Sprouted Grains

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"Sprouted grains" are defined by the American Association of Cereal Chemists (AACC) with the endorsement of the United States Department of Agriculture (USDA) as follows: "malted or sprouted grains containing all of the original bran, germ, and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labelled as malted or sprouted whole grain".

Keywords: whole grain ; germination ; sprout ; elicitation ; phytochemical ; health ; microbiological safety

1. Introduction

Nowadays consumer lifestyle has shift towards "healthy living and healthier foods", consequently food demand is more oriented towards diets rich in fruits and vegetables, characterized by high content of bioactive molecules. A particular role is played by ready-to-eat vegetables harvested at the initial and very earliest plant growth stages, which are commonly known as sprouted seeds. The term "sprouted seeds" involves different types of products obtained from seeds, depending on the part of the plant collected and consumed—in particular whether the seed is comprised or removed—and on the growing substrate and environmental conditions during sprouting. For each of these products several ambiguous commercial definitions occur (i.e., microgreens, shoots, babygreens, cress, wheatgrass), widespread even in the scientific literature, and the same term could refer to different types of product.

This frequently leads to misunderstanding, depriving specialists of the basic terminology on which it is necessary to point out, since the only legal definition in Western countries is given for "sprouts" and "sprouted grains".

"Sprouts" (Regulation (EC) No 208/2013) are "the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed". "Sprouted grains" are defined by the American Association of Cereal Chemists (AACC) with the endorsement of the United States Department of Agriculture (USDA) as follows: "malted or sprouted grains containing all of the original bran, germ, and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labelled as malted or sprouted whole grain" [1].

Basically, it is essential to clarify that: (i) shoots are intended to come from the germination of seeds in water, to produce a green shoot with very young leaves and/or cotyledons—the final product does not include the seed teguments and the root [2][3]; (ii) cress is the shoots grown in soil or hydroponic substrate, sold as the entire plants [2][3]; (iii) microgreens is the most common and equivocal market term, it may be referred to as seedlings at fully expanded cotyledon stage [4], or at the first true leaf stage, sold with stem, cotyledons, and first true leaves [5]; (iv) wheatgrass is the youngest stage of wheat plant which grows from the wheat grain and takes 6–10 days to germinate [6]. In general, wheatgrass refers to shoots of *Triticum aestivum* Linn. [7] but it could be extended to other species of *Triticum* genus as well as to species from *Poaceae* family, although several authors have utilized specific terms such as "ricegrass" [8] and "barleygrass" [9]. For this reason, the generic term "cereal grass" could be utilized.

1.1. Use of Sprouted Seeds in Human Nutrition

Sprouting of seeds has been known for a very long time, mainly in the Eastern countries where seedlings are traditionally consumed as an important component of culinary history. Starting from the 1980s, the consumption of sprouted seeds raised popularity also in the Western countries due to the consumer demand for dietetics and exotic healthy foods; in the latest years the interest around sprouted seeds has been focusing principally on low processing and additive-free. Given their peculiar characteristics such as unique color, rich flavor and appreciable content of bioactive substances, they could be used to enhance the sensorial properties of salads, or to garnish a wide variety of high-quality products [5]. Moreover, sprouting is a simple and inexpensive process which can be done without sophisticated equipment, has a quick production cycle (two to three weeks at most), occupies very little space in greenhouse production [10][11] and provides fairly high yields [12].

Beside malting—which represents a special kind of germination used for the production of alcoholic beverages—cereal seedlings might be consumed in the form of ready-to-eat sprouts or further processed, e.g., dried or roasted [13]. A possible trend is the supplementation of wheat bread in flour from sprouted cereals and pseudocereals [14]. However, the high accumulations of enzymatic activity under uncontrolled germination conditions may adversely affect the physical properties of dough and the resulting baking performance, making the use of sprouted cereals for baking more challenging [15]. The dehydrated sprouted cereals can be also used for making noodles, pasta, laddu, unleavened bread and porridge [16]. Functional beverages—obtained by lactic acid fermentation of mixture based on sprouted grains and flour [17]—represent a possible future perspective. Indeed, cereals contain water-soluble fiber, oligosaccharides and resistant starch, and thus have been suggested to fulfill the probiotic formulations. At least, wheatgrass is mostly consumed as fresh juice or as tablets, capsules and liquid concentrates [18]. Further perspectives could be given by the use of cereal sprouts as supplements in animal feeding, as it has been proposed for non-grain species [19][20].

