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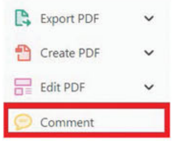

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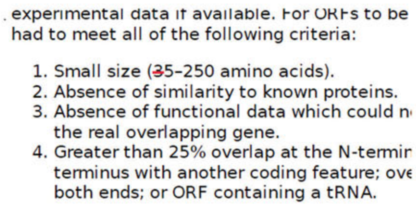
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3. Absence of functional data which could n
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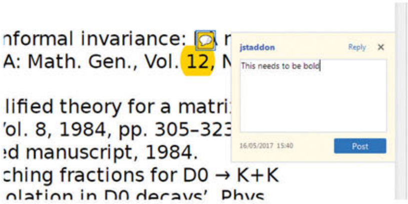
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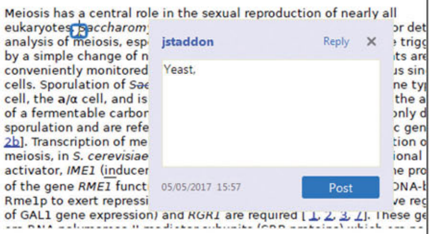
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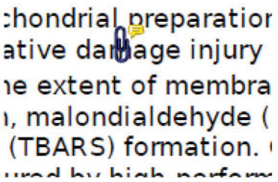

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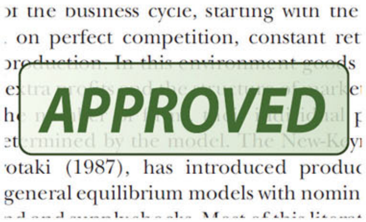

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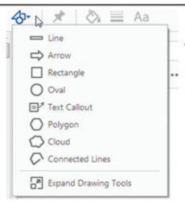

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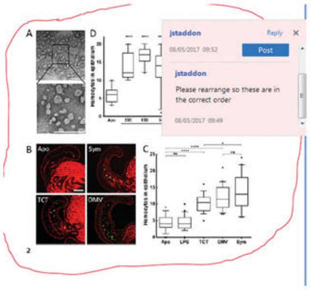

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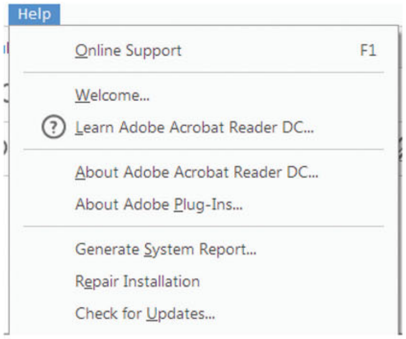

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Comprehensive
REVIEWS
in Food Science and Food Safety

Impact of Cereal Seed Sprouting on Its Nutritional and Technological Properties: A Critical Review

Q1 Elien Lemmens, Alice ~~L.~~ Moroni, Jennifer Pagand, Pieter Heirbaut, Anneli Ritala, Yann Karlen, Kim-Anne ~~LÊ~~, Hetty C. Van ~~Den~~ Broeck, Fred J.P.H. Brouns, Niels De Brier, and Jan A. Delcour

Q2

Abstract: Sprouting induces activation and *de novo* synthesis of hydrolytic enzymes that make nutrients available for plant growth and development. Consumption of sprouted grains is suggested to be beneficial for human health. Positive consumer perceptions about sprouted cereals have resulted in new food and beverage product launches. However, because there is no generally accepted definition of "sprouting," it is unclear when grains are to be called sprouted. Moreover, guidelines about how much sprouted grain material food products should contain to exert health benefits are currently lacking. Accordingly, there is no regulatory base to develop appropriate food labeling for "sprouted foods." This review describes the nutritional and technological properties of sprouted grains in relation to processing conditions and provides guidelines to optimize sprouting practices in order to maximize nutritive value. Relatively long sprouting times (3 to 5 days) and/or high processing temperatures (25 to 35 °C) are needed to maximize the *de novo* synthesis and/or release of plant bioactive compounds. Nutrient compositional changes resulting from sprouting are often associated with health benefits. However, supportive data from clinical studies are very scarce, and at present it is impossible to draw any conclusion on health benefits of sprouted cereals. Finally, grains sprouted under the above-mentioned conditions are generally unfit for use in traditional food processing and it is challenging to use sprouted grains as ingredients without compromising their nutrient content. The present review provides a basis for better defining what "sprouting" is, and to help further research and development efforts in this field as well as future food regulations development.

Keywords: cereal, germination, malting, sprouting, sprouts

Introduction

Cereals are members of the family of the grasses, called Gramineae. They produce dry, 1-seeded fruits called grains, which consist of a fruit coat (pericarp) and a seed. The seed itself consists of the embryo (germ), the endosperm, the nucellar epidermis and the seed coat. The chemical constituents of cereal grains are localized in compartments that are separated from each other by cell walls or other barriers (Delcour & Hoseney, 2010).

Sprouted grain usually designates a seed with a visible radicle. In recent years, the food industry has increasingly launched products containing sprouted grains or flours made thereof. Such products vary from baked goods to pasta, breakfast cereals, snacks, and beverages. An increasing positive attitude of consumers toward such products has been linked to expectations about "natural", "better taste", "more nutritious", and "healthier" (Mattucci, 2015). In addition, sprouted grains have been communicated as organic, vegan, genetically modified organism free, and whole grain (Mattucci, 2015). These positive connotations are in some territories combined with a "whole grain content" claim (~~2018; 2018~~). Q4

Most of the "sprouted grains" product launches took place in Europe and North America, mainly bakery products and in North America also snacks. In the Asia-Pacific region, most launches of products containing sprouted grains have been ready-to-drink products. Quinoa (a pseudocereal) and wheat are the most frequently used sprouted grains. Sprouted buckwheat (also a pseudocereal), barley, and millet have been used, but much less frequently (Mattucci, 2015).

To date, there is no globally recognized definition and regulation of "sprouting" (what is the underlying process? What are the criteria of a "sprouted whole grain"?). The lack of proper definition and regulation indicates that there is also no base to use "sprouted grain" content or benefit claims.

This paper provides an overview of the biochemical and physicochemical changes that take place during sprouting of sorghum, millet, barley, wheat, brown rice, rye, and oats, thereby taking into account different processing conditions. These changes are

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CRF3-2018-0193 Submitted 8/24/2018, Accepted 10/31/2018. *Authors Lemmens, ~~Brier~~, and Delcour are with Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20, B-3001, Leuven, Belgium. Authors Moroni, Karlen, and ~~LÊ~~ are with Nestlé Research Centre, Route du Jorat 57, 1000, Lausanne, Switzerland. Authors Pagand and Heirbaut are with Puratos Group, Industrialaan 25, B-1702, Groot-Bijgaarden, Belgium. Author Ritala is with VTT Technical Research Centre of Finland, Vuorimiehentie 3, 02150, Espoo, Finland. Author ~~Den Broeck~~ is with Wageningen Univ. & Research, Business unit Bioscience, Droevendaalsesteeg 1, 6708, PB Wageningen, The Netherlands. Author Brouns is with Dept. of Human Biology School of Nutrition and Translational Research in Metabolism Faculty of Health, Medicine and Life Sciences, Maastricht Univ., Universiteitssingel 40, 6229, ER Maastricht, The Netherlands. Direct inquiries to author Lemmens (E-mail: elien.lemmens1@kuleuven.be).* Q3

doi: 10.1111/1541-4337.12414

placed in perspective of health effects that may result from regular consumption. Furthermore, the technological implications and potential pitfalls of the use of sprouted cereals during product making are discussed. In addition, an attempt is made to bring more clarity to the definition of "sprouting" and the regulatory context of foods containing "sprouted grain."

Definition and Regulatory Status of the Sprouting Process and Products Thereof

Various definitions of germination, sprouting, and malting exist. Their exact wordings vary with the discipline and the product application (Old Whole Grain Council, 2017). The terms "germination," "sprouting," and "malting" are frequently used as synonyms (Hassani, Procopio, & Becker, 2016; Hübner & Arendt, 2013; A. Singh & Sharma, 2017). In this section, we propose to harmonize the terminology based on plant physiology and the potential applications of the products obtained by such processes. In a final part, we present an overview of the regulatory status of "germinated grains," "sprouted grains," and "malt" in different countries.

From a plant physiological point of view, germination of a seed starts with the uptake of water and is completed with the appearance of the radicle (Nonogaki, Bassel, & Bewley, 2010). The complex physical and metabolic events during germination can be grouped into 3 phases mainly linked to water uptake by the seed (Figure 1). In phase I, the seed is steeped in water and cell material and matrices become fully hydrated (imbibition). Further water uptake is only limited in phase II, which consists of the activation of the endogenous metabolism necessary for mobilizing reserve material and radicle growth. The radicle emerges at the end of phase II and the germination "sensu stricto" is terminated. In phase III, the seed takes up further water, major mobilization of reserve material occurs, and the seedling starts to grow (Nonogaki et al., 2010). During this phase, nutrients are made available for, for example, respiration, fueling the seedling growth. Initially, reserve components, for example, carbohydrates and triacylglycerol, mainly concentrated in the scutellum of the embryo, are hydrolyzed and metabolized. In a next step, the reserve components in the endosperm are utilized for plant development and growth. The consumption of the reserve components by the developing embryo during sprouting leads to respiratory losses, and thus, to a decrease in grain dry matter ranging from 3.5% to 5.0% (Briggs, Hough, Sevens, & Young, 1971). These losses mainly depend on the sprouting conditions applied with higher temperatures and longer times directly leading to higher respiration losses (see section "Biochemical and Physicochemical Changes in Cereal Grains as a Result of Sprouting") (Bewley, Bradford, Hilhorst, & Nonogaki, 2013; Briggs et al., 1971). The reader interested in learning more about the physiology of the germination process is referred to the work of Nonogaki et al. (2010).

> The process of germination hence forms the basis for producing sprouted grains, shoots, and cress, as well as malt. The European Sprouted Seeds Association (ESSA) provides the following definitions for sprouts, shoots and cresses: "Sprouts means 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"; "Shoots are sprouted seeds obtained from the germination and the development of seeds to produce a green shoot with very young leaves and/or cotyledons. The shoots and the leaves are harvested at the end of the production process and the final product does not include the seed integuments and the roots"; "Cresses are sprouted seeds obtained from the germination and development of true seeds in soil or in hydroponic substrate, to produce a green shoot with very young leaves and/or cotyledons. Cress is sold as the entire plants in its substrate or soil." (ESSA, 2016)

Malting is a specific form of sprouting. It produces fermentable extracts for the brewing and distilling industries. The malting process is highly industrialized and controlled. It consists of 3 subsequent steps: steeping to hydrate and activate the seed (germination), sprouting, and kilning. The latter stops seedling growth and forms flavor compounds and color (Kunze, 2010). The malting industry focuses on the length of the acrospires for defining the length of the sprouting process, which is considered to be complete when the acrospire is about 33% to 75% of the length of the grain (Delcour & Hoseney, 2010).

Both nonkilned malts and sprouted grains are products in the above-mentioned phase III. The main differentiation resides in (i) the (bio)chemical changes induced in the grains, (ii) the acrospire, which is removed in malts, and (iii) the typical way the malted grains are kilned (Kunze, 2010). A distinction can be made between nondiastatic and diastatic malts. The former have no residual enzyme activity and are mainly used in the bakery industry for coloring and flavoring, while the latter have enzymatic activity to, for example, hydrolyze starch into fermentable sugars during the mashing process (Ezeogu, 2008; Manley, 2011). Malts used for beer brewing have to meet well-defined quality criteria such as extract yield, diastatic power, total, soluble, and free amino nitrogen contents, and Kolbach index. Furthermore, the malt has to result in wort of a specified color. Finally, the residual β-D-glucan content in the malt has to be low enough to ensure efficient wort filtration (Briggs et al., 1971; Jin, Du, Zhang, Xie, & Li, 2012; Karababa, Schwarz, & Horsley, 1993; Kunze, 2010; Lee & Bamforth, 2009; Ullrich, 2011).

Surprisingly, in contrast to what is the case for malting, there are guidelines neither for the (bio)chemical changes, nor for the physical removal of acrospires for sprouting grains for food production.

In practice, both sprouted grains (meeting the above ~~European Sprouted Seeds Association [~~ESSA~~]~~ definition) and malts are used for food and beverages. A search on the regulatory status of both terms revealed a lack of clarity in the guidelines and definitions (see Table 1). Indeed, sprouting and germination are used as synonyms and no specific indications on how to approach the processes and products thereof are provided.

According to the Codex Alimentarius (1978, Appendix IV), a germinated grain is one which has been sprouted or in which the process of germination is visible within the embryo. This definition has specifically been proposed as a draft standard for maize. However, it does not clarify which modifications should be visible in the embryo for calling it "germinated" and one can only assume that this definition maintains that "a grain which has been sprouted" shows the radicle.

