reduction–pathogen dependent	↑↓ treatment time dependent	↓↑ yield – treatment time dependent; not affected reducing sugars, total phenolics, radical scavenging activity; ↑ slightly "flavor"; ↓ slightly "appearance", "color", "taste", "overall acceptance"–treatment time dependent	[201]
Nonthermal plasma	Corona discharge plasma jet, 3 min	Pak choi	Aerobic bacteria *Bacillus cereus Escherichia coli Salmonella* spp.	On seeds: 1.3–2.1 log CFU/g reduction–pathogen dependent; on sprouts: ~2.0 log CFU/g reduction–pathogen dependent	↑↓ treatment time dependent	↓↑ yield – treatment time dependent; not affected reducing sugars, total phenolics, radical scavenging activity; ↑ slightly "texture"; ↓ slightly "appearance", "taste", "overall acceptance"–treatment time dependent	[202]
Ozone water	2 ppm, 5 min	Alfalfa	*Listeria monocytogenes*	On seeds: not effective; on sprouts: not effective	–	–	[184]

Table 5. *Cont.*

Disinfection Method	Disinfection Conditions	Plant Species	Pathogen/s	Treatment Effectiveness	Effects on Seed Germination	Effects on Sprouts Yield/Quality	References
Plasma-treated water (PTW)	10 mL air-PTW	Mung bean	Natural population of total bacteria	On sprouts: 5.2 log CFU/g reduction	↑	↑ superoxide dismutase (SOD) activity, indole acetic acid (IAA) content; ↓ malondialdehyde (MDA) and abscisic acid (ABA) contents.	[203]
Ozone	2000 ppm, 4 h; 4000 ppm, 2 h	Wheat	Coliforms	On seeds: >1.5 log CFU/g reduction	Not tested	Minimum effect on antioxidant properties, lipid oxidation, moisture content	[204]
Ozone	5 ppm, 20 min	Alfalfa	*Salmonella* spp. Shiga toxin-producing *Escherichia coli*	On seeds: 2.1 log CFU/g reduction; on sprouts: up to 3.6 log CFU/g reduction–pathogen dependent	Not affected	Not affected yield and color	[205]
Ultraviolet irradiation, krypton-chloride excilamp	222-nm, 261 mJ/cm^2	Alfalfa	*Salmonella* Typhimurium *Escherichia coli* O157:H7	On seeds: 2.8–3.0 log CFU/g reduction–pathogen dependent	↓ slightly or not affected	–	[206]
Chemical + physical							
Dry heat + hydrogen peroxide	80 °C, 24 h; H$_2$O$_2$ (30%) solution (2% *v/v*), 10 min	Alfalfa	*Salmonella* Typhimurium	On seeds: 1.7–3.6 log CFU/g reduction	↑	–	[207]
Mild heat + hydrogen peroxide + acetic acid	60 °C, 20 min; 4% (30% *w/w* in H$_2$O), 15 min; 0.2% (5% distilled white vinegar), 15 min	Mung bean	*Escherichia coli* O157:H7 *Listeria monocytogenes* *Salmonella enterica*	On seeds: >4.0 log CFU/g reduction–pathogen dependent; on sprouts: not effective	Not affected	–	[208]
Mild heat + hydrogen peroxide + AcOH sanitizing solution	50 °C, 10 min; 2.0% H$_2$O$_2$ + 0.1% AcOH (Pure White Vinegar), 10 min	Alfalfa, radish	*Escherichia coli* O157:H7 *Listeria monocytogenes* *Salmonella enterica*	On seeds: 4.6–5.0 log CFU/g reduction – pathogen dependent	Not affected	Yield not affected	[209]
Biological							
Bacteriocins from *Enterococcus hirae* (Eh9)	Eh9 - Lactic acid bacteria (LAB) cell-free supernatant (CFS), 0.5 g in 30 mL, 10 min	Butterhead lettuce	*Escherichia coli* O157:H7	On seeds: 2.0 log CFU/g reduction	Not affected	↑ slightly weight and sprouts length	[210]
Bacteriophages	*Salmonella* phage, seed soaking, 2 h, room temperature	Alfalfa	*Salmonella enterica*	On seeds: 2.5 log CFU/g reduction; on sprouting seeds: reduced without significance	Not affected	Not affected	[211]
Bacteriophages	*Escherichia coli* O104:H4 phage cocktail, (i) free phage application: 2 mL (2.5 × 10^{10} plaque-forming units (PFU)/mL), 1 h; (ii) impregnated phage application: filter paper in 7 cm diameter blotting with 6 mL (2.5 × 10^{10} PFU/mL); (iii) encapsulated phage application: seeds placed onto the paper coated with encapsulated phage in alginate beads.	Alfalfa	*Escherichia coli* O104:H4	On seeds: below the limits of detection (< 1.0 log CFU/g); on sprouts: 1-log cycle reduction	–	–	[212]
Non-antagonistic bacterium	*Erwinia persicina* EUS78 strain, 10^8 colony-forming unit (CFU)/mL seed, dwelling	Alfalfa	*Salmonella enterica*	Not affected	Not affected	–	[213]
Lactobacillus plantarum, *Pediocaccus acidilactici* and *Pediococcus pentosaceus* mixture	7 log CFU/g, 20 °C, 5 d	Alfalfa	*Listeria monocytogenes* *Salmonella* spp.	1.0–4.5 log CFU/g reduction–depending on pathogen and initial concentration	Not affected	Not affected	[214]