2. Changing in Chemical Composition during Germination

By definition, germination incorporates those events that begin with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryo axis, usually the radicle, which extends to penetrate the structures that surround it [21]. The subsequent mobilization of the major storage reserves is associated with the growth of seedling [21]. Therefore, physical and biochemical events underlie this process, i.e., weakening of seed covers, turning on of metabolic activity, activation of gene transcription, relaxation of the embryonic cell walls, and reassembly and biogenesis of organelles [22].

Briefly, during a first phase (Phase I) there is a rapid imbibition of water by the dry seeds until all of the matrices and cell contents are fully hydrated. Then, a second phase (Phase II) involves a limited water uptake (plateau phase) but a strong metabolic reactivation. The increase in water uptake associated with Phase III is associated with cell elongation leading to completion of germination [21]. Upon imbibition, the quiescent dry seeds rapidly restore their metabolic activity, including remobilization, degradation and accumulation, which imply important biochemical, nutritional and sensorial changes in the edible products [23]. The outcoming primary and secondary metabolites exert differential biological health effects when consumed compared with non-germinated seeds [24][25][26].

The extent of the changes of the principal metabolites, observed during whole grains germination, as well as the involved enzymatic activity are reported along this section. However, it should be underlined that most of the reported results refer to seedlings observed in the early germination stages and strictly depend on the species, seedling growth stage, germination conditions and laboratory techniques, which can greatly differ among experiments.

3. Factors Influencing Nutritional Quality of Germinated Whole Grains

3.1. Genotype and Seed Source

The most significant role in the determination of nutritional value of sprouted grains is played by the genotype. In the last years, several studies focused on the characterization of grains from different ancient and modern cereals genotypes [27][28][29][30][31][32] as well as pseudocereals species [33][34], principally in terms of bioactive compounds.

As is known, the biochemical composition of whole grains is also conditioned by the environmental condition during crop growth, especially during grain development. Bellato et al. [35] have investigated on the content of total polyphenols, antiradical activity and 5-n alkylresorcinols of 30 Italian commercial varieties of durum wheat, grown in two different geographical areas located in Central and Southern Italy. Results showed that the contribution of genotype (G) x environment (E) interaction to the total variability was lower than that due to the separate effects (G and E), and E accounted for the highest proportion of the variation. In particular, alkylresorcinols concentration increased in environment with dry conditions during grain filling, while high water availability during grain development was favorable to free phenols accumulation. Environment has been also indicated as the main factor contributing to the total variation in some quality parameters in the case of winter and spring wheat varieties [36]. In the same location, phenolic and flavonoid contents in durum and soft wheat were higher in years characterized by lower temperatures and higher rainfall during the 30 days before harvest [37]. Different effects of abiotic stresses during ripening (i.e., drought or high temperatures) have been observed [38], with increases in protein [39][40] and carotenoid content [41], and contrasting effects on starch quality [42]. This is generally because dehydration stress shortens the grain filling period.