The European Union (EU) regulation defines sprouted grains as grains in which the embryo has undergone clearly visible changes (EU 1272/2009), thereby focusing on the agricultural aspect of sprouting as a quality characteristic in intervention trade (EU 742/2010). Neither a specific reference to which changes should be observed is given nor are clear guidelines for approaching the

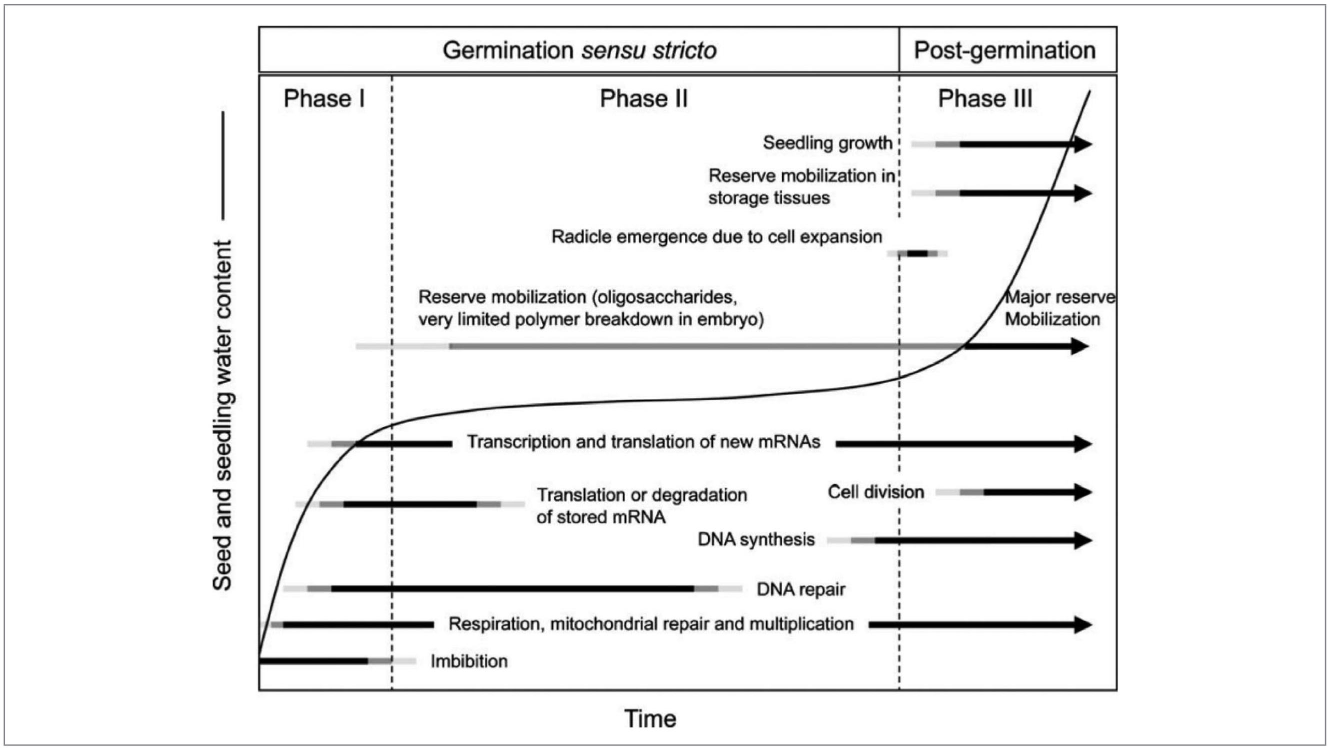

Figure 1–Water uptake by seed or seedling as a function of physical and metabolic events occurring during germination (phase I and phase II) and during early seedling growth (sprouting) (phase III) (Nonogaki et al., 2010, with permission from Plant Science).

process of obtaining sprouted grains presented. The main technical aspect of the EU regulation refers to guidelines for producing sprouts in a safe and hygienic manner (No 208/2013). For example, in the U.S.A., Turkey, and Russia, malt is described as a grain that has been germinated "under controlled conditions" and dried.

A further point of attention is whether sprouted grains comply with the whole grain definition put forward by AACC International (2008), which reads as follows: "Malted or sprouted grains containing all of the original bran, embryo, 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 labeled as malted or sprouted whole grain." This definition provides no clear guidelines on the extent to which the biochemical modifications have impacted the nutritional composition of the grain. Evidently, it is not possible to sprout grains that do not contain the original embryo, bran, and endosperm tissues. The extent of sprouting and the grain treatments after sprouting (for example, partial removal of sprouts and/or bran) together determine whether or not the sprouted material can be considered whole grain. In addition, the comprehensive HEALTHGRAIN definition of whole grain is similar to the one of AACC International. However, the former is more closely in line with industrial milling practices, since it includes recombination of milling streams (for example, refined flour and bran) as well as the opportunity of removing some of the outer part of grain kernels for sanitation reasons. This definition reads as follows: "Whole grains shall consist of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk. The principal anatomical components: the starchy endosperm, germ and bran are present in the same relative proportions as they exist in the intact kernel. Small losses of components that is, less than 2% of the grain or 10% of the bran that occur through processing methods consistent with safety and quality are allowed" (van der Kamp, Poutanen, Seal, & Richardson, 2014). A more recent definition of the Healthgrain Forum of the term "whole grain food" underlines that there is no need for restricting the type of processing for whole grains, unless it leads to a reduction in the dietary fiber content by more than 10%. The latter is then taken to be as an indicator of the amount of beneficial components in the whole grain (Ross et al., 2017). Therefore, this "cutoff" of 10% fiber reduction may well be applied to define the extent of modifications allowed during germination for still considering a sprouted grain as a sprouted whole grain. However, an inherent difficulty associated with this approach is that—in commercial trade—the dietary fiber content of the nonsprouted cereal would have to be specified. In addition, the "whole grain food" definition does not contain any criteria on changes in other cereal nutrients such as those relating to vitamin content, antioxidant activity, and mineral bioaccessibility.

Biochemical and Physicochemical Changes in Cereal Grains as a Result of Sprouting

Impact of sprouting on the starch content in cereal grains and its digestibility

Sprouting initiates the *de novo* synthesis of ~~starch degrading~~ enzymes, such as α-amylase and α-glucosidase, in the scutellum and aleurone cells (Ayernor & Ocloo, 2007; Duke, 2009; Saman, Vazquez, & Pandiella, 2008; J. Xu, Zhang, Guo, & Qian, 2012). β-Amylase is in part bound by disulfide linkages to the protein in the starchy endosperm of mature grains, and is released, and hence, activated during sprouting (Agu & Palmer, 1997; Buttimer & Briggs, 2000; Duke, 2009). As a result of sprouting, the total

Table 1–Regulatory definitions of sprouted grains and malt in various governmental agencies.

Territory	Sprouted grains	Malt
U.S.A.	NA (not available)	Code of Federal Regulations Title 21 - Sec. 184.1445: “Malt syrup (malt extract): Malt is the product of barley (*Hordeum vulgare L.)* germinated under controlled conditions”
Australia	Grain Trade Australia – Guideline N°3: Sprouted grains are those grains in which the covering of the embryo is split. It includes any further advanced stage of growth of the embryo.	NA
China	NA	NA
Malaysia	NA	P.U.(A) 437 (1985) - FOOD ACT 1983 - FOOD REGULATIONS 1985. Definition: ““Malt" means the grain of barley, or of any other cereal that has germinated and has been subsequently dried”
Russia	NA	Grain sprouted and dried under specific conditions
Turkey	NA	NA Definition for beer malts: “Dried and roasted form of grains, mainly barley, used in beer production which is germinated with water under controlled conditions”
EU	• No 208/2013 Safety and hygiene for production Sprouts (Article 2(a)) (standard EN 15587) • No 1272/2009, Part III, Annex I “Sprouted grains:. [. . .] embryo has undergone clearly visible changes which make it easy to distinguish the sprouted grain from the normal grain”	NA
Denmark, Estonia, Finland, Germany, Poland	Follow EU	Follow EU (NA)
Spain	Follow EU	Royal Decree No. 678/2016 “Malt: final product obtained from grains of barley or other cereals once subjected to the malting process: soaking, germination and subsequent drying and toasting in technologically suitable conditions. It must be designated with the name of the origin cereal”
United Kingdom	Follow EU Local Q&A guidance (Agency, 2013)	Follow EU (NA)

Not available (NA).

starch content decreases by 5% to 15% in brown rice sprouted for 1 to 3 days at 30 to 35 °C (Chungcharoen, Prachayawarakorn, Tungtrakul, & Soponronnarit, 2015; J. Xu et al., 2012), in barley sprouted for 4 days at 17 °C (Vinje, Duke, & Henson, 2015), and in oat sprouted for 6 days at 16 °C (Peterson, 1998). Moreover, a much higher decrease in total starch content (35% to 50%) has been observed in sorghum (Elmaki, Babiker, & El Tinay, 1999) and millet (Mbithi-Mwikya, Van Camp, Yiru, & Huyghebaert, 2000) sprouted for 4 days at 30 °C, in rice sprouted for 5 days at 25 to 35 °C (Ayernor & Ocloo, 2007), and in oat sprouted for 4 days at 16 °C (Tian et al., 2010). The enzyme actions finally lead to the partial hydrolysis of starch into glucose, maltose, and maltotriose and a wide range of dextrins (Ayernor & Ocloo, 2007; Saman et al., 2008), and hence, increase the sugar content (Agu & Palmer, 1997; Ayernor & Ocloo, 2007; Coulibaly & Chen, 2011; Duke, 2008; J. Xu et al., 2012). The sugars formed during sprouting of cereals serve as an energy source for the developing embryo (see section “Definitions and Regulatory Status of the Sprouting Process and Products Thereof”) (T.Y. Chung, Nwokolo, & Sim, 1989; Coulibaly & Chen, 2011; Elmaki et al., 1999).

Sprouting not only impacts starch content but also its nutritional properties that cannot be assessed based on its content alone. Based on its digestibility, starch can be categorized into rapidly digestible, slowly digestible, and enzyme-resistant starch. The latter escapes digestion in the small intestines and is fermented by the colonic bacteria to provide energy for the colonocytes (Perera, Meda, & Tyler, 2010). However, there is no consensus on the impact of sprouting on *in vitro* starch digestibility in the literature. J. Xu et al. (2012) stated that the process of sprouting increases the proportion of slowly digestible starch with 14%, and Cornejo, Caceres, Martínez-Villaluenga, Rosell, and Frias (2015) even that the *in vitro* starch digestibility decreases by 58% in sprouted brown rice bread. The authors hypothesized that the sprouting process increases the relative portion of starch crystalline regions since the amorphous ones are more susceptible to amyloytic action. The former are less accessible for pancreatic amylase, and thus, hydrolyzed at a slower rate in the gastrointestinal tract (Cornejo et al., 2015; J. Xu et al., 2012). In contrast, many studies have shown that starch digestibility increases as a result of sprouting. Brown rice sprouted for 2 days at 30 °C and subsequently boiled contains 20% to 30% more rapidly digestible starch than its nonsprouted counterpart (H. Chung, Cho, Park, Kweon, & Lim, 2012; You et al., 2016). Damaged granules and partially degraded starch molecules are more susceptible to enzymatic attack during digestion (H. Chung et al., 2012; Noda et al., 2004; You et al., 2016). Further, the starch digestibility increases from 17% to 36% in sprouted (3 days at 25 to 30 °C) and boiled millet (Archana, Sehgal, & Kawatra, 2001). In millet, this has mainly been attributed to the breakdown of antinutrients such as amylase inhibitors, phytic acid, and polyphenols that inhibit α-amylase action. Sprouted cereals are generally better digestible because of their enzymatically damaged starch granules, thin cell walls, and higher content of readily available sugars (Yan et al., 2010), making them especially suitable for the production of foods for infants and elderly (Correia, Nunes, Barros, & Delgadillo, 2008; Srivastava, Singh, & Shamim, 2015; Tizazu, Urga, Abuye, & Retta, 2010).

Impact of sprouting on the protein content in cereal grains and its digestibility

The protein content of different cereals typically ranges from 8% to 16% of dry mass (dm) (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012). Sound cereal grains have low endogenous peptidase activity levels, which ~~increases~~ substantially after 1 day of sprouting (Elmoneim, Elkhalifa, & Bernhardt, 2010; Faltermaier, Zarnkow, Becker, Gastl, & Arendt, 2015; Kolodziejczyk & Michniewicz, 2004). A 2- to 5-fold increase in peptidase activity has been observed in sorghum (Elmoneim et al., 2010), barley (Osman et al., 2002), rye (Kolodziejczyk & Michniewicz, 2004), oats (Mäkinen, Zannini, & Arendt, 2012), and wheat (Faltermaier et al., 2015; Seguchi, Uozu, Oneda, Murayama, & Okusu, 2010) when sprouted for 3 to 7 days at 15 to 27 °C. Endopeptidases are produced and secreted from the aleurone layer and scutellum during sprouting. They are essential for seedling development as they degrade storage proteins and activate functional proteins (for example, β-amylase) (Faltermaier et al., 2015; Osman et al., 2002). Endopeptidases have an optimal activity at pH 3.5 to 6.5 and at 40 to 50 °C (Osman et al., 2002; Schwalb, Wieser, & Koehler, 2012). Proteolysis is more pronounced after longer sprouting times (minimum 2 days) and at higher temperatures (20 to 28 °C) (Agu & Palmer, 1997; Cáceres, Martínez-Villaluenga, Amigo, & Frias, 2014; Koehler, Hartmann, Wieser, & Rychlik, 2007). In general, while sprouting results in protein hydrolysis, it does not cause relevant changes in total protein content (Cáceres et al., 2014; T.Y. Chung et al., 1989; Kaur, Singh, & Singh, 2002; Ogbonna, Abuajah, Ide, & Udofia, 2012; Swieca & Dziki, 2015; Van Hung, Maeda, Yamamoto, & Morita, 2011; Vis & Lorenz, 1998; J. Xu et al., 2012). However, some studies have reported a significant decrease of 3% to 10% of the protein content in sprouted wheat (Seguchi et al., 2010), sorghum (Afify, El-Beltagi, El-Salam, & Omran, 2012; Elmaki et al., 1999), or rice (Esa, Kadir, Amom, & Azlan, 2011; Mohan, Malleshi, & Koseki, 2010; Veluppillai, Nithyanantharajah, Vasantharuba, Sandrasegarampillai, & Arasaratnam, 2009). In contrast, other studies have reported an increase in protein content of 5% to 10% in sprouted barley (Donkor et al., 2012; Teixeira, Nyman, Andersson, & Alminger, 2016), oats (Donkor et al., 2012), wheat (Donkor et al., 2012), sorghum (Donkor et al., 2012), rye (Donkor et al., 2012), rice (Donkor et al., 2012; Ohtsubo, Suzuki, Yasui, & Kasumi, 2005; Pal et al., 2016; Watanabe, Maeda, Tsukahara, Kayahara, & Morita, 2004), and millet (Mbithi-Mwikya et al., 2000). The decrease in protein content was attributed to leaching of water-soluble peptides in the steeping water (Afify et al., 2012; Elmaki et al., 1999), while the increase can probably be explained by loss of carbohydrates through respiration (Mbithi-Mwikya et al., 2000; Tizazu et al., 2010). However, the relative differences in protein content between sprouted and nonsprouted cereals have been reported to be smaller than 10%, indicating that, in general, sprouting does not substantially affect total protein content (see above).