Table 5. *Cont.*

Disinfection Method	Disinfection Conditions	Plant Species	Pathogen/s	Treatment Effectiveness	Effects on Seed Germination	Effects on Sprouts Yield/Quality	References
Plant extracts							
Benzyl isothiocyanate (BIT)	1.5–2.0% (v/v), 15 min, 22 ± 2 °C	Alfalfa	*Salmonella enterica* serotypes	On seeds: >1.5 and 0.7 log CFU/g reduction–serovar dependent; on sprouts: 1.2–2.3 log CFU/g reduction–serovar dependent	Not affected	Not tested	[215]
Grapefruit seed extract	0.05% aqueous solution	Lettuce	Natural population of total bacteria	Strong seed sterilization effects	Not affected	No effects on texture and color	[216]
Allspice, thyme and rosemary essential oils	Concentrations variable among species and pathogens	Alfalfa	*Salmonella* Typhimurium *Listeria monocytogenes*	Strong seed sterilization effects	Not affected	No effects on sensory properties	[217]
Savory, bay leaf and thyme hydrosols	40 min	Wheat, lentil, mung bean	*Salmonella* Typhimurium *Staphylococcus aureus*	Strong seed sterilization effects–pathogen dependent	Not affected	–	[218]

5. Sprouts and Human Health: Rewards and Riddles

Numerous studies have been conducted to investigate the role of sprouts as functional foods or nutraceuticals [219]. These two terms carry slightly different connotations depending on the jurisdiction of sale, regulatory considerations, and marketing strategies, but largely overlap and are frequently used interchangeably [220]. While functional foods generally imply health benefits in addition to nutritional value, nutraceuticals tend to denote a food (or a part of it) with an impact on prevention and/or treatment of a specific disease or disorder [221].

Sprouts are a good fit for nutrition research due to their wealth of phytochemical content combined with reduced antinutrient levels, and the expression of distinct cohorts of secondary metabolites that are subject to spatiotemporal modes of expression, which may largely diverge from patterns and profiles displayed in seeds and mature plants [219,222,223]. Furthermore, research offers the prospect of substantial rewards, given that products aimed at benefitting human health have progressively gained traction in recent years in response to increasing information and awareness about better eating habits and plant-forward diets, coupled with concerns associated with an aging population at risk of nutrition frailty [224]. Accordingly, markets for functional foods and nutraceuticals are growing globally at a stunning pace, accounting for revenues of US $174 billion in 2019 (forecasted to US $275 billion in 2025) for the former and US $383 billion in 2017 (forecasted to US $561 billion in 2023) for the latter (https://www.statista.com/statistics/591619/global-market-size-nutraceuticals/).

Whether sold for general health purposes or medical applications, claims about health benefits must be supported by rigorous and convincing methods of investigation prior to market launch, which may entail review and subsequent approval by governmental agencies in specific jurisdictions [225]. In this regard, nutrition science can face challenges, given the tension between scientific rigor, profitable business, and conflicting interests in relation to issues such as safety, health benefits, intellectual property, regulatory formats, marketing strategies, and financial affairs [226]. As for other complex sources of macro- and micro-nutrients, and secondary metabolites, the assessment of biological and nutraceutical effects of sprouts on human health is typically based on studies involving cellular systems representing a given phenotypic trait(s), preclinical studies in relevant animal models and, ultimately, human clinical trials. Investigational procedures, in particular cell-based studies, can be greatly facilitated by fast turnaround times to obtain samples, and the ability to enhance the content of specific metabolites using variable elicitation conditions, either abiotic (e.g., temperature, humidity, soil salinity, light intensity) or biotic (e.g., plant hormones, amino acids) [81]. On the other hand, metabolic intensity, biomechanical plasticity, and rapid physiological changes may negatively affect standardization and robustness of experimental conditions and, in turn, reproducibility and accurate interpretation of data.

Cell models generally serve as the biological systems for investigating phenotypic effects, or mechanistically identifying biologically relevant targets and related molecular networks. Within this context, sprouts provide the opportunity for implementing quick, flexible, and versatile procedures to concurrently develop metabolic variants for subsequent characterizations, comparisons to controls, and application of multivariate statistical analyses to discriminate the chemical entities exercising an active role on observed biological effects. By way of example, this rationale was applied by Ferruzza et al. [227] to show a protective effect by *Brassica oleracea* sprouts against a human model of gut inflammation developed in Caco-2 cells.