The nutritional value of wheat grains is also influenced by the exposure of the mother plant to biotic stress (i.e., pathogens, weeds) and nutrient shortage. Many studies, focusing on the differences in nutritional values between organic and conventional cereals, have led to contradictory results [43]. In general, organic products have a lower content of proteins; however, old wheat varieties show a more efficient nutrient use in low-N environments compared to the modern ones, which are strictly dependent on high-level of available N [44]. Although phenolic compounds accumulate more under biotic stress conditions, and consequently organic crops are often thought to contain more phenolic compounds, the effect of cultivation system on secondary metabolites content is often not significant, also in terms of single phenolic acids [43]. The influence of different agronomic practices or environmental stresses on secondary metabolites in the field is naturally modified by the effects of other potential co-variables. Under greenhouse conditions, three Chilean landraces of quinoa exposed to two levels of salinity (100 and 300 mM NaCl) starting from 34 days after sowing showed a deep change in the amino acid composition and protein profiles of the main seed storage proteins as well as in the contents of bioactive molecules [45].

3.2. Germination Conditions

Biochemical changes during sprouting occur depending on germination conditions as well as on the "seed invigoration" treatments applied to the grains in order to improve the germination and post-germination seedling growth. Seed priming is a pre-sowing treatment during which seeds are hydrated with a solution that allows them to imbibe and go through the first reversible stage of germination but does not allow radicle protrusion through the seed coat [46]. Common priming techniques include osmopriming (soaking seeds in osmotic solutions such as polyethylene glycol, PEG), halopriming (soaking seeds in salt solutions) and hydropriming (soaking seeds in water) [46].

Recently, researches were addressed to identify the optimal combination of temperature and time during pre-sowing and sprouting treatments, to obtain higher quality sprouts, especially in terms of phytochemical content. These goals are achieved through the use of sophisticated statistical techniques including the response surface methodology approach. For example, in Ecuadorian brown rice cultivars, soaked grains (deionized water, 28 °C, 24 h) were introduced in a germination cabinet at 28 and 34 °C in darkness for 48 and 96 h [47]. The multiple linear regression predicted optimal germination conditions for accumulation of GABA and antioxidant activity after soaking followed by germination at 34 °C for 96h, while the highest total phenolic content was obtained in the combination of 28 °C for 96 h, although differences between genotypes were recorded [47]. In foxtail millet the highest total phenolic content, total flavonoid content and antioxidant activity were obtained with 15.84 h of soaking in tap water at room temperature and 40 h of germination at 25 °C [48]. The optimum germination conditions for sorghum suitable for supplementary food formulations (i.e., low tannin and high protein contents) were established to be steeping for 24 h at 31 °C plus germination for 4.5 days at 30 °C [49]. The highest antioxidant concentrations in wheat sprouts were achieved following 7 days of germination at 16.5 °C [50] while in purple corn sprouts it was possible to maximize the content of GABA, total phenolic compounds and antioxidant activity using a germination temperature of 26 °C for 63 h [51]. The same conditions were guaranteed by kiwicha (Amaranthus caudatus) sprouts richer in GABA and phenolic compounds [52], whilst the highest phenolic content in sprouted quinoa was obtained at 20 °C for 42 h [53]. The results reported here refer to specific experiments conducted in controlled environments, under specific laboratory conditions and germination times, so that the stage of cereal grass is not always reached. Accordingly, the optimization of seedling growth parameters needs to be deeply investigated.

Not always pre-sowing treatments induce a greater accumulation of bioactive compounds. During the 24 h soaking period, an increase of total phenolic compounds was observed in brown rice [54] and wheat [50]. The pH of the soaking solution can affect the enzymatic activities in seeds allowing to enhance or reduce the phytochemical content in sprouted grains. In brown rice the optimal pH for higher GABA content ranged from 3.0 to 5.8 [55], since a lower cytosolic pH stimulates GAD activity [56]. GABA content of germinated barley grains after steeping in a buffer solution (pH 6.0) was slightly higher than that in water [57].

Sub-optimal conditions during germination process can lead to an accumulation of phytochemicals in seedlings due to the activation of secondary metabolism [58]. Rehydration involves high levels of oxidative stress, so that abiotic stress induced during seed germination can intensify the production of reactive oxygen species (ROS), which could damage the structures of DNA, protein, lipid, and other macromolecules in the seeds. Therefore, ROS scavenging is pivotal for seed germination under stress conditions and comprises non-enzymatic components, mainly linked with overproduction of antioxidants (e.g., phenolics) [59]; an induced environmental stress during germination can be classified as abiotic elicitor.