Sprouting of brown rice (Mohan et al., 2010) or wheat (Koehler et al., 2007) for 3 to 7 days at 20 to 25 °C does not significantly change the level of albumins and globulins (less than 35 kDa). However, when sprouting oats, an increase in albumins, which are rich in essential amino acids, has been observed (Klose & Arendt, 2012; Tian et al., 2010). One can assume that the oat storage proteins are degraded and solubilized during sprouting (Tian et al., 2010). Furthermore, malting wheat has been reported to cause a 3-fold increase in water-extractable protein levels (12 to 44 kDa). This increase especially occurs during the 1st and 2nd day of sprouting (Xie, Jin, Du, & Zhang, 2014). It may be caused by partial degradation of globulins, prolamins, and glutenins. Indeed, substantial degradation of the high-molecular-weight (HMW) storage proteins, consisting of glutenin subunits for wheat (80 to 120 kDa) (MacRitchie, 1992) and (α-β)glutelin subunits (19 to 39 kDa) (Van Der Borght et al., 2006) for rice, has been observed when sprouting wheat (Boukid, Prandi, Buhler, & Sforza, 2017; Koehler et al., 2007; Ohm, Lee, & Cho, 2016) and brown rice (Mohan et al., 2010) for 3 to 7 days at 20 to 25 °C.

The storage proteins are not only degraded into peptides with varying MW, but also into free amino acids of which the content increases by a factor of 5 to 10 when sprouting oats (Klose, Schehl, & Arendt, 2009), wheat (Ohm et al., 2016), brown rice (Ohtsubo et al., 2005), and sorghum (Afify et al., 2012; Correia et al., 2008; Tian et al., 2010) for 3 to 5 days at 13 to 30 °C. In particular, the levels of essential amino acids isoleucine, leucine, lysine, threonine, valine, and phenylalanine were highly increased (Klose et al., 2009; Moongngarm & Saetung, 2010; Van Hung et al., 2011).

Protein solubility and digestibility is higher as a result of sprouting. In this respect, a 1.2- to 2.0-fold increase in protein solubility has been noted in barley (Osman et al., 2002) and sorghum (Afify et al., 2012; Elmoneim et al., 2010) when sprouted for 3 to 5 days at 17 to 27 °C. The protein digestibility increased from 34% to 55% in millet when sprouted for 4 days at 30 °C (Mbithi-Mwikya et al., 2000) and from 50% to 65% to 65% to 80% in sorghum (Afify et al., 2012; Elmoneim et al., 2010) and in barley (T.Y. Chung et al., 1989) when sprouted for 3 to 6 days at 22 to 27 °C. In contrast, Swieca et al. (Swieca & Dziki, 2015) did not observe any changes in protein digestibility when sprouting wheat for 4 days at 20 to 25 °C. This fact was related to the increase in the levels of free phenolic compounds (see section ~~“Impact of sprouting on the phytate content, the mineral redistribution, and the mineral bioaccessibility in cereals”)~~ during sprouting, which can form insoluble complexes with proteins and reduce their digestibility. Also, some phenolic compounds can adversely affect the activity of the enzymes of the digestive tract and the affinity for their substrates (Kroll, Rawel, & Rohn, 2003; Swieca & Dziki, 2015; Swieca, Gawlik-Dziki, Dziki, Baraniak, & Csyz, 2013).

In cereals, also “antinutrients” can reduce the digestibility of proteins. Sorghum protein is 15% to 25% less digestible than other cereal proteins because it contains condensed tannins and trypsin inhibitors (Afify et al., 2012; Albarracín, González, & Drago, 2013; T.Y. Chung et al., 1989; Swieca & Dziki, 2015), which limit digestibility of proteins by forming complexes with proteolytic enzymes (Elmaki et al., 1999; Ogbonna et al., 2012). There is no consensus on the impact of sprouting on the tannin content in cereals. A decrease in tannin content of 8% to 60% has been noted when sprouting sorghum (Elmaki et al., 1999; Ogbonna et al., 2012; Osuntogun, Adewusi, Ogundiwin, & Nwasike, 1989) and millet (Hemalatha, Platel, & Srinivasan, 2007; Mbithi-Mwikya et al., 2000) for 2 to 7 days at 20 to 30 °C. This has been attributed to leaching of tannin into the steeping medium, to the activity of polyphenol oxidases, and to polymerization of tannins, the latter resulting in loss of solubility (Mbithi-Mwikya et al., 2000; Osuntogun et al., 1989; A.K. Singh, Rehal, Kaur, & Jyot, 2015). In contrast, the tannin content increased 25% to 300% when sprouting wheat (Hithamani & Srinivasan, 2014) and sorghum for 3 to 5 days at 20 to 32 °C (Ahmed, Mahgoub, & Babiker, 1996; Hithamani & Srinivasan, 2014; R. Yang, Wang, Elbaloula, & Zhenxin, 2016). This increase in tannin content has been explained as resulting from *de novo* synthesis (Ahmed et al., 1996; R. Yang et al., 2016). In addition, degradation of proteins and polysaccharides results in the release of earlier undetectable tannins (R. Yang et al., 2016). Sprouting sorghum for 4 days at room temperature (RT) reduced its trypsin inhibitor content by about 40% (Ogbonna et al., 2012), probably due to increased action of specific peptidases (Mbithi-Mwikya et al., 2000; McGrain, Chen, Wilson, & Tan-Wilson, 1989; Murugkar, 2014; Wilson, 1988).

Impact of sprouting on the lipid content in cereal grains

The lipid catabolism provides energy and carbon sources needed for the biochemical and physicochemical modification during seedling growth (T.Y. Chung et al., 1989; Coulibaly & Chen, 2011; Elmaki et al., 1999; Inyang & Zakari, 2008; Kubicka, Grabska, & Jedrychowski, 2011; Moongngarm & Saetung, 2010). In this context, a 1.2- to 2.3-fold increase in lipase and lipoxygenase activity levels occurs during sprouting of cereal grains due to their *de novo* synthesis in the aleurone and/or scutellum (Kubicka et al., 2011; Mäkinen et al., 2012; Uvere & Orji, 2002). However, regular mature oat grains already have high levels of lipase activity that remain unchanged or even decline during sprouting, which is unique among the cereals (Mäkinen et al., 2012; Peterson, 1999). As a result of the lipase activities, a 8% to 15% decrease in the lipid contents occurs in millet sprouted for 3 days at RT (Suma & Urooj, 2014), in barley sprouted for 5 days at 22 °C (T.Y. Chung et al., 1989), and in oat sprouted for 6 days at 16 °C (Peterson, 1998). Increasing the sprouting temperatures leads to a higher lipid breakdown. A decrease in total lipid content of 18% to 28% has been observed in millet sprouted for 2 days at 32 °C (Inyang & Zakari, 2008), in wheat sprouted for 2 days at 30 °C (Van Hung et al., 2011), and in brown rice sprouted for 1 to 5 day at 25 to 30 °C (Mohan et al., 2010; Watanabe et al., 2004). Lipase catalyzes the degradation of triglycerides to glycerol and free fatty acids (Kubicka et al., 2011; Peterson, 1998). The glycerol and free fatty acids are mainly converted to ~~hexose and then~~ sucrose, which is sent to the scutellum for use by the rootlet and shoot (Bewley & Black, 1994).

Impact of sprouting on the dietary fiber matrix and properties in cereal grains

A wide range of health effects are ascribed to dietary fiber consumption, some of which related to their solubility, viscosity, particle size, and/or water-holding capacity (Guillon & Champ, 2000). A high intake of insoluble dietary fiber improves stool bulk and decreases gut transit time due to its relatively high water-holding capacity (M.A. Eastwood, Robertson, Brydon, & MacDonald, 1983), while especially soluble dietary fiber is one of the principal substrates for microbial fermentation in the colon (Cummings & Bingham, 1987; Damen et al., 2011). Principal fermentation products include short-chain fatty acids (mainly acetate, propionate, and butyrate), which can, in turn, promote colonic health by providing energy for the colonocytes and by decreasing gut permeability and motility (Bränning & Nyman, 2011). Furthermore, some dietary fibers, particularly viscous soluble fiber, may impact on postprandial glycemic responses (Weickert et al., 2006) and lower plasma lipid concentrations (Keenan et al., 2007; Queenan et al., 2007).

Dietary fiber components are present in plant cell walls of cereal grains. The cell walls of wheat and rye are mainly built up of arabinoxylan (AX), while those of barley, oats, sorghum, and millet are mainly built up of β-D-glucan (Autio et al., 2001; Narsih, Yunianta, & Harijono, 2012). As a result of sprouting, these cell wall polysaccharides are hydrolyzed by *de novo* synthesized enzymes (Hübner, O'Neil, Cashman, & Arendt, 2010; Krahl, Zarnkow, Back, & Becker, 2010; Mohan et al., 2010; Teixeira et al., 2016). Degradation of these components facilitates the passage of, for example, α-amylases and peptidases, which are synthesized in the scutellum and aleurone, to their substrates (starch and protein, respectively) in the starchy endosperm cells (Bewley et al., 2013; Ranki & Sopanen, 1984).

Sprouting induces changes in composition and contents of insoluble and soluble dietary fiber (Hübner et al., 2010; Krahl et al., 2010; Mohan et al., 2010; Teixeira et al., 2016), which can be used to modify the dietary fiber content. In general, the impact of process conditions on solubilization of dietary fiber depends on the cereal cultivar, since differences in hydration rate affect enzyme synthesis and activity (Cáceres et al., 2014; Teixeira et al., 2016).

While according to Teixeira et al. (2016) sprouting does not significantly affect the total fiber content in barley grains when sprouted for 3 days at 15 °C, Koehler et al. (2007) showed that it decreases in wheat during the first 2 days of sprouting at 15 or 20 °C, while it remains fairly constant at higher temperatures (25 and 30 °C). When sprouting wheat for 4 days, the total dietary fiber content expressed as a proportion of total grain mass decreases (partly) due to the loss of reserve compounds (mainly starch) (Koehler et al., 2007; Van Hung et al., 2011). The soluble dietary fiber content increases 3- to 4-fold as a result of sprouting, while the insoluble dietary fiber content decreases, especially when long sprouting times are applied (5 to 7 days) (Koehler et al., 2007). Below, the changes in content and properties of AX and β-D-glucan as a result of sprouting are discussed in more detail.

Arabinoxylan. Endo-1,4-β-D-xylanases (EC 3.2.1.8, further referred to as endoxylanases) are key enzymes in the breakdown of AX. They are involved in the progressive degradation of aleurone and endosperm cell walls during grain sprouting (Autio et al., 2001; Corder & Henry, 1989; Hrmova et al., 1997). Low endoxylanase activities have been detected in wheat (De Backer, Gebruers, Van den Ende, Courtin, & Delcour, 2010), rye (Autio et al., 2001; Kolodziejczyk & Michniewicz, 2004), and barley (Autio et al., 2001; Li, Lu, Gu, Shi, & Mao, 2005; Sungurtas, Swanston, Davies, & McDougall, 2004) in the early stages of sprouting, increasing steeply until 5 to 6 days of sprouting (De Backer et al., 2010; Li et al., 2005). However, the total AX contents do not change (De Backer et al., 2010; Krahl et al., 2010; Teixeira et al., 2016) or only slightly decrease (Li et al., 2005) during the sprouting process. The water-extractable AX (WEAX) level in wheat strongly increases during the first 4 to 6 days of sprouting, while the water-unextractable AX (WUAX) level decreases, indicating solubilization of the latter (De Backer et al., 2010; Krahl, Müller, Zarnkow, Back, & Becker, 2009; Li et al., 2005). The increase in WEAX levels upon germination is accompanied by a shift in its MW distribution. Arabinoxylan-oligosaccharides (AXOS) and WEAX (11 to 300 kDa) may provide human health benefits by improving colon fermentation that is associated with a proliferation of probiotic bifidobacteria and lactobacilli (Broekaert et al., 2011; Hughes et al., 2007; Zhong, Nyman, & Fak, 2015). During the malting process (15 °C for 6 days at a grain moisture content of 45%), the level of WEAX increases from 0.8% of dm in regular spelt kernels to 1.6% of dm in their malted counterparts (Dervilly et al., 2002). Krahl et al. (2009) found the WEAX content during malting to increase from 0.8% of dm to 1.5% of dm in wheat and from 1.4% of dm to 2.9% of dm in rye. Hydrolysis of WEAX is boosted by steeping conditions allowing optimal endoxylanase activity. Teixeira et al. (2016) showed that 40% to 85% of the WEAX in barley is degraded into either simple sugars or oligosaccharides by steeping at 35 °C in 0.4% v/v lactic acid. Finally, the total AX level and the WUAX level in wheat shoots increased as a result of 4 days of sprouting due to deposition of AX in the newly synthesized cell walls of the developing seedling (De Backer et al., 2010).