Although routinely carried out in many laboratories, tissue culture requires special attention to ensure a phenotypically relevant model for contextual interpretations of experiments, as well as robust and reproducible data. In this regard, the following are important considerations: (1) a specific cell line may exhibit significantly phenotypic variability across different laboratories, and should therefore be authenticated as a first step prior to experimentation [228,229]; (2) culture conditions and passage number should adhere to recommended protocols to avoid or at least minimize genomic and molecular alterations, while recognizing that cell populations are, to some degree, heterogeneous due to the likely presence of slow-cycling stem or progenitor cell subpopulations [230]; (3) primary

cells grown on plastic (2D cultures) are rapidly subject to phenotypic changes such that they no longer reflect the biology of parental tissues [231]; and (4) cancer cell lines generally double at much higher rates (e.g., 18–24 h) than native tissues and do not represent the phenotypic multiplicity shown by the corresponding patient cohorts [232]. However, co-cultures can offer valid models to assess the effects of nutraceuticals and functional foods in that cell-cell contacts as well as paracrine or juxtacrine interactions are relevant to physiological and pathological environments, and may markedly influence sensitivity to external challenge [233]. This was clearly illustrated when quercetin and genistein, two polyphenols with proven anti-inflammatory properties, were used as positive controls to counteract the inflammatory response provoked by interleukin 1β in human pluripotent stem cell-derived endothelial cells (hPSC-EC). Notably, both polyphenols were inactive when tested in these cells, but displayed a powerful anti-inflammatory response when hPSC-EC were co-cultured with their hepatocytic counterpart hPSC-HEP that, unlike hPSC-EC, retains a metabolic capacity to breakdown quercetin and genistein into bioactive molecules [234]. Finally, it should be noted that organoids and 3D cell-based assays, despite technical challenges [235], represent the most recent advance in tissue culture to recapitulate the cellular and biological complexities of tissues and organs. Although already used for screening purposes [236], this technology is still in its infancy and, to our knowledge, not yet employed in nutraceutical screens.

Cruciferous, grain, and legume sprouts have been tested in preclinical and human studies for their preventive and therapeutic benefits across a variety of health and pathological conditions. These investigations, extensively described and critically reviewed [27,237,238], illustrate the typical complexities of food-based trials, particularly with regard to considerations and concerns over regulatory and scientific matters, and debates about the functional and biological significance of experimental data [225,239]. In short, the same dilemma exists today as was raised by Clare M. Hasler almost 20 years ago following a 1999 guidance by the Food and Drug Administration [240]; namely, the distinction between *emerging evidence* and *strong scientific agreement* by which a product can be marketed as a dietary supplement to enhance nutrition and achieve physiological benefits, or a medical treatment to prevent or attenuate symptoms, or treat a condition. However, scientific rigor can often be questionable and not sufficiently strong to permit accurate conclusions or comparisons. For plant-derived food, including sprouts, the lack of standardized protocols for the preparation and manipulation of the natural matrix is a crucial limitation. Sprouts, in fact, have been used in animal and human studies as a whole, in the form of extracts, and juices, using different amounts and inconsistent dietary regimens within the population appraised [241,242]. Variable conditions can impact bioavailability, while elusive information about liberation, absorption, distribution, metabolism, and excretion (LADME) phases of pharmacokinetics can complicate decisions as to when and for how long a treatment should be considered [243]. For example, when ingestion of broccoli sprouts (100 g/day) was compared to the same daily amount of alfalfa sprouts (used as the placebo) in 40 (1:1 randomized) asthmatic individuals to evaluate anti-inflammatory and physiological improvements, Sudini et al. [244] used a 3-day intervention protocol that, despite having been purposely developed to mitigate variability, proved to be essentially inconclusive. Similarly, no protective effect was shown in a small-case study by Duran et al. [245], where 16 healthy individuals (1:1 randomized) followed a 3-day diet including 200 g/day of either broccoli or alfalfa sprouts (200 g/day in both cases) prior to exposure to ozone-induced neutrophilic airway inflammation. Conversely, Brown et al. [246] showed that a sulforaphane-enriched extract from broccoli sprouts taken for 14 days by 45 moderately asthmatic patients beneficially impacted the methacholine bronchoconstriction challenge in 60% of cases, with 20% of patients negatively affected and the remaining 20% unresponsive. Notably, while the study from Duran did not identify any change in the expression of NRF2 (Nuclear factor erythroid 2-related factor 2)-dependent genes (known to be induced by sulforaphane), Brown et al. [246] found instead that activation of the NRF2 pathway correlated with bronchoprotective benefits. Overall, these studies show that divergence of key experimental factors, such as quality and quantity of the material used, time of intervention, size of population tested, and endpoint measurements, can lead to inconsistent