It is important to highlight that the stressful conditions during sprouting, as well as the pre-sowing grain treatments, may reduce germination percentage and/or dry matter production. It follows that the commercial use of those manipulations of environmental conditions during germination should be appropriately set up for each species, to obtain sprouts characterized by higher nutritive and health promoting values, without or slightly affecting the production levels.

3.3. Biofortification

Germinated whole grains would be a promising vehicle for food biofortification programs. In brown rice, a recent research by Wei et al. [60] has shown the possibility to increase Fe concentration in germinated grains (24 h of sprouting) by soaking kernels in solutions of $FeSO_4$, just before germination process. Fe fortification increased Fe concentration of 1.1–15.6 times in brown rice sprouts, due to the penetration of Fe solutions across the aleurone layers via the dorsal vascular bundle present in the endosperm [61], as well as Fe solubility, which was nearly 4-fold higher than in non-fortified sprouts [60]. However, the relatively low permeability of some seed coats does not allow obtain fortification with Fe enriched solutions. This was the case for broccoli and radish soaked with Fe(III)-EDTA and Fe(III)-citrate solutions [62]. Conversely, the same authors observed significantly higher iron concentrations in 5-day old alfalfa sprouts obtained from Fe-soaked seeds. This increase was associated with a significant decrease in Ca, Mg, Na and/or Mn concentrations, due to the leakage during imbibition, and with a significant induction of phenolic compounds concentration [62].

Plant seeds are able to accumulate Se and to transform it from inorganic to organic form (i.e., Se-containing proteins) during germination. In this way, Se-biofortification during sprouting could represent a valid strategy to improve Se concentration in seedlings [63]. In 3-day old tartary buckwheat sprouts, the total Se concentration tended to rise up with increasing external selenite treatments [64]. In brown rice, both total Se and protein-bound Se contents in seedlings significantly increased with increasing external selenite concentration (up to 60 μmol/L Na_2SeO_3) and germination time (4-day old sprouts); moreover, germination time promoted the transformation of inorganic Se to protein-bound Se, despite a very non-uniform distribution in Se-containing proteins was observed [65]. In wheat, Se enriched kernels in combination with some enzymatic and performance traits (i.e., α-amylase activity) can be obtained with 35 mg/L Na_2SeO_3 in germination medium for 24 h at 25 °C [66].

Fortification programs can also be applied during crop cycles, representing a suitable approach to ameliorate the concentration of macro- and micro-elements in whole grains, thus influencing their dynamics during the subsequent germination process. Foliar Zn fertilization, applied over panicle initiation and grain filling stages, represents an effective agronomic practice to promote rice grains Zn concentration, especially when supplied as Zn-amino acid and $ZnSO_4$ [67]. Application of urea containing Zn increased Zn and protein levels in maize grains, showing better results in poor Zn soil [68]. Both foliar spray and soil application of Se significantly increased Se uptake in common buckwheat, with Se content in grains showing the highest correlation coefficient with soil Se application treatments [69].

4. Post-Harvest Storage and Processing Effects on Sprouts Safety and Quality

Sprouts are commonly harvested and freshly consumed, since they are naturally characterized by a rapid quality loss at relatively low temperature. As a consequence, there is the need to optimize the storage conditions through temperature control and modified atmosphere or active packaging which allow to finely manage the chemical composition of the package headspace during their shelf life. At the same time, sprouts consumption has been involved in several foodborne outbreaks due to the lack of a post-germination kill step. Many studies have been conducted over these topics even though little has been found on cereal and pseudo-cereal sprouts. Anyway, the technologies used on other species are based on common assumptions, although the genotypic variation in biochemical composition of grains may need further optimization protocols.