β-D-Glucan. Cereal β-D-glucans are nonstarch polysaccharides composed of linear chains of glucose residues polymerized through β-1-3 and β-1-4 linkages (Ahmad, Gani, Shah, Gani, & Masoodi, 2016). In 2003, the U.S. Food and Drug Administration (FDA) had authorized a health claim that recognized the relationship between β-D-glucan soluble fiber from whole oat sources and reduced risk of coronary heart disease (CHD) (FDA, 2003). In 2006, the FDA published an interim final rule to include β-D-glucan-soluble fiber from barley into the above-mentioned health claim listing (FDA, 2006). The European Food Safety Authority (EFSA) has also approved 2 claims regarding β-D-glucans from these cereals. One is related to reducing low-density lipoprotein (LDL) blood cholesterol, which may decrease the risk of (coronary) heart disease. Another is related to reducing postprandial glycemic responses at doses of about 4 g of β-D-glucan per 30 g of available carbohydrates in bread and pasta products when consumed alone or in the context of a meal (EFSA, 2011a, 2011b), which may reduce diabetes risks.

As a result of sprouting, β-D-glucans are degraded by endo-β-D-glucanases (EC 3.2.1.6) into oligosaccharides (Autio et al., 2001; Bamforth & Martin, 1983; Rimsten et al., 2002; Wang, Zhang, Chen, & Wu, 2004). Indeed, endo-β-D-glucanase activity increases 5- to 8-fold when sprouting barley for 4 days at 15 °C (Ellis et al., 1997; Rimsten et al., 2002; Wang et al., 2004). As a result, the MW of β-D-glucan substantially decreases along with its content (Ahmad et al., 2016; Autio et al., 2001; Bamforth & Martin, 1983; Hoj, Slade, Wettenhall, & Fincher, 1988; Lee & Bamforth, 2009; Marconi, Tomasi, Dionisio, Perretti, & Fantozzi, 2014; Peterson, 1998; Sungurtas et al., 2004). Strategies have been developed to maintain the β-D-glucan content and its MW distribution as much as possible during sprouting to meet the above-mentioned health claims. They mainly focused on limiting the β-D-glucanase activity during germination and/or sprouting. For instance, Haraldsson et al. (2004) and Teixeira et al. (2016) reported that the barley β-D-glucan content does not change significantly when steeped in 0.4% to 0.8% lactic acid solution at 35 to 48 °C and subsequently sprouted for 3 to 4 days at 15 °C. Moreover, Rimsten et al. (~~2011~~) found that the β-D-glucan content only decreases by 11% to 14% when barley is steeped at 48 °C and then sprouted at 15 to 18 °C for 4 days, while a 40% decrease in β-D-glucan content occurs in barley grains steeped at 15 °C and then sprouted at 15 to 18 °C for 4 days. This has been explained by the much slower development of β-D-glucanase when steeping at the higher temperature. Finally, Wilhelmson et al. (2001) found the β-D-glucan content and its average MW to decrease by about 10% when oat grains were sprouted for 6 days at 5 °C.

Impact of sprouting on the phytate content, the mineral redistribution, and the mineral bioaccessibility in cereals

About 85% of phosphorus (P) in bran is stored as phytic acid (myoinositol 1,2,3,4,5,6-hexa*kis*phosphate) that occurs as granules embedded in protein-rich globoid structures found mainly in aleurone cells (Raboy, 2003; Schlemmer, Frolich, Prieto, & Grases, 2009). Most phytic acid is chelated by (divalent) cations, such as those of iron (Fe), zinc (Zn), calcium (Ca), manganese (Mn), magnesium (Mg), and copper (Cu), to result in phytates (Schlemmer et al., 2009). The bioavailability of these phytates and their availability for absorption in the human gastrointestinal tract (that is, bioaccessibility; Anson, van den Berg, Havenaar, Bast, & Haenen, 2009), as well as their distribution to human organs and tissues through the blood stream (Persson, Türk, Nyman, & Sandberg, 1998) only amounts to 5% to 25% (Bouis, Hotz, McClafferty, Meenakshi, & Pfeiffer, 2011; Fredlund et al., 2003), since they are poorly digested and absorbed, as humans lack intestinal phytase enzymes (Iqbal, Lewis, & Cooper, 1994; Sandberg & Andersson, 1988). Not only inositol hexaphosphate (IP6) exists, but also some so-called lower inositol polyphosphates. These include inositol pentaphosphate (IP5), tetraphosphate (IP4), and triphosphate (IP3). In plant-based diets, IP6 and IP5 are the main inhibitors of Fe and Zn absorption from the gut (Hurrell, 2004). Inositol phosphates IP1 to IP4 do not have a negative effect on Zn absorption, whereas inositol phosphates IP1 and IP2 do not inhibit Fe absorption (Gibson, Bailey, Gibbs, & Ferguson, 2010). In 2006, World Health Organization and Food and Agriculture Organization jointly published guidelines on food fortification with micronutrients (Allen, de Benoist, Dary, & Hurrel, 2006), which maintain that in order to achieve a meaningful increase in Fe absorption from food, the molar ratio of phytic acid to Fe needs to be maximally 1:1 or even lower than 0.5:1 if no compounds enhancing Fe absorption are used.

Dephytinization is a very promising strategy for improving mineral bioaccessibility in whole grain cereal products. During germination of cereal seeds, phytases are activated, *de novo* synthesized, and secreted to make phosphate, mineral elements, and myo-inositol available for plant growth and development (Miransari & Smith, 2014; Platel, Eipeson, & Srinivasan, 2010; Raboy, 2003). Thus, controlled grain sprouting increases the bioaccessibility of mineral elements. Most of the studies in this area focus on total phytate degradation and not on hydrolyzing different lower inositol polyphosphates. Here, we only discuss phytate (IP6) reduction.

Mature cereal seeds have relatively low endogenous phytase activity levels, which substantially increase during sprouting. These enzyme activity levels vary with the cereal and the conditions of sprouting. A 3- to 10-fold increase in phytase activity levels has been found in rice (Azeke, Egielewa, Eigbogbo, & Ihimire, 2011), rye (Bartnik & Szafranska, 1987), wheat (Azeke et al., 2011; Bartnik & Szafranska, 1987; Lemmens et al., 2018), barley (Bartnik & Szafranska, 1987), sorghum (Azeke et al., 2011), and oats (Bartnik & Szafranska, 1987) when sprouting for 4 to 5 days at 15 to 25 °C. The phytase activity levels in sprouted rye are 2, 5, and even 12 times higher than those in sprouted wheat, barley, and oats, respectively, when sprouting for 3 days at 20 °C (Bartnik & Szafranska, 1987). Maximum phytase activity levels are reached in brown rice and barley when sprouted for 2 to 4 days at 20 to 25 °C. However, after reaching its maximum, the phytase activity decreases relatively fast (by 20% to 50%) at steeping and/or sprouting temperatures exceeding 20 °C. When sprouting at 15 °C, the maximum phytase activity is obtained only after 7 days, after which the activity decreases more slowly (Ou et al., 2011; Sung et al., 2005). The latter can be ascribed to enzyme degradation by activated peptidases and/or by product inhibition due to the liberated phosphates (Haraldsson et al., 2004; Ou et al., 2011; Sung et al., 2005).

The increase in endogenous phytase activity as a result of sprouting directly leads to phytate hydrolysis (Table 2), and thus, to release of bound minerals and improved mineral bioaccessibility. Bartnik and Szafranska (1987) measured a phytate breakdown of 12% in barley, 16% in wheat, and 28% in rye as a result of 3 days of sprouting at 20 °C, while Centeno et al. (2001) reported a 37% and 68% phytate breakdown in barley and rye, respectively when sprouted for 3 days at 22 °C. These findings are in line with

Table 2–The influence of steeping and sprouting conditions on phytate hydrolysis in sorghum, millet, barley, wheat, brown rice, rye, and oats.

	Steeping conditions		Sprouting conditions			
Cereal	Temperature (°C)	Time[a] (in water[b]) (hours)	Temperature (°C)	Time (days)	Phytate breakdown (%)	Reference
Sorghum	30	24 (24)	None	None	4	(Lestienne et al., 2005)
	RT (room temperature)	20 (20)	RT	3	25 to 35[c]	(Afify et al., 2011)
	22 ± 2	22 (22)	22 ± 2	2	52 to 56[c]	(Tizazu, Urga, Belay, Albuye, & Retta, 2011)
	NA (not available)	48 (45)	NA	4	66	(Ogbonna et al., 2012)
	RT	Overnight	RT	4	70 to 85[c]	(Mahgoub & Elhag, 1998)
Millet	NA	16 (16)	25	2	13	(Hemalatha et al., 2007)
	30	24 (24)	None	None	28	(Lestienne et al., 2005)
	32	12 (12)	32	2	45	(Inyang & Zakari, 2008)
	NA	12 (11)	32 ± 3	1	57 to 72[c]	(Badau et al., 2005)
	NA	12 (11)	32 ± 3	4	87 to 91[c]	(Badau et al., 2005)
Barley	RT	14 (14)	22	1	5	(Centeno et al., 2001)
	15	Until desired moisture content of 38% was reached	15	4	3 to 5[c]	(Rimsten et al., ~~2011~~)
	15	Until desired moisture content of 38% was reached	18	4	6 to 10[c]	(Rimsten et al., ~~2011~~)
	15	32 to 37 (32 to 37)	15	3	12 to 19[c]	(Teixeira et al., 2016)
	NA	42 (21)	10	2	21	(Hübner et al., 2010)
	NA	42 (21)	20	2	24	(Hübner et al., 2010)
	NA	42 (21)	10	6	27	(Hübner et al., 2010)
	NA	42 (21)	20	6	23	(Hübner et al., 2010)
	RT	14 (14)	22	3	37	(Centeno et al., 2001)
	RT	14 (14)	22	5	58	(Centeno et al., 2001)
Wheat	15	29 (17)	15	1	6	(Lemmens et al., 2018)
	15	29 (17)	15	3	14	(Lemmens et al., 2018)
	15	29 (17)	15	5	15	(Lemmens et al., 2018)
	NA	NA	20	1	17 to 19[c]	(Bartnik & Szafranska, 1987)
	NA	NA	20	3	19 to 23[c]	(Bartnik & Szafranska, 1987)
Brown rice	25	24 (24)	30	1	9	(Liang et al., 2008)
	29 ± 1	12 (12)	29 ± 1	1	13	(Moongngarm & Saetung, 2010)
	30	24 (24)	None	None	17	(Lestienne et al., 2005)
	NA	NA	30	1	17	(Watanabe et al., 2004)
	28	24 (24)	28	2	38 to 55[c]	(Cáceres et al., 2014)
	25	24 (24)	30	3	54	(Liang et al., 2008)
	28	24 (24)	34	2	46 to 63[c]	(Cáceres et al., 2014)
	28	24 (24)	28	4	56 to 74[c]	(Cáceres et al., 2014)
	28	24 (24)	34	4	61 to 80[c]	(Cáceres et al., 2014)
Rye	RT	14 (14)	22	1	11	(Centeno et al., 2001)
	NA	NA	20	1	19 to 22[c]	(Bartnik & Szafranska, 1987)
	NA	NA	20	3	28 to 29[c]	(Bartnik & Szafranska, 1987)
	RT	14 (14)	22	3	68	(Centeno et al., 2001)
	RT	14 (14)	22	5	84	(Centeno et al., 2001)
Oat	NA	42 (21)	10	2	6	(Hübner et al., 2010)
	NA	NA	20	1	13	(Bartnik & Szafranska, 1987)
	NA	NA	20	3	16	(Bartnik & Szafranska, 1987)
	NA	42 (21)	20	2	18	(Hübner et al., 2010)
	16	24 (24)	16	1	20	(Tian et al., 2010)
	NA	42 (21)	20	6	24	(Hübner et al., 2010)
	16	24 (24)	16	3	31	(Tian et al., 2010)
	NA	42 (21)	10	6	32	(Hübner et al., 2010)
	16	24 (24)	16	6	69	(Tian et al., 2010)

[a] Total steeping time.

[b] Time in which grains were submerged in water; Room temperature (RT).