conclusions, further exacerbated by population heterogeneities [247], as well as bi-phasic or hormetic responses commonly raised by bioactive agents including phytochemicals [248]. Landberg et al. [249], for example, advanced the importance of a methodological approach to stratify clinical trial participants based on the integration of different parameters such as genetic (polymorphisms), phenotypic (age, gender, body mass index (BMI), and the gut microbiome) and ADME profiles to define metabolic representations, namely metabotypes, to ultimately strengthen the association between the effect of plant-based food or any of its (semi)purified components and a specific group of individuals, thus, implementing what it could be referred to as *personalized or precision nutrition*.

6. Antinutrients and Allergens in Sprouts

Antinutrients in plant-based foods have been for years a subject of much interest in human health and malnutrition. Briefly, antinutritional factors are defined as compounds or substances, which negatively interfere with the absorption of other nutrients in the diet, causing reduced nutrient intake, digestion, and utilization, and the occurrence of adverse effects. However, like any other bioactive compounds, antinutritional factors can be responsible for a variety of effects, mainly depending on concentrations and the physiology of the subjects exposed [250,251]. For example, tannins are generally considered to be antinutrients, but Kumari and Jain [252] observed a useful role in diabetes management. A similar circumstance was shown for phytic acid [253]. Thus, these two cases support Calder's [254] statement "*nutrition science would be wise to adopt practices more akin to pharma when evaluating the functional properties and health impacts of foods, nutrients and non-nutrient food components*". For a more complete list of major antinutrients and their role in human nutrition, which goes beyond the scope of this review, we recommend the work by Akande et al. [255].

In general, the germination process is known to decrease the concentration of antinutrients in the resulting sprouts. This has been extensively highlighted in the literature for many different species, i.e., green gram, cowpea, lentil and chickpea [256,257], buckwheat [258], quinoa [259], millet [260,261] barley [262], and flaxseeds [263]. In particular, Pal et al. [264] compared the effects of germination, dehulling, and cooking in horse gram, and showed different patterns of nutrient/antinutrient ratios in relation to treatments. In particular, cooking outperformed the natural germination process in lowering the levels of tannins, phytic acid, and trypsin inhibitors in lentils [264]. We must say that while a rich source of literature has shown antioxidant profiles in sprouts, little information is instead available about modifications of antinutrients profile during the germination process. For example, during the germination of common black beans, it was found that while trypsin inhibitors and phytates decrease, levels of phenols, saponins and tannins trend higher [265]. In the same study, the application of a Controlled Pressure-Drop demonstrates to further lowers trypsin inhibitors and extra raises saponin concentration, while it counters the decrease of phytates and the increase of phenols and tannins [265]. Since the biochemical characteristics of sprouted seeds are strictly related to sprouting conditions, different techniques, alone or in combination, could be performed to easily impact antinutritional levels. For example, lowest phytic acid contents were observed in 48h-old chickpea sprouts grown under blue and red lights [266]. Moreover, advances in molecular breeding raise prospects for future breeding programs aimed to select varieties characterized by low levels of antinutrients or toxins.

The allergenic potential of sprouts and its management is an issue of major concern in food science as well. Allergic reactions are generally mediated by proteins that act as antibodies. During sprouting, a deep modification of proteins profile occurs, thus potentially reducing the concentration of allergenic storage proteins. The peanuts case has been well dug: the results of the in-vitro immunoreactivity of the protein Ara h 1 showed that it remains stable in cotyledons and decreases in others epigeic tissues during the germination process [267]. In the meanwhile, whilst the Ara h 2 antigen is decomposed, resveratrol—a bioactive compound that, in-vitro, interferes with the immune reaction to both Ara h 1 and 2—is newly synthesized [268]. A similar process appears in lentils and mung bean, where germination, associated with cotyledon removal reduced immunoreactivity in-vitro by 97% and 99%, respectively [269]. Resveratrol is a secondary metabolite produced in response to biotic

or abiotic stresses. Ultrasound application on peanut seeds proved to be an effective solution to increase resveratrol content in related sprouts, as shown by Yu et al. [94], who found amounts that, compared to controls, ranged 1.7–3.3-fold higher. Moreover, ultrasound applications resulted in an accelerated degradation of allergenic proteins, leading concentrations to undetectable levels after 3 days of germination [94]. Ultrasounds at different power levels (from 0 W to 300 W) have also been tested on soybeans seeds, showing that concentrations of allergenic proteins decrease with increasing power of applications after 5 days of sprouting [95]. Soybeans have been also treated with HHP, and the concentration of allergenic proteins resulted lower in sprouts in relation to raw seeds [270].