It is important to specify that “microgreens” in their commercial definition do not include the consumption of radicles, so that they are considered as fresh cut vegetables, and microbiological risks can be managed with the existing technologies and packaging, prior and during commercialization [70] (see also Reference [71]). It follows that the “Microbiological safety” section refers principally to the freshly consumption of sprouts (including seeds) (see also Reference [2]).

5. Sprouted Seeds and Human Health

In the past many health benefits have been argued by motivating the strong in vitro antioxidant potential, although the relationship between those data and the redox status measurable in vivo is very weak. Furthermore, this approach ignores other biological effects, that perhaps could be related to compounds which may not be detected by an in vitro assay, but, thanks to a good bioavailability, can activate/deactivate an oxidative-related metabolic pathway or interfere with gene regulation or with other signaling pathways, etc. [72]. In this chapter only studies that deal with the bioavailability concept will be considered.

Nowadays, the most important preclinical and clinical studies have been focused on germinated brown rice; anyway, a recent more in-depth review focusing on health benefits of sprouted grains, including also other cereal species, is provided by Lemmens et al. [73].

Germinated brown rice has proven strong potentials for better glycemic control, correction of dyslipidemia, amelioration of oxidative stress, reduced type 1 tissue plasminogen activator inhibitor (PAI-1), enhanced adiponectin concentration, and increased sodium potassium adenosine triphosphatase and homocysteine thiolactonase activities, which are reviewed in detail in Imam et al. [74] and Nelson et al. [24]. These effects cannot be attributable to a single individual bioactive compound, but rather to a synergic interaction between all the bioactive compounds induced also by the germination process (i.e., GABA, oryzanol, phenolics, dietary fibers and others), so obtaining a greater functional effect when consumed as a whole food [75]. The effects of white rice, brown rice and germinated brown rice in the dietary management of cardiovascular diseases have been recently investigated by Imam et al. [76] who highlighted the modulation of the lipid metabolism and oxidative stress in rats. In pregnant rats the exposure to high-fat diet plus germinated brown rice until 4 weeks post-delivery, influenced metabolic outcomes in offspring of rats with underlying epigenetic changes and transcriptional implications, that led to improved glucose homeostasis and reduced the risk of insulin resistance manifestations [77]. Germinated brown rice supplied in the diet also reduced obesity complications in high-fat diet induced-obese rats, through the improvement of lipid profiles and reduction of leptin level and white adipose tissue mass [78]. Germinated brown rice enhanced insulin levels, insulin receptor, glucose transporters and glucose metabolism in induced-hyperglycemia mice [79]. In addition, in human SH-SY5Y cells, a neuro-protective effect by gene modulations has been found to be provided by germinated brown rice extracts [80]. These evidences represent only a preliminary indication on the potential beneficial effects of germinated brown rice, and more research is still required.

Tartary buckwheat sprouts have been used as ingredient for a new “functional” pasta, which has been proven to reduce cardiovascular risk and metabolic disorders, such as hypertension in rats [81]. Authors have shown that spontaneously hypertensive rats fed with pasta containing tartary buckwheat sprouts (30% vs. 70% durum wheat semolina) exhibited improved levels of blood pressure-related biochemical parameters, probably attributable to the higher levels of rutin and its aglycone quercetin.

The consumption of wheatgrass juice has received particular attention in the case of thalassemics, principally due to its high chlorophyll content. Patients fed with wheatgrass juice during transfusion therapy showed up to 25% reduction in transfusion volume requirement, without compromising hemoglobin levels and a 29.5% increase on the mean time interval between transfusions [18]. In addition, wheatgrass juice is currently under investigation as a possible therapy for ulcerative colitis as it is rich in apigenin, which is possibly correlated with the normalization during oxidative stresses [18].

There is room for a lot of research on this subject: overall there must be a reason why diet supplementation with wheatgrass on Drosophila melanogaster improved flies’ longevity to 58 days as compared to 52 days in control flies [6].

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