[c] Range of phytate breakdown is given if different varieties were used in the same study; Not available (NA).

the higher phytase activity levels in rye than in barley and wheat (Bartnik & Szafranska, 1987). In general, higher endogenous phytase activity levels in a cereal result in more extensive phytate hydrolysis. Relatively long sprouting times (3 to 5 days) are needed to lower the phytate concentration by more than 30% (Azeke et al., 2011; Badau, Nkama, & Jideani, 2005; Bartnik & Szafranska, 1987; Cáceres et al., 2014; Centeno et al., 2001; Mahgoub & Elhag, 1998; Tian et al., 2010). Azeke et al. (2011) indeed found only a 5%, 10%, 9%, and 9% decrease in phytate content as a result of 2 days of sprouting at 24 to 28 °C, while the content decreased by 57%, 47%, 34%, and 37% in rice, sorghum, wheat, and millet, respectively, when sprouted for 5 days. Also, Hübner et al. (2010) reported only a 6% decrease in phytate content in oats as a result of 2 days of sprouting at 10 °C, while the phytate content decreased by 32% when sprouted for 6 days at this temperature.

Cereal phytases have temperature and pH optima of 37 to 55 °C (Guo et al., 2015; Konietzny & Greiner, 2002; Larsson & Sandberg, 1992; Türk, Carlsson, & Sandberg, 1996) and 4.5 to 6.0, respectively (Konietzny & Greiner, 2002; Lei & Porres, 2003; Peers, 1953). Thus, increasing the steeping and/or sprouting temperatures to those optimal for phytase action can enhance phytate breakdown. Cáceres et al. (2014) reported a 15% to 50% higher phytate breakdown in brown rice as a result of 4 days of sprouting at 34 °C than at 28 °C. Hübner et al. (2010) showed that increasing the sprouting temperature from 12 to 18 °C improved phytate breakdown in oats from 14% to 26% when sprouted for 4 days. It is reasonable to assume that drying of sprouted grains also affects their phytate contents, since endogenous phytase can be active during the initial drying or kilning phases (for example, at about 40 to 60 °C and sufficient moisture levels). However, to the best of our knowledge, no systematic study on the impact of different drying/kilning conditions on phytase action and phytate breakdown has been executed. However, it has been reported that phytate breakdown can be achieved by hydrothermal processing of cereals at high temperature (38 to 45 °C) and low pH (2.0 to 6.0) during steeping. In brown rice, 90% of the phytate is hydrolyzed when the grains are first steeped in 0.6% v/v lactic acid at 45 °C for 48 hours and subsequently sprouted for 2 days at 28 °C (Albarracín et al., 2013). In contrast, Teixeira et al. (2016) obtained a phytate reduction by only 8% in barley steeped in 0.4% v/v lactic acid solution for about 35 hours at 35 °C and then sprouted for 3 days at 15 °C.

During steeping, minerals can be lost due to leaching (Afify, El-Beltagi, El-Salam, & Omran, 2011; Hübner et al., 2010; Lintschinger et al., 1997). The loss is relatively high (about 30%) when the cereal grains are steeped at 30 °C prior to germination (Afify et al., 2011; Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005). In addition, minerals are translocated to the developing embryo leading to loss of minerals from the sprouted grain when the developing rootlets and acrospires are removed (Bashir, Ishimaru, & Nishizawa, 2010; Hübner et al., 2010; Ozturk et al., 2006; Takahashi et al., 2009). However, the understanding of this transport mechanism to date is limited (L. Lu et al., 2013; Takahashi et al., 2009). The elements passing to the seedling originate mainly from the scutellum and aleurone, while the concentration of minerals in the starchy endosperm remains largely unchanged (D. Eastwood & Laidman, 1971). The most mobile mineral ions during seed sprouting are those of K, Ca, and Zn, while Mn and Fe ions are less mobile (L. Lu et al., 2013). Lemmens et al. (2018) found that Zn was also detected in the pericarp tissues after sprouting, but it especially accumulates in the developing coleoptile and radicle, suggesting that Zn ions play a key role in their meristemic tissues (Ozturk et al., 2006).

Several *in vitro* digestion studies simulating the human gastrointestinal tract have been carried out to assess the potential impact of phytate degradation on mineral bioaccessibility. While the outcomes of such studies are rather diverse, it can be concluded that a decrease in phytate content leads to a significant increase in mineral bioaccessibility. A 15% decrease in phytate content in wheat as a result of sprouting has been found to lead to an increase in Fe and Zn bioaccessibility from 4.6% to 14.1% and from 2.5% to 14.6%, respectively (Lemmens et al., 2018). A 30% decrease in phytate content in sorghum as a result of sprouting has been found to increase the Fe and Zn bioaccessibilities from 10% to 20% and from 8% to 15%, respectively (Afify et al., 2011). Platel et al. (2010) and Luo et al. (~~2016~~) showed that Fe and Zn bioaccessibility amounts to 20% to 30% in sprouted wheat, rice, millet, and barley grains. Liang, Han, Nout, and Hamer (2008) did not observe a significant increase in Zn bioaccessibility in brown rice, even if the phytate content decreased by 54% as a result of 3 days of sprouting at 30 °C. Q5

As discussed above, controlled sprouting of cereals under conditions optimal for phytase action paves the way for reducing and potentially improving *in vitro* mineral bioaccessibility. Moreover, to limit the loss of minerals, it is advisable to use moderate steeping temperatures (15 to 20 °C) and to retain the shoots and rootlets.

Impact of sprouting on the vitamin content in cereals

Cereal grains contain vitamin E (which consists of tocopherols and tocotrienols) in levels ranging from 0.9 to 4.1 mg/100 g (EFSA, 2015; Fardet, Rock, & Rémésy, 2008; Haraldsson et al., 2004; Moongngarm & Saetung, 2010; Plaza, de Ancos, & Cano, 2003; Watanabe et al., 2004). Consuming 100 g cereals per day contributes 8% to 34% of the recommended dietary allowance (RDA) of this vitamin (EFSA, 2015). Also, thiamine (B1), riboflavin (B2), and pyridoxal (B6) contents range from 0.2 to 0.5 mg/100 g (Coulibaly & Chen, 2011; Hucker, Wakeling, & Vriesekoop, 2012; Lebiedzinska & Szefer, 2006; Malleshi & Klopfenstein, 1998; Watanabe et al., 2004), from 0.02 to 0.14 mg/100 g (Hucker et al., 2012; Lebiedzinska & Szefer, 2006; Malleshi & Klopfenstein, 1998; Plaza et al., 2003; Watanabe et al., 2004), and from 0.25 to 0.76 mg/100 g, respectively, depending on the cereal (Coulibaly & Chen, 2011; Lebiedzinska & Szefer, 2006; Plaza et al., 2003). Finally, the niacin (B3) content in cereals amounts to 2.7 to 7.6 mg/100 g (Asiedu, Lied, Nilsen, & Sandnes, 1993; Lebiedzinska & Szefer, 2006; Malleshi & Klopfenstein, 1998; Moongngarm & Saetung, 2010) and that of folate (B9) to 0.016 to 0.143 mg/100 g (Fardet et al., 2008; Gujska & Kuncewicz, 2005; Kariluoto et al., 2006; Katina, Liukkonen, Kaukovirta-Norja, et al., 2007; Koehler et al., 2007). Consuming 100 g cereals per day contributes 20% to 50% of the RDA of thiamine and niacin and 10% to 30% of the RDA of pyridoxal. The impact of cereal riboflavin on the RDA is less pronounced and amounts to 2% to 11%. Adults meet 5% to 11% of the RDA values of folate when consuming 100 g of rice or oat grains, 13% to 29% of the RDA by consuming 100 g of wheat and barley grains, and even up to 20% to 47% by consuming 100 g of rye grains (EFSA, 2006).

The vitamin content in cereal grains is important for seedling development (Hucker et al., 2012) and increases during sprouting as a result of biosynthesis. Differences in the vitamin contents of sprouted grains (Table 3) depend on the type of grain and conditions of steeping and sprouting. Care needs to be taken to avoid excessive leaching of vitamins during steeping, for example, by using a minimal amount of steeping water. In addition, tailoring the drying procedure can avoid thermal degradation (Ariahu & Ogunsua, 2000; Hucker et al., 2012; Moongngarm & Saetung, 2010; Vercauteren, 2014; Watanabe et al., 2004). The synthesized vitamins mainly accumulate in the rootlets, and removing them after the process is, hence, accompanied by a substantial loss of vitamins (Haraldsson et al., 2004; Hucker et al., 2012; Kariluoto et al., 2006; Malleshi & Klopfenstein, 1998). The next paragraphs focus on the impact of sprouting on the contents of specific vitamins in cereal grains (Table 3).

Vitamin E is a well-known antioxidant. It scavenges free radicals within cell membranes and is mainly located in the embryo of cereals (Fardet et al., 2008). Tocopherols are synthetized and stored within the embryo and transported to the rootlets and acrospires during seed sprouting (Fardet et al., 2008; Hucker et al., 2012;

Table 3–The impact of steeping and sprouting of sorghum, millet, barley, wheat, brown rice, rye, and oats on the vitamin B, E, and C contents and their contribution to the recommended dietary allowance (RDA).

	Steeping conditions		Sprouting conditions		Vitamin B, E, or C content			
Cereal	Temperature (°C)	Time^a(in water^b) (hours)	Temperature (°C)	Time (days)	Type	Content (mg/100 g of dm^c)	RDA (%)/100 g of dm	Reference
Sorghum	25	16 (16)	25	1	Thiamine	0.22	20	(Malleshi & Klopfenstein, 1998)
					Riboflavin	0.13	10	
					Niacin	3.0	22	
					Vitamin C	0.14	<1	
	25	12 (12)	25	3	Thiamine	0.92	83	(Asiedu et al., 1993)
					Niacin	3.5	26	
					Pyridoxal	0.23	9	
	25	16 (16)	25	4	Thiamine	0.17	15	(Malleshi & Klopfenstein, 1998)
					Riboflavin	0.20	16	
					Niacin	4.7	35	
					Vitamin C	1.9	3	
Millet	25	16 (16)	25	1	Thiamine	0.22	20	(Malleshi & Klopfenstein, 1998)
					Riboflavin	0.11	9	
					Niacin	2.3	17	
					Vitamin C	0.99	2	
	RT (room temperature)	24 (24)	RT	1	Riboflavin	0.16	13	(Coulibaly & Chen, 2011)
					Pyridoxal	0.76	30	
					Vitamin E	0.18	2	
					Vitamin C	0.99	2	
	25	16 (16)	25	4	Thiamine	0.19	17	(Malleshi & Klopfenstein, 1998)
					Riboflavin	0.25	20	
					Niacin	2.9	22	
					Vitamin C	3.2	5	
	RT	24 (24)	RT	4	Riboflavin	0.40	32	(Coulibaly & Chen, 2011)
					Pyridoxal	2.8	100	
					Vitamin E	0.30	3	
					Vitamin C	3.2	5	
Barley	15	23 to 28 (23 to 28)	15	4	Vitamin E	4.1 to 4.4^d	34 to 37	(Haraldsson et al., 2004)
	17	24 (12)	17	4	Thiamine	0.30	27	(Hucker et al., 2012)
					Riboflavin	0.05	4	
Wheat	20 ± 1	12 (12)	20 ± 1	3	Vitamin C	7.9	14	(Lintschinger et al., 1997)
	16.5	24 (24)	16.5	3	Vitamin C	16	28	(F. Yang et al., 2001)
	13 and 20	48 (9)	20	4	Folate	0.20	67	(Koehler et al., 2007)
	28	16 (16)	28	4	Thiamine	0.08	7	(Plaza et al., 2003)
					Riboflavin	0.07	6	
					Pyridoxal	0.67	27	
					Vitamin E	0.20	2	
					Vitamin C	1.8	3	
	16.5	24 (24)	16.5	6	Vitamin C	42	72	(F. Yang et al., 2001)
Brown rice	29 ± 1	12 (12)	29 ± 1	1	Thiamine	0.12	11	(Moongngarm & Saetung, 2010)
					Niacin	4.5	33	
					Pyridoxal	0.66	26	
					Vitamin E	0.86	7	
	NA (not available)	NA	30	1	Thiamine	0.49	45	(Watanabe et al., 2004)
					Riboflavin	0.04	3	
					Vitamin E	1.1	9	
Rye	15	30 (14)	15	4	Folate	0.95	>100	(Kariluoto et al., 2006)
	25	30 (14)	25	5	Folate	0.25	83	(Kariluoto et al., 2006)
	18	28 (12)	18	5	Folate	0.22	73	(Katina et al., 2007)
Malted oat	NA	NA	NA	NA	Thiamine	0.23	21	(Hucker et al., 2012)
					Riboflavin	0.04	3	

[a] Total steeping time.
[b] Time in which grains were submerged in water.
[c] Dry mass (dm); room temperature (RT).
[d] Range of vitamin content is given if different varieties were used in the same study; Not available (NA).

Lampi, Nurmi, & Piironen, 2010). Haraldsson et al. (2004) found no changes in the total level of all tocopherol and tocotrienol compounds in barley first sprouted for 4 days at 15 C and from which in a next step, the rootlets were removed prior to analysis. The duration of sprouting is the main determinant for the increase in vitamin E content. For example, Moongngarm and Saetung (2010) and Watanabe et al. (2004) observed no significant changes in α-tocopherol or vitamin E content in brown rice when sprouted for 1 day at 28 to 30 °C, indicating that relatively long sprouting times are needed to allow *de novo* synthesis of this vitamin. Esa et al.