Although in some cases the consumption of germinated seeds does not cause an allergic reaction as severe as that associated to raw seeds, sprouts can manifest unexpected cross-reactions in sensitive individuals. In mung bean sprouts Vig r 6 and Vig r 1 proteins can activate basophils high concentrations, and cause the same allergic reaction caused by a protein responsible for pollen allergy, namely Bet v 1 [271]. Cross-reactions can also underlie allergic conditions, as shown for peanuts and sprouts of different *Leguminosae* species. Jensen et al. [272] compared in-vitro the proteic extract of soybean, green pea, blue lupine, mung bean, alfalfa, broad bean and adzuki bean sprouts with peanut proteic extract and showed that the concentration necessary to achieve the same level of histamine release varied by 3 to 6 orders of magnitude, thereby posing a high risk to sensitive subjects.

7. Sprouts: A Compelling Case for Drug Discovery?

The biological and pharmacological activities of whole sprouts or their extracts have been extensively investigated at different levels, including preclinical models, and human studies centered on multiple conditions [27,237,241]. Despite such a wealth of information, sprouts were never systematically employed to generate natural product collections aimed at selecting bioactive entities applicable to chemical genetics and medicinal chemistry programs. Instead, it would seem that this could be a valuable pursuit as explained below.

Firstly, sprouts can grow quickly, under consistent and controlled conditions within relatively small footprints and confined layouts, and potentially scaled-up for industrial production based on FDA-recommended guidelines [273]. Notably, the opportunity to obtain natural products from sprouts produced via local greenhouse arrangements offers environmental and ethical advantages, by easing concerns around possible insults to sustainable biological diversity arising out of traditional approaches to drug discovery. In fact, after the 1992 United Nations Convention on Biological Diversity (integrated a decade later by the Nagoya Protocol) [274], traditional approaches based on collections of living organisms from different geographical regions and ecosystems have posed challenges in terms of securing international access to natural products as well as sharing intellectual property rights with source countries [275].

Secondly; sprouts, such as plants, stand out for chemical diversity, which may arise as a result of (1) the influence that genetic and environmental factors, and agronomic setups, may have on metabolic homeostasis [276]; (2) chemical sensitivity to elicitation techniques that may be established through a wide array of combinations between germination conditions and abiotic and/or biotic stimuli [4]; and (3) the occurrence of alternative biosynthetic routes in relation to changes in gene expression profiles [277]. Together with the vast number of structural modifications exhibited by plant metabolites [278], such metabolic adaptability can potentially contribute to the expansion of natural product chemistry into the uncharted regions of druggable space, namely, the realm of chemicals that specifically interact with, and modulate, disease-relevant targets [279–283].

Thus, sprouts appear to be a good candidate for the OSMAC (One Strain MAny Compounds) concept, which refers to the rational, systematic modification of culture conditions aimed at maximizing the range of secondary metabolites produced in a given system. Initially described in 2002 [284], the OSMAC rationale was developed to trigger, or unlock, metabolic gene clusters that consistently remain unexpressed under common or standard culture conditions, and successfully applied to fungi, bacteria, and marine microorganisms as primary sources of chemicals for drug research [285].

This approach can also be relevant to sprouts, given that genetically clustered metabolic pathways exist in plants, and have been linked to the biosynthesis of bioactive chemicals, such as terpenes, alkaloids, diketones, cyanogenic glucosides, and hydroxamic acid [286]. The investigation of biosynthetic gene clusters in plants using sequencing technologies, bioinformatics tools, and predictive computational solutions [287–291] is a rapidly expanding field leading towards unprecedented possibilities for tapping into unexplored metabolic pathways, identifying new molecules, and uncovering mechanisms of metabolic diversification. In this regard, the dynamic rearrangement of neighboring genes was recently shown in *Brassicaceae* to rewire triterpene biosynthesis and, consequently, account for triterpene metabolic diversity [292].

Thirdly, the systematic investigation of sprouts in drug discovery programs can be strongly facilitated by major advances in the production of natural product collections [293]; these include, for example, methods of sample extraction, pre-fractionation and drug-like enrichment [294–296]; NMR, MS and related hyphenated techniques for dereplication and unambiguous identification of novel chemicals [297–299]; effective and versatile solutions in high-throughput technologies [300,301]; design, optimization, and miniaturization of cell-free and cell-based screening assays [293]; and, finally, progressively easier access to integrated *omics* platforms applicable, for example, to target identification programs and mechanistic elucidation of a drug's biological impact, efficacy, and side or adverse effects [302] (Figure 1).
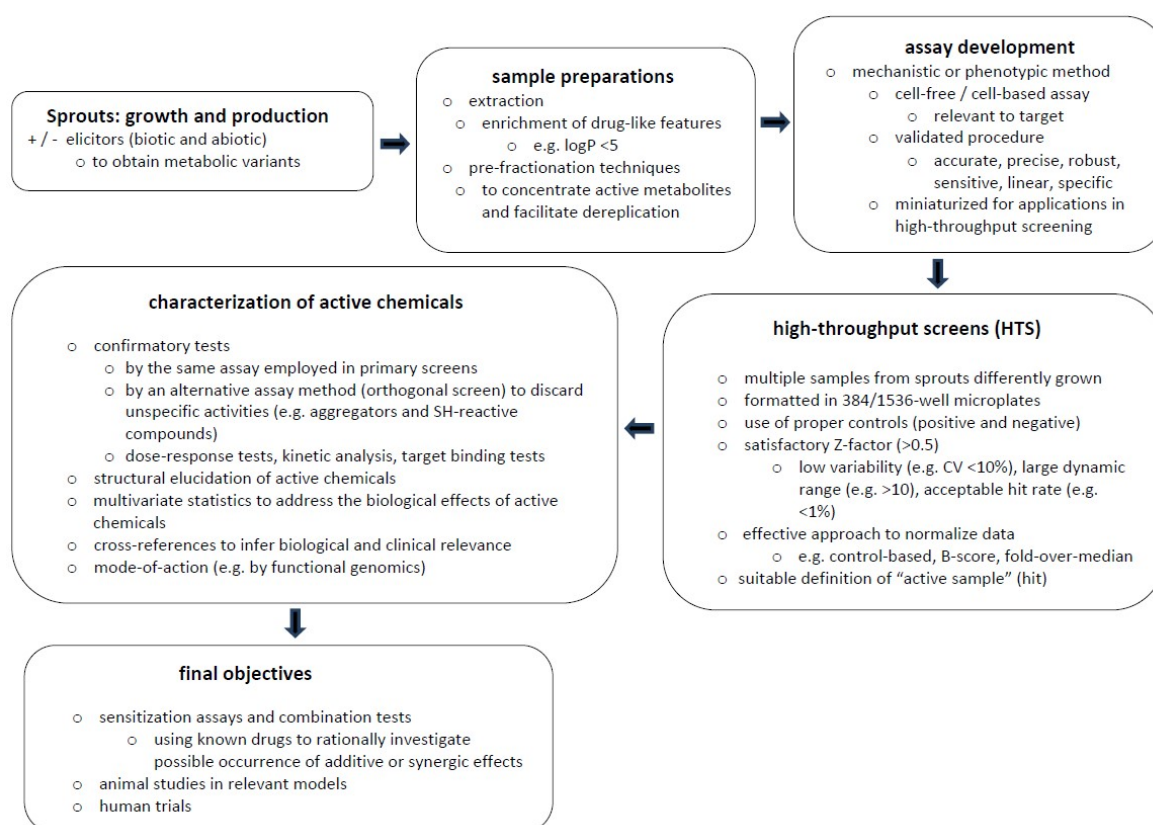
Figure 1. Schematic example of an experimental workflow aimed to select and identify pharmacologically active chemicals from sprouts.

It is emblematic that in-vitro and in-vivo studies have almost exclusively been carried out using whole sprouts or, at best, fractionated extracts performed by non-standardized protocols [27,241]. This approach, while offering the possibility of uncovering health benefits and nutraceutical properties of functional foods, gives rise to significant challenges in drawing definitive conclusions due, for example, to variability in the production and preparation of the food matrix, dietary regimens and, importantly, the effects caused by interactions between active nutrients, as in the case of polyphenols where a fine line runs between additive, synergic, and antagonistic effects [303–305].

Fourthly, sprouts contain significant amounts of phytochemicals, which have historically been studied in human clinical trials. In this regard, phenolics (and, particularly, flavonoids [306]) and the breakdown products of glucosinolates, isothiocyanates, and indoles [27,242], have been investigated, either singly or combined with other treatments. Although many of these chemicals are very well known and have been comprehensively reviewed, one must note that phenolics account for more than 8000 widely and differentially distributed entities [307], and glucosinolates (both fully and partially characterized) are represented by at least 137 different structures [308] subject as well to genetic and environmental variability [309]. Such extraordinary diversity, combined with a multi-target chemistry shown by polyphenols [310], flavonoids [311], and isothiocyanates [312], may represent novel and significant opportunities to explore, and possibly extend, druggable space [313].