(2011) found that the tocopherol concentration increases from undetectable levels to 60 mg/100 g in brown rice when sprouted for 3 days at 30 °C, while the tocotrienol concentration remains fairly constant. F. Yang, Basu, and Ooraikul (2001) reported a 2.5-fold increase in tocopherol content in wheat when sprouted for 7 days at 16.5 °C.

The vitamin B content in cereals generally increases as a result of sprouting and supports the seedling development and growth. Indeed, a 1.2- to 5.5-fold increase in thiamine content was found in sorghum (Asiedu et al., 1993), wheat (Plaza et al., 2003), and rice (Trachoo, Boudreaux, Moongngarm, Samappito, & Gaensakoo, 2006) when sprouted for 3 to 4 days at 25 to 30 °C. Similarly, the riboflavin content in sorghum (Malleshi & Klopfenstein, 1998), barley (Hucker et al., 2012), millet (Malleshi & Klopfenstein, 1998), and wheat (Plaza et al., 2003) when sprouted for 4 days at 17 to 28 °C was doubled and accounted for 0.07 to 0.25 mg/100 g of dm. Also, a 1.3- to 1.5-fold increase in niacin and pyridoxal contents was observed in wheat (Plaza et al., 2003), millet (Coulibaly & Chen, 2011; Malleshi & Klopfenstein, 1998), and sorghum (Asiedu et al., 1993; Malleshi & Klopfenstein, 1998) when sprouted for 3 to 4 days at 25 °C. However, Watanabe et al. (2004) and Moongngarm et al. (2010) observed that the thiamine, riboflavin, niacin, and pyridoxine contents remain unchanged or even decrease when brown rice is steeped or sprouted for only 1 day. The authors concluded that *de novo* synthesis of vitamins is only initiated in later sprouting stages and that water-soluble vitamins can leach into the steeping water (Moongngarm & Saetung, 2010; Watanabe et al., 2004). B vitamins are also transported to the rootlets and acrospires during sprouting, which, in turn, means that discarding them decreases the vitamin B content of sprouted grains (Hucker et al., 2012; Kariluoto et al., 2006; Malleshi & Klopfenstein, 1998). For instance, a 40% to 50% loss in thiamine concentration is observed in sprouted sorghum (Malleshi & Klopfenstein, 1998) and brown rice (Moongngarm & Saetung, 2010) when the developing embryo is removed. It is also possible that thiamine, one of the most heat labile B-vitamins, is degraded during the drying process (Ariahu & Ogunsua, 2000; Hucker et al., 2012).

Sprouting stimulates *de novo* synthesis of folate because of the increased demand for methyl groups as the metabolic activities of the developing seedling increase (Jabrin, Ravanel, Gambonnet, Douce, & Rébeillé, 2003; Jägerstad et al., 2005; Kariluoto et al., 2006). Various researchers showed a 4- to 6-fold increase in folate to a final content ranging from 1.5 to 3.0 mg/kg in wheat (Hefni & Witthöft, 2012; Koehler et al., 2007) and rye (Hefni & Witthöft, 2012; Kariluoto et al., 2006; Katina,Liukkonen, Kaukovirta-Norja, et al., 2007; Liukkonen et al., 2003) when sprouted for 4 to 6 days at 18 to 25 °C.

The vitamin C content in cereal grains is usually undetectable or very low. During sprouting, however, vitamin C is synthesized *de novo* (Coulibaly & Chen, 2011; Lintschinger et al., 1997; Malleshi & Klopfenstein, 1998; F. Yang et al., 2001) leading to 5 to 55 mg/100 g in sprouted wheat (Lintschinger et al., 1997; F. Yang et al., 2001), millet (Coulibaly & Chen, 2011; Malleshi & Klopfenstein, 1998), and sorghum (Malleshi & Klopfenstein, 1998). Lintschinger et al. (1997) noted that rinsing wheat sprouts with hot water at 70 °C decreased vitamin C content by 40% to 60%. Accordingly, process conditions need to be chosen carefully to preserve this vitamin as it is one of the most heat- and light-unstable vitamins.

When consumed, the relatively high levels of vitamins E, B, and C in sprouted cereals can significantly contribute to daily intakes of these vitamins (Table 3). Adults can meet the RDA value of folate by daily consuming 100 to 150 g of sprouted wheat or rye grains. Sprouted grains contribute less to the daily intake of niacin, riboflavin, pyridoxal, and vitamin E than folate, but their contribution is still substantial. Indeed, 100 g of sprouted cereals can account for 20% to 35% of the RDA of niacin, 5% to 30% of the RDA of riboflavin, and pyridoxal and to 3% to 36% of the RDA of vitamin E (EFSA, 2006, 2015). However, there is great variation in the thiamine content in sprouted cereals. Consuming 100 g of sprouted wheat, brown rice, oats, millet, or barley contributes up to 10% to 50% of the RDA of thiamine, while consuming 100 g of sprouted sorghum contributes up to 20% to 80% of its RDA. Sprouted grains only contribute to the daily intake of vitamin C to a limited extent, since consuming 100 g of sprouted cereals meets about 3% to 10% of its RDA.

To conclude, short steeping times, using a limited amount of water, followed by long sprouting times, mild drying, and retaining rootlets and acrospires are most favorable to obtain an elevated vitamin content in sprouted grains.

Impact of sprouting on the antioxidant capacity in cereals

In general, sprouting increases the antioxidant activity of cereals. Various researchers have shown a 1.2- to 2.9-fold increase in antioxidant activity in wheat (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Zilic et al., 2014), barley (Ha, Jo, Mannam, Kwon, & Apostolidis, 2016), brown rice (Cáceres et al., 2014; Cornejo et al., 2015; Ti et al., 2014), and oats (J.G. Xu et al., 2009) when sprouted for 2 to 5 days at 15 to 28 °C. The higher antioxidant activity in sprouted grains is mainly attributed to accumulations of vitamin E (see section “~~Impact of sprouting on the dietary fiber matrix and properties in cereal grains”~~) and polyphenols (Ahmad et al., 2016; Kim et al., 2013). The latter secondary plant metabolites play a role in the protection of plants against environmental stresses (Alvarez-Jubete et al., 2010; Ha et al., 2016; Pal et al., 2016). Their antioxidant activity is associated with their ability to scavenge free radicals, break radical chain reactions, and chelate metals (Fardet et al., 2008; Ha et al., 2016; J. Lu et al., 2007). The main (poly-)phenols in cereal grains are p-hydroxybenzoic, ferulic, sinapic, vanillic, and p-coumaric acids, and in oats also avenanthramides (Cáceres et al., 2014; Fardet et al., 2008; Hithamani & Srinivasan, 2014; Kim et al., 2013; J. Lu et al., 2007; Skoglund, Peterson, Andersson, Nilsson, & Dimberg, 2008; J.G. Xu et al., 2009). Generally, 60% to 90% of the polyphenols in cereals occur in a bound form (Fardet et al., 2008).

A 1.2- to 3.6-fold increase in total polyphenol content has been measured in wheat (Alvarez-Jubete et al., 2010; Hithamani & Srinivasan, 2014; Swieca & Dziki, 2015), barley (Ha et al., 2016; J. Lu et al., 2007), sorghum (Hithamani & Srinivasan, 2014), rye (Katina, Laitila, Juvonen, et al., 2007; Liukkonen et al., 2003), oats (Tian et al., 2010; J.G. Xu et al., 2009), and brown rice (Cáceres et al., 2014; Cornejo et al., 2015; Pal et al., 2016) when sprouted for 2 to 6 days at 15 to 28 °C. Furthermore, a 1.5- to 1.7-fold increased concentration of bound phenolic compounds has been observed in brown rice (Ti et al., 2014) and wheat (Ohm et al., 2016) when sprouted for 2 to 5 days at 20 °C, while a 1.3- to 3-fold increase in free phenolic compounds has been noted in brown rice (Ti et al., 2014) and wheat (Benincasa et al., 2014; Ohm et al., 2016; Zilic et al., 2014) when sprouted for 2 to 5 days at 18 to 20 °C. The free phenolic compounds are more effective antioxidants than their bound counterparts (Ti et al., 2014). Phenolic acids are biosynthesized during sprouting and the synthesized cell wall-degrading enzymes, such as cellulases and endoxylanases in combination with cinnamoyl esterases and feruloyl esterases, can hydrolyze phenolic compounds bound to cell walls. This increases the levels of free phenolic compounds, mainly ferulic acid, and thus increases the antioxidant capacity (Benincasa et al., 2014; Cáceres et al., 2014; Cornejo et al., 2015; Katina, Liukkonen, Kaukovirta-Norja, et al., 2007; Kim et al., 2013; J. Lu et al., 2007; Pal et al., 2016; Ti et al., 2014; J.G. Xu et al., 2009). Especially the developed shoots had relatively high total phenolic acid contents, since they were 11 times higher in the shoot than in the remaining brown rice kernel after 5 days of sprouting at 30 °C (Cho & Lim, 2018). The increased antioxidant capacity is concerned to be important for the protection of the sprouting grain. However, it is debatable to link this increase to possible benefits for human health (Bast & Haenen, 2013; Pompella et al., 2014).

Impact of sprouting on γ-aminobutyric acid content in brown rice

γ-Aminobutyric acid (GABA), an important nonprotein amino acid, functions as the predominant inhibitory neurotransmitter in the central nervous system (Charoenthaikij, Jangchud, Jangchud, Prinyawiwatkul, & Tungtrakul, 2010; Kinnersley & Turano, 2000), and it is also effective at decreasing blood pressure (Hayakawa et al., 2004; Inoue et al., 2003) and in treating epilepsy (Treiman, 2001). It is formed by transamination of α-ketoglutarate to glutamic acid, which is then decarboxylated by glutamic acid decarboxylase to GABA (Kinnersley & Turano, 2000; Treiman, 2001).

The GABA concentration in barley and wheat is less than 2 mg/100 g of dm (Ohm et al., 2016), while in regular brown rice grains it ranges from 3 to 7 mg/100 g of dm (Banchuen, Thammarutwasik, Ooraikul, Wuttijumnong, & Sirivongpaisal, 2009; Charoenthaikij et al., 2010; Chen et al., 2016). It is present in higher concentrations in sprouted barley and wheat (7 to 25 mg/100 g) (H. Chung, Jang, Cho, & Lim, 2009; Ohm et al., 2016), and especially sprouted brown rice (35 to 80 mg/100 g of dm) (Banchuen et al., 2009; Chungcharoen et al., 2015; Cornejo et al., 2015; Ohm et al., 2016; Ohtsubo et al., 2005; Roohinejad et al., 2011; Thitinunsomboon, Keeratipibul, & Boonsiriwit, 2013). Charoenthaikij et al. (2010), Cornejo et al. (2015), and Roohinejad et al. (2011) observed a 8- to 12-fold increase in GABA content in brown rice when sprouted for 2 to 4 days at 27 to 35 °C. Relatively high sprouting temperatures are needed to stimulate GABA synthesis. Donkor et al. (2012) and Kim et al. (2012) found only a 2-fold increase in GABA content in brown rice when sprouted for 3 to 6 days at about 15 °C. Acidic conditions during steeping can also potentiate GABA synthesis. The GABA content in sprouted brown rice increases up to 120 to 130 mg/100 g of dm when the kernels are steeped at 30 °C in lactic or citric acid solutions (pH 3.0 to 5.6) prior to germination for 1 to 2 days at 30 to 35 °C. Indeed, an acidic pH is more optimal for rice embryo glutamic acid decarboxylase action (Banchuen et al., 2009; Thitinunsomboon et al., 2013; Zhang et al., 2014). It is noteworthy that GABA is very heat-stable and that it is not degraded during kilning/drying (Chungcharoen et al., 2015; Ohtsubo et al., 2005). However, Baranzelli et al. (2018) observed a 75% to 90% loss in GABA content in bread baked (175 °C) from sprouted wheat, which was attributed to the degradation of free amino acids that are used in the Maillard reaction.

Health Benefits of Sprouted Grains

We found only a limited number of preclinical and clinical studies when combining the search terms "sprouted grains" or "germinated grains" with the term "health." While most articles provide a thorough description of the biochemical changes as a result of sprouting, and make connections with potential health benefits, the direct effect of such changes on *in vivo* health markers has only been measured infrequently. Many articles describing the biochemical changes do extrapolate the potential health benefits of the specific nutrients based on the cause-effect links described in the literature. In many studies, the types of grains used and the sprouting treatments are diverse and sometimes the processing conditions are not fully detailed. Accordingly, at present, it is impossible to draw consistent conclusions on the impact of the consumption of sprouted grains on human health and the regulating mechanisms behind it.

Most of these studies have been performed with rodents, while only a few with humans. The main grains used were rice (6 in rodents, 1 in humans) and barley (3 in rodents, 1 in humans) with, to the best of our knowledge, wheat, millet, and oats each used in only 1 (human) study. Brown rice has been used by various researchers to investigate the effect of sprouting on lipid metabolism or symptoms linked to it (Imam et al., 2014; Imam, Ismail, Omar, & Ithnin, 2013; Miura et al., 2006; Roohinejad et al., 2010; Wu, Yang, Touré, Jin, & Xu, 2013). However, the sprouting conditions used differed from 1 study to another and so did the levels of bioactive compounds such as tocopherol, oryzanol, and GABA (Miura et al., 2006; Roohinejad et al., 2010). Furthermore, the reference sample used was not always a nonsprouted counterpart, making it difficult to compare sprouted with regular grains (Hagiwara, Seki, & Ariga, 2004; Trachoo et al., 2006). Zhong, Teixeira, Marungruang, et al. (2015) used commercial barley malt thoroughly characterized in terms of its dietary fiber and β-D-glucan contents and MW distributions to study the impact of sprouting on the rodent gut microbiota. Unfortunately, the heat treatment (leading to Maillard and/or caramelization reactions) and the removal of rootlets (and thus also of the B and E vitamins) as part of the industrial malting process affected the overall nutrient content. Interesting in this context is that Trachoo et al. (2006) reported that these vitamins affect the *in vitro* growth of probiotics. In the next paragraphs, we discuss the health effects of sprouted grains investigated in the above-mentioned *in vivo*/*in vitro* studies.

Lipid metabolism

The effect of sprouted brown rice on plasma lipid profiles has been the subject of numerous studies. These profiles are associated with hypercholesterolemia and high blood pressure, which themselves are linked to cardiovascular disease (CVD). Wu et al. (2013) compiled 5 *in vivo* studies suggesting that sprouted brown rice may have a greater effect on reducing blood serum cholesterol and blood pressure than unprocessed brown rice. A similar conclusion was drawn by Roohinejad et al. (2010). They showed that total cholesterol in rats fed with high-cholesterol diets is reduced to a greater extent by brown rice when sprouted for 2 days, than by such rice either nonsprouted or sprouted for just 1 day. Imam et al. (2013), with an *in vivo* rat study, showed that, although the effects on total cholesterol were similar for brown rice and sprouted brown rice, the lipid composition was more favorable for decreasing the CVD risk after consumption of the latter. A corrective effect on hypercholesterolemia of (sprouted) brown rice was observed with hepatoma-bearing rats by Miura et al. (2006). The authors attributed this positive effect to the increase in γ-oryzanol and GABA levels and to the higher soluble fiber contents in brown rice and sprouted brown rice than in white rice. However, they did not demonstrate a significant difference between the results obtained with either brown rice or sprouted brown rice. It has been suggested that γ-oryzanol decreases blood serum cholesterol levels by suppressing its absorption (Miura et al., 2006; Rong, Ausman, & Nicolosi, 1997), while the dietary fiber in rice bran may favor fecal bile acid and total bile acid excretion and stimulate the activity of cholesterol 7 α-hydroxylase.

To the best of our knowledge, apart from the above work on rice, only sprouted barley and wheat have been investigated for their effects on lipid profiles. Jackson, Suter, and Topping (1994) found no significant difference between the effect of barley and malted barley on blood serum lipids of rats fed either a low- or a high-cholesterol diet. In Nelson et al.'s (2016) double-blind cross-over intervention study, 10 overweight or obese people were given breakfast cereals based either on whole wheat or on sprouted wheat. A similar significant reduction of blood pressure was observed for both interventions, thus not supporting the hypothesis that sprouted wheat would have a stronger impact than its nonsprouted counterpart. Analysis of the blood lipids showed that the LDL cholesterol level was affected by both interventions in a similar way, whereas total and high-density lipoprotein (HDL) cholesterol levels remained unchanged. In this study, the authors mainly focused on the link between the polyphenol content and antioxidant activity of the breakfast flakes and the obesity-related metabolic disorders, including unfavorable blood lipid profiles, while other characteristics of the grains, such as the fiber type or their solubility, were not described.

Glucose metabolism

Only very few studies are available on the effect of sprouted grain consumption on glucose metabolism and type II diabetes. Most of these have focused on sprouted rice and used rodents. Hagiwara et al. (2004) showed that ingestion of sprouted brown rice by diabetic rats results in lower blood glucose levels and a tendency to decrease the plasma lipid peroxide concentrations. Ito et al. (2005) complemented this work by research with healthy humans. They confirmed the positive effect of sprouted brown rice on plasma glucose but not on insulin concentrations. Moreover, they noted no significant differences between brown rice and sprouted brown rice, concluding that, in this case, the lower glucose response may not be linked to a stimulation of insulin secretion by the higher GABA content of the sprouted brown rice as earlier suggested by Hagiwara et al. (2004). The effect was rather attributed to the high fiber content of the 2 types of rice (Ito et al., 2005). S.I. Chung, Ryu, and Kang (2016) showed that ovariectomized rats fed sprouted brown rice had lower plasma glucose and insulin concentrations than the group fed nonsprouted counterparts (S.I. Chung et al., 2016). The results obtained in these studies confirm that whole grain rice may have a hypoglycemic effect, but they allow to draw no conclusions on the mechanism behind the effect, neither do they demonstrate the additional benefit of sprouting the whole grain to manage glucose metabolism. Finally, an acute study with overweight and obese humans (Mofidi et al., 2012) exposed to commercial breads (of which nutritional values but not the exact grain composition were given) showed some positive effect of the breads with sprouted grains on glycemic response. Surprisingly, however, there was no significant difference in postprandial glucose response between the whole grain (11-grain) and white bread (Mofidi et al., 2012). It hence appears that it is impossible to claim any benefits of sprouted grains over those of whole grains.

Mineral absorption

As discussed in section “~~Impact of sprouting on the lipid content in cereal grains,~~” phytic acid chelates are broken down by endogenous phytases that release minerals that are supposedly available to be metabolized. To understand the effect of phytate degradation as a result of sprouting, Larsson, Rossander-Hulthén, Sandström, and Sandberg (1996) fed oat porridge to 2 groups of 9 or 10 healthy subjects who either received a reference porridge containing unprocessed oats or a test product based on sprouted oats. A significant increase of the Zn and Fe absorption in the subjects who received the test porridge was observed and related to a significant reduction in phytate (76%) between the reference and test porridges. A final experiment in which sprouting led to a 67% phytate degradation showed no significant difference in Fe absorption. This was potentially due to too high residual phytate levels in the test porridge. Fredlund et al. (2003) obtained similar results for Zn absorption with a group of 10 healthy subjects when giving them breakfast cereals either based on malted barley that contained 30% less phytate than nonprocessed barley or breakfast cereals based on the latter. An increase by 15% to 23% in Zn absorption was observed with the former breakfast cereals. The same experiment to measure Ca absorption resulted in no significant differences, probably because barley is not the main source of this mineral in the breakfast cereals. The results by Tatala, Ndossi, Ash, and Mamiro (2007) unfortunately did not confirm the observations made on the small groups by Larsson et al. (1996) and Fredlund et al. (2003). The objective of their randomized control study (Tatala et al., 2007) with a group of 300 infants over a 6-month period was to assess the effect of porridge prepared from nonsprouted or sprouted finger millet on the Fe status of children in a region where food is traditionally plant-based and where Fe absorption thus depends on the extent to which mineral absorption is inhibited. No significant improvement of the hemoglobin and anemia status among the 2 groups was noted. This may have been due to the limited difference in phytate content between the reference and the test recipes, which, although it was significant, was only 10%. Albarracín et al. (2016) studied Ca absorption in 16 male Wistar rats fed with extruded brown rice and extruded sprouted brown rice diets for 60 days and did not observe any differences in its absorption (about 70%) among these diets. Based on the information given above, we suggest that a reduction in phytate as a result of sprouting may contribute to better mineral bioavailability in humans, but that, as of now, no specific health benefit statement can be made.

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Celiac disease

Celiac disease is an inflammatory disease of the upper small intestine in genetically susceptible individuals (approximately 1% of the population worldwide) triggered by the storage proteins of wheat, rye, or barley (Catassi, Gatti, & Lionetti, 2015; Lebwohl, Sanders, & Green, 2018; P. Singh et al., 2018). Gluten proteins, including α-gliadins, γ-gliadins, and low-molecular-weight (LMW) glutenins, are not completely hydrolyzed by human gastrointestinal enzymes resulting in peptides that stimulate T-cells in the intestinal mucosa of people suffering from celiac disease (Kerpes, Knorr, Procopio, Koehler, & Becker, 2016; Schwalb et al., 2012). Most of these immunogenic peptides are prolamine-rich and glutamine-rich and contain at least 9 amino acid residues required for T-cell recognition (Sollid, Qiao, Anderson, Gianfrani, & Koning, 2012). Gluten protein degradation to levels lower than 20 ppm can make these cereals suitable for the manufacture of gluten-free products.

Sprouting has been investigated as a means to reduce the immunogenicity of gluten. Indeed, it is likely that cereal endogenous peptidases synthesized during sprouting can extensively hydrolyze gluten, since the seedlings need amino acids for their development (Hartmann, Koehler, & Wieser, 2006). Immunogenic peptides are extensively degraded when incubated for 2 hours at 37 °C and pH 6.5 with peptidases extracted from bran of rye sprouted for 7 days at 15 °C (Hartmann et al., 2006). In addition, 40% to 80% of celiac disease-stimulating peptides are hydrolyzed by endogenous peptidases of barley and wheat sprouted for 4 to 8 days at 12 to 25 °C (Boukid et al., 2017; Kerpes et al., 2016). Such durations may be difficult to achieve in economically feasible food production. However, when water-soluble peptidases from sprouted wheat were incubated with a gliadin digest containing immunogenic epitopes for 24 hours, these epitopes (peptides) were significantly degraded. Although the *in vitro* immunogenicity of the resulting peptides was markedly decreased, residual reactivity was still present. The sole use of peptidases for sprouted wheat to digest gliadin did not result in safe food for people suffering from celiac disease (Stenman et al., 2009). Further improvements have been proposed (Schwalb et al., 2012; Stenman et al., 2009) and might be addressed by optimizing the conditions of sprouting along with the type of cereal used and the cultivar (Scherf, Wieser, & Koehler, 2018). Schwalb et al. (2012) indeed showed that enzymes extracted from different sprouted cereals have different specificities toward different peptides (Schwalb et al., 2012; Stenman et al., 2009). This can potentially be done either using extracted and purified enzymes or by adding grain fractions rich in enzymes, such as bran, to food recipes (Gebendorfer, Hartmann, Wieser, & Koehler, 2011).

Sprouted grains and gut health

Sprouting affects the fibers and other nutrients of grains and therefore also their capacity to be fermented by the gut microbiota (Cummings & Bingham, 1987; Damen et al., 2011). Zhong, Teixeira, Marungruang, et al. (2015) showed that malt material, but not barley whole grain in combination with a high-fat diet, fed to rats resulted in an increase in the levels of butyric and other short-chain fatty acids in the gut. This suggests that sprouting generates molecules such as fructo-oligosaccharides, AXOS, and depolymerized AX and β-D-glucan, which exert a positive impact on the gut microbiota. The same group in a 2nd *in vivo* study with rats showed that using barley malt, rather than barley whole grain, can stir the gut microbiota toward species producing more butyric acid (Zhong, Nyman, et al., 2015).

In conclusion, only very few studies have investigated the effect of sprouted grains on health-relevant parameters. Most studies have focused on brown rice and used rodent models. It is therefore not possible to extrapolate any finding to cereals or to potential health benefits in general.

Techno-Functional Implications of Sprouting Grains

The biochemical changes induced by sprouting not only modify the nutritional properties of the cereals, but they can also affect the physicochemical properties and functionality that play a role during product making. This section deals with the techno-functional properties of sprouted cereals, and thus, how sprouting affects the quality of flour and especially bread and tortilla products made therefrom. ~~As~~ ~~cereal~~ grains contain a complex microbial population that is active and evolves during sprouting. Therefore, we here first discuss changes in microflora and the microbial activity as a safety factor. Noots, Delcour, and Michiels (1999) and Justé et al. (2011) already extensively reviewed the influence of microbial activity on safety and quality aspects of malted grains. The microflora is mainly concentrated in the outer layers of the grain and in the embryo (Berghofer, Hocking, Miskelly, & Jansson, 2003). Diverse microbial communities naturally colonize cereal grains. These include bacteria, yeast, and molds. Gram-negative bacterial genera (such as Enterobacter and Pseudomonas) and gram-positive bacterial genera (such as Lactobacillus and Leuconostoc) are dominantly present. Also, diverse yeast and molds genera, such as Ascomycota, Basidiomycota, Zygomycota, and Mitosporic fungi occur in grains (Justé et al., 2011). Steeping is the most critical stage of the sprouting process regarding safety, since it induces microbial proliferation. Bacteria and yeasts rapidly grow and mold mycelium develops, while at the same time, dormant spores are activated. The viable numbers of bacteria and yeasts reach a maximum during sprouting. A 7- to 15-fold increase in the microbial counts occurs during steeping and sprouting and they typically amount to 10^6 to 10^9 colony-forming units (CFU)/g dm for bacteria, and 10^3 to 10^6 CFU/g dm for yeast in sprouted grains (Douglas & Flannigan, 1988; Haraldsson et al., 2004; Justé et al., 2011; Noots et al., 1999). Extensive mold growth, especially of the Fusarium species, is associated with production of mycotoxins that are toxic for humans at concentrations exceeding 4 to 20 μg/kg food (Justé et al., 2011; Zain, 2011). In past efforts, strategies have been developed to limit microbial activity during the sprouting process and/or reduce the microbial counts to 10^5 to 10^7 CFU/g dm for bacteria and to 10^3 to 10^4 CFU/g dm for yeast and molds in sprouted grains. Products having such microbial counts are generally recognized as safe for human consumption (Berghofer et al., 2003). Gamma irradiation can be used for sterilizing cereal grains prior to sprouting (Noots et al., 1999; Ramakrishna, Lacey, & Smith, 1991), but also grain surface sterilization during the first or last steeping step with formaldehyde, with dilute solutions of lactic acid, or with inorganic acids such as sulfuric acid, phosphoric acid, or hypochloric acid limits the growth of microorganisms (Agu & Palmer, 1999; Haraldsson et al., 2004; Noots et al., 1999; Ramakrishna et al., 1991). Furthermore, the microbial activity during sprouting may be limited by appropriate sprouting temperature control, since the use of a relatively high sprouting ~~temperatures~~ (25 °C) results in a 10^4 higher amount of CFU/g dm of bacteria and filamentous fungi than sprouting at 15 °C (Noots et al., 1999; Wilhelmson et al., 2001). Evidently, kilning procedures can reduce the microbial counts of sprouted grains by a factor of 10 to 1000 (Douglas & Flannigan, 1988; Jiamyangyuen & Ooraikul, 2008; Justé et al., 2011; Wilhelmson et al., 2001). Finally, sulfur dioxide can be added to the air stream during kilning to decrease the microbial load (Noots et al., 1999).

Sprouting is generally associated with grain softening due to reduced interaction between the protein matrix and the starch granules in the endosperm as a consequence of peptidase and amylase action (Dziki & Laskowski, 2010). Liu, Hou, Cardin, Marquart, and Dubat (2017) observed that wholemeal flour from sprouted wheat had a 20% lower damaged starch content than did regular whole wheat flour. The water absorption of flour from wheat sprouted for 1 to 2 days at 15 to 25 °C was 4% to 14% lower than that of control flour due to its lower damaged starch content and differences in the properties of dietary fiber and protein (Baranzelli et al., 2018; Kaur et al., 2002; Liu et al., 2017; Marti, Cardone, Pagani, & Casiraghi, 2018; Morad & Rubenthaler, 1983). For instance, water-extractable dietary fiber binds substantially less water than its unextractable counterpart (Courtin & Delcour, 2002).

Furthermore, gluten proteins in flour from sprouted wheat cannot form a viscoelastic network due to partial protein hydrolysis and modified water-binding properties (Liu et al., 2017; Marti et al., 2018).

The pasting properties of cereals influence their functionality in some applications. They are mainly determined by their starch and dietary fiber (mainly β-D-glucan and AX) characteristics. Suspensions of starch (10% w/w) isolated from flour from preharvest sprouted wheat or from brown rice sprouted for 1 day at 30 °C had a 20% to 60% lower peak viscosity in a Rapid Visco Analyzer model system than that isolated from their nonsprouted counterparts because the increased endogenous α-amylase action hydrolyzed α-1,4-glycosidic linkages of starch molecules (Noda et al., 2004; J. Xu et al., 2012). In addition, suspensions of β-D-glucan (10% w/v) extracted from barley sprouted for 1 day at 25 °C had 40% lower viscosity readings than a β-D-glucan suspension made from unprocessed barley. Sprouting reduced the average MW of β-D-glucan by 22%, and hence, its thickening ability (Ahmad et al., 2016). As a result of starch (and β-D-glucan) hydrolysis, sprouted wheat, sorghum, oat, and rice flour resulted in 50% to 90% lower peak viscosity values in a Rapid Visco Analyzer model system upon heating than did their nonsprouted counterparts (Doehlert & McMullen, 2003; Marengo et al., 2015; Moongngarm, 2011; Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011; Wichamanee & Teerarat, 2012; J. Xu et al., 2012; Zilic et al., 2016). Finally, the cold paste viscosity levels were 60% to 85% lower for suspensions made from flour of sprouted (1 to 2 days) rice (Wichamanee & Teerarat, 2012; J.G. Xu et al., 2012) or sorghum (Phattanakulkaewmorie et al., 2011; Tizazu et al., 2010) than those from their nonsprouted counterparts. As a result of sprouting, amylase activity decreased amylose chain lengths, causing limited amylose gelation (Phattanakulkaewmorie et al., 2011; Wichamanee & Teerarat, 2012; J.G. Xu et al., 2012). Too low batter and dough viscosities result in poor-quality attributes of wheat-based products (Delcour & Hoseney, 2010), as will be discussed in the next paragraph.

Overall, using flour from sprouted wheat in a bread recipe is detrimental for product quality. Various authors have observed that dough development is negatively impacted when part or all of wheat flour is substituted (2% to 100%) by flour from wheat sprouted for 1 to 4 days at 15 to 25 °C. Farinograph and mixograph analyses have shown that doing so decreases dough development time and stability by 16% to 75% and 25% to 70%, respectively. Partial gluten protein hydrolysis and, more in particular, the decrease in the level of HMW-glutenin macropolymers, is a main cause for these poor dough viscoelastic properties (Baranzelli et al., 2018; Kaur et al., 2002; Liu et al., 2017; Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017; Marti et al., 2018; Morad & Rubenthaler, 1983; Ohm et al., 2016; Watanabe et al., 2004). Furthermore, synthesized α-amylases in sprouted flour are active during dough making and fermentation and impair its processing characteristics, making doughs slack and sticky (Lorenz, Roewe-Smith, Kulp, & Bates, 1983). Substitution of high levels of regular flour by flour from sprouted cereals (more than 20% to 30%) and/or when longer sprouting times are applied (4 to 5 days, 15 to 28 °C) results in breads with much lower overall quality and acceptability. Indeed, the resultant breads have lower volumes (15% to 40%) mainly due to loss of structure by partial gluten hydrolysis (Gawlik-Dziki, Dziki, & Pietrzak, 2017; Hugo, Rooney, & Taylor, 2000; Ichinose et al., 2001; Kaur et al., 2002; Mäkinen & Arendt, 2012; Mäkinen et al., 2012). In addition, the bread crumb is sticky and wet as a result of the high α- and β-amylase levels, as extensive starch hydrolysis reduces flour water-holding capacity (Hugo et al., 2000) and leads to high levels of water-soluble dextrins (Every & Ross, 1996). Moreover, hydrolysis of gelatinized starch during early baking (55 to 75 °C) strongly reduces the viscosity in the system leading to coalescence of gas cells and large holes in the crumb structure (Mäkinen & Arendt, 2012; Mäkinen et al., 2012). These observations are in line with those obtained when flour from wheat that has undergone preharvest sprouting is used in bread making (Ibrahim & D'Appolonia, 1979; Lorenz et al., 1983; Moot & Every, 1990).

Using sprouted cereals in a bread recipe also affects product crumb and crust color. Generally, the crumb and crust are darker (L*, lightness in the CIELAB color space)], more reddish (a*, green–red color component), and yellowish (b*, blue–yellow color component) (Baranzelli et al., 2018; Charoenthaikij et al., 2010; Mäkinen & Arendt, 2012; Marti et al., 2017; Marti et al., 2018; Phattanakulkaewmorie et al., 2011; Tian et al., 2010). Indeed, substantially more reactions between free amino groups and reducing sugars occur during baking of dough made with flour from sprouted wheat than with regular flour (Lasekan, Lasekan, & Idowu, 1997; Tian et al., 2010; van Boekel, 2006). Furthermore, longer sprouting times increase the level of undesirable flavors and odors due to increased lipase and lipoxygenase action leading to aldehydes, free phenolic compounds, heterocyclic substances, and dimethyl sulfide (Dong et al., 2013; Heiniö, Oksman-Caldentey, Latva-Kala, Lehtinen, & Poutanen, 2001; Wu, Yang, Chen, Jin, & Xu, 2011). When present in too high concentrations, these compounds impart rancid, fatty, cabbage-like, and beany flavors to products (Wu et al., 2011).

On the other hand, with short sprouting times (20 to 36 hours), or with a substitution of 10% to 20% of regular wheat by sprouted wheat, high-quality bread can be produced since some α-amylase in flour is desired (Moot & Every, 1990; Toups, 2017). As observed by rheofermentometer analysis, *de novo* synthesized α-amylases can effectively hydrolyze the damaged starch into fermentable sugars promoting yeast fermentation, and hence, increase carbon dioxide production by about 20% (Charoenthaikij et al., 2010; Kaur et al., 2002; Marti et al., 2017; Marti et al., 2018; Struyf, Verspreet, & Courtin, 2016) and maximal dough height by 18% to 22% (Marti et al., 2017; Marti et al., 2018). In addition, decreased dough viscosity leads to increased gas cell expansion (Kim, Maeda, & Morita, 2006; Rouillé, Della Valle, Devaux, Marion, & Dubreil, 2005), which may also contribute to increases in dough height. During baking, residual α-amylase activity levels further decrease the viscosity during starch gelatinization and, by doing so, prolong the oven spring and increase loaf volume (Goesaert et al., 2005; Goesaert, Slade, Levine, & Delcour, 2009). The use of sprouted cereals for baking can also positively impact bread shelf-life and sensory properties. The crumb firming rate of bread produced from sprouted wheat was lower than that of control bread. This may be mainly explained as resulting from the action of amylases on starch, which ultimately reduces amylopectin retrogradation (Liu et al., 2017; Marti et al., 2017; Marti et al., 2018; Phattanakulkaewmorie et al., 2011; Watanabe et al., 2004). Breads from recipes containing sprouted wheat are in general appreciated by the consumer due to their 50% higher total sugar content (Marti et al., 2018; Ohtsubo et al., 2005), giving them a pleasant sweet note (Marti et al., 2018; Ohtsubo et al., 2005; Seguchi et al., 2010). However, the additional nutritional benefits of these types of products are low since it is clear from previous sections that sprouting times of at least 3 days are needed to increase the nutritional value of wheat.

However, for some specific product applications, such as in the production of tortillas, sprouting can make wholemeal products more acceptable for consumers. Fully or partially substituting wheat flour with flour from wheat sprouted for 2 days at 25 °C significantly increased the size of tortillas (Liu et al., 2017; Zhu, Adedeji, & Alavi, 2017). Dough produced from sprouted wheat flour readily expands during extrusion resulting in puffy tortillas, which are valued by consumers. Indeed, such extruded products have an increased sensory score and overall good acceptability (Liu et al., 2017; Pawar, Shere, & Khapre, 2016). Tortillas from sprouted wholemeal have a less grainy texture, bitterness, and better opacity scores than their counterparts from nonsprouted wheat wholemeal (Liu et al., 2017). Furthermore, during storage, the latter more easily crack or break, probably because their starch component retrogrades less than that in tortillas produced from nonsprouted wholemeal (Liu et al., 2017).

Conclusion

Recent growing positive consumer perceptions about sprouted cereals have resulted in new food and beverage launches, in particular in the U.S.A. and in Europe. Such products have a "positive health" halo. Indeed, the nutritional value of cereal grains increases upon sprouting, especially when long sprouting times (3 to 5 days) and/or high processing temperatures (25 to 35 °C) are applied. During sprouting, minerals are released from their phytate chelates and become available for intestinal absorption, and vitamins and GABA are synthesized and accumulate. It is critical to optimize process conditions and to choose the desired type of cereal when one is to maximize the nutritional properties of sprouted grains.

At the moment, there is no accepted definition of the characteristics and requirements for whole grains to be considered as sprouted. In the context of maximizing the cereal nutritive value, we recommend that a whole grain can only be considered as sprouted if it has a clearly visible radicle. Indeed, only in phase III of germination, the major mobilization of reserve material occurs for seedling growth and development. Moreover, we urge the existence of a globally recognized definition and regulation of "sprouting" to ease research on this topic and support the production of ~~sprouted based~~ products.

In the literature, the (bio)chemical changes occurring during sprouting have frequently been extrapolated to potential health benefits. However, the outcome of clinical studies to support specific health benefit statements is very limited. Initial studies have suggested that sprouted grains may have a beneficial effect on blood cholesterol and glucose levels, blood pressure, and mineral absorption. However, much work has been done using *in vitro* and animal models. In the few available human intervention studies, the materials were poorly characterized. It is hence difficult to draw any definitive conclusions about cause and effect. Interesting observations stem from *in vitro* research showing that sprouting can reduce the content of and bioreactivity to celiac disease-stimulating peptides. However, the implications of these observations are hard to interpret, because the quantity of sprouted material used in food determines the effectivity of the food in terms of reducing disease risk factors and improving tolerance. Therefore, there is a clear need for well-designed randomized clinical trials to study the impact of well-characterized sprouted grains and quantities on physiological parameters and health status.

Sprouted nutrient-dense grains are deleterious for bread making. Including flour from sprouted wheat in a bread recipe negatively affects its end-quality due to excessive peptidase and α-amylase actions during dough making, leading to a loss of structure and sticky and wet crumb with large holes, respectively. The food industry tackles these shortcomings by using short sprouting times and/or by blending flour from sprouted wheat with its regular counterpart to obtain good end-products. Unfortunately, these strategies substantially compromise the cereal nutrient content and potential health-beneficial effects of the sprouted grains. Innovative strategies need to be developed to inactivate hydrolyzing enzymes in sprouted grains before or during product making, and their functionality in a wide range of staple foods such as bread, pasta, noodles, and breakfast flakes need to be explored.

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Author contributions

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List of Abbreviations

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~~R&D~~	~~Research and development~~
~~GMO~~	~~Genetically modified organism~~
ESSA	European Sprouted Seeds Association
EU	European Union
HMW	High molecular weight
kDa	Kilodalton
MW	Molecular weight
RT	Room temperature
WHO	World Health Organization
AX	Arabinoxylan
WEAX	Water-extractable arabinoxylan
WUAX	Water-unextractable arabinoxylan
AXOS	Arabinoxylan oligosaccharides
FDA	Food and Drug Administration
CHD	Coronary heart disease
EFS	European Food