An example of such an opportunity is illustrated by sulforaphane, an isothiocyanate resulting from the hydrolysis of its glucosinolate precursor, glucoraphanin, which is 10–100 times more abundant in broccoli sprouts than the mature plant [314]. Sulforaphane was isolated in 1992 as a cancer chemoprotective inducer of Phase II detoxifying enzymes and used in >2000 studies to investigate (1) mechanistic function over the NRF2/ARE axis and downstream activation of hundreds of cytoprotective genes; (2) chemical reactivity and structure-activity relationship (SAR) analyses; (3) pleiotropic roles; (4) biological significance in disparate cellular and animal models; and (5) disease prevention and therapeutic effects in humans (reviewed in [241,315]). In the last 15 years, sulforaphane from broccoli sprouts (introduced in the diet as a whole, or administered in the forms of homogenates, extracts, beverages or caplets) was investigated in >30 human trials targeting a large variety of conditions including cancer, inflammation, diabetes, obesity, cardiovascular disease, bacterial and viral infections, respiratory diseases, and neurological disorders [241]. Currently (as of August 24, 2020), 14 such trials are in the *recruiting* or *not-yet-recruiting* stage (https://clinicaltrials.gov).

Such keen interest in the molecule's potential can most likely be attributed to its excellent bioavailability [316,317] and an overt plurality of biological effects evidenced by over 200 high-confidence targets observed in breast cancer cells [318] and the potential for hermetic behaviors, as shown in several cell models [319]. While other isothiocyanates have not been studied as extensively as sulforaphane, its polypharmacological behavior may reflect a general signature of these chemicals, based on their chemical structure, lipophilicity, and reactivity [312]. The same can be said for indoles, such as indole-3-carbinol, a glucobrassicin derivative that displays differential effects depending on timing of administration, and its condensation product, 3,3 diindolylmethane, which impacts multiple pathways associated with cancer and inflammation. Notably, both indoles have been tested in human clinical trials, either alone or in combination with other treatments [242].

Polypharmacology is an intriguing point that merits special attention, given a growing consensus in recent years towards a drug discovery shift from the traditional "magic bullet" concept of *one drug/one target*, to a preference for chemicals that act on multiple molecular targets and effect various downstream functions. This approach, while potentially risky in terms of drug promiscuity and side effects, may nonetheless provide solutions for optimizing drug safety/efficacy and/or conceiving target/therapeutic reposition strategies by *omics* technologies in system biology (e.g., functional genomics) and cheminformatics [320,321].

In summary, sprouts can be pivotal in drug discovery research, well beyond nutritional and nutraceutical prerogatives. Their chemical content is a rich and versatile source of functional ingredients that, when mixed in extracts, display biological activities which, however, may not necessarily reflect the biological potential of purified or semi-purified entities. With the latter, selection of lead candidates can be pursued using either classical or reverse pharmacology (i.e., phenotypic- or target-based, respectively), whereas sample extracts are generally limited to phenotypic bioassays where technical difficulties often arise due to the occurrence of false negatives.

As a preliminary step, we carried out a high-throughput screening campaign, using HPLC-prefractionated extracts, to assess whether and to what extent different species of sprouts had an impact on the effects shown by approximately 1000 marketed drugs and 640 kinase inhibitors in a variety of cancer cells lines. Using cytotoxicity as the primary measurement, we found in several instances that phenolics-enriched fractions displayed either synergic or antagonistic effects in presence of several drugs and that, notably, these effects were frequently associated with specific cell types. For example, a markedly cytotoxic effect by Tofacitinib observed in HeLa cells (but not LNCaP cells) was virtually abolished in the presence of specific fractions of maize sprout extract, similar to what known antioxidants (i.e., resveratrol and quercetin), included for comparison purposes, had shown. On the other hand, synergic effects were observed when the same extract was combined with Dovitinib in HeLa cells, and Tivozanib in LNCaP cells (Datti, unpublished results). No effects, instead, were observed when whole extracts were used as a reference, thereby suggesting, in this case, new unexplored avenues to assess the integration of nutritional supplements and dietary habits with anticancer chemotherapeutic treatments.

8. Sprouts in Animal Feeding

The use of sprouts to supplement animal feeding can be aimed at maintaining or enhancing animal health as well as transferring phytochemicals to livestock products (milk, meat, eggs), which are subsequently ingested by humans. Through this approach, it is possible to bypass the risks to human health posed by microbiological contamination of raw sprouts. Moreover, sprouts can be integrated into feed rations in seasons when fodder production is limited [322].

Despite a mutual relevance to zootechnics and human nutrition, literature on this field is scant (Table 6).

Table 6. Livestock species fed with micro-scale vegetables to improve quality and nutritional features of derived products.

Livestock	Sprout Species	Sprout Family	Days after Sowing (DAS)	Effects on Livestock Health and Products	Phytochemicals Transferred to Livestock Products	References
Poultry						
Betagro laying hens	*Brassica oleracea* var. *Alboglabra*–Chinese kale	*Brassicaceae*	15	↑Se content in eggs and tissues	N/A	[323]
Japanese quails	*Hordeum vulgare*–barley	*Poaceae*	7	↑egg laying rate; ↑relative weights of gizzard and testis; ↑ fertility; ↑ number of hatched chicks/female; no effect on egg quality indices	N/A	[324]
	Brassica oleracea var. *alboglabra*–Chinese kale	*Brassicaceae*	7	No effects on performances and carcass characteristics; ↑Se content in tissues	N/A	[325]
			15	Se from sprout does not affect performances and egg quality; ↑Se content in eggs	N/A	[326]
Leghorn hens	*Medicago sativa*–alfalfa	*Leguminosae*	3	↓cholesterol content in eggs	↑ isoflavones, antioxidants and vitamin content in eggs	[327]
	Linum usitatissimum–flax	*Linaceae*	3	↓cholesterol content in eggs	↑ isoflavones, antioxidants and vitamin content in eggs	[327]
Lohmann Brown hens	*Brassica oleracea* var. *alboglabra*–Chinese kale	*Brassicaceae*	7	↑ Se bioavailability and Se content in eggs, tissues and plasma	N/A	[328]
Rabbits						
New Zealand white rabbits	*Medicago sativa*–alfalfa	*Leguminosae*	3	↑ n-3 fatty acid contents of meat	↑phytoestrogens in muscles	[329]
	Linum usitatissimum–flax	*Linaceae*	3	↑ n-3 fatty acid contents of meat	↑phytoestrogens in muscles	[329]
	Hordeum vulgare–barley	*Poaceae*	12	No adverse effects on performances and meat characteristics	N/A	[330]
Ruminants						
Awassi male lambs	*Hordeum vulgare* – barley	*Poaceae*	7	↑some rumen characters; ↑most nutrient digestibility; ↑ feeding efficiency	N/A	[331]
Barky ewes	*Hordeum vulgare*–barley	*Poaceae*	15	↑NDF and ADF digestibility; ↑fat, total solid, ash and energy of milk	N/A	[332]
Rams	*Hordeum vulgare*–barley	*Poaceae*	8	↓DM intake; ↑enzyme activity and digestive function	N/A	[332]

ADF = acid detergent fiber; N/A = not assessed; NDF = neutral detergent fiber.

Studies about sprouts for livestock feeding initially dealt with Se-enriched species aimed at transferring Se to poultry meats and eggs [323,325,326,328]. Early research aimed at studying nutritional properties of rabbit meat was carried out by Dal Bosco et al. [329], who used alfalfa and flax sprouts, and by Mohsen et al. [330], who used barley sprouts. Both studies demonstrated an increase in rabbit meat quality. Barley sprouts were also used by Helal et al. [332] to feed ewes. Mattioli et al. [327] employed alfalfa and flax sprouts to supplement hen diet, with positive outcomes on hen plasma and egg nutritional quality (lower cholesterol and higher phytochemical content). These first results encourage further studies on (1) combinations between various sprout species and livestock species; (2) the effect of different levels of supplementation; (3) the effect on animal health indicators; and (4) the effect on livestock product quality (meat, milk, eggs).

No studies have been performed relating to sprout supplementation in pigs; however, the positive effects of a flavonoid-enriched diet on pig growth and health give reason to encourage investigations in this species [333].

As regards supplementation levels, since high amounts of fresh sprouts might reduce feed consumption and energy intake, studies are needed to evaluate their use in the form of dried powder or pellet. Mattioli et al. [173] demonstrated that drying, in particular freeze-drying, did not compromise the bioactive compound contents of alfalfa and flax sprouts, and considered this evidence as promising for future research on the effect of different levels of supplementation.

An intriguing research prospect is the potential use of sprouts and microgreens to supplement the diet of pets. Pets tend to be considered family members, thereby inducing owners to search for healthy and nutraceutical foods, both for disease prevention and improving quality of life [334]. As an example, the grass (i.e., 10–15 Days After Sowing, DAS) obtained from barley, wheat, oat, or rye, improperly called "catnip", is said to facilitate cat digestion and regurgitation of hairballs. True catnip (*Nepeta cataria*), instead, decreases the stress of homebound cats, by stimulating their olfactory system and causing an apparently euphoric reaction [335]. Further research is needed in all pet species using multiple varieties of sprouts. Another possible avenue of study is the use of sprouts to feed insects. The sector of edible insects is rapidly expanding and shows wide research possibilities, including in relation to rearing [336]. All of the foregoing subjects deserve to be investigated using a multidisciplinary approach involving sprout scientists, food technologists, animal dieticians, animal growers, and veterinarians.

9. Conclusions

Sprouts and microgreens have been extensively studied in recent years, however several aspects remain under-investigated or unexplored. New research prospects may be focused on the production stage (e.g. genotypic analyses, seed treatments, elicitation techniques, biofortification), post-harvest processing (e.g. seed sanitation, shelf-life extension), the impact on human nutrition and animal feeding and, most intriguingly, drug discovery. Since these areas of investigation may overlap, successful research will be contingent on multi- and inter-disciplinary collaborative efforts.

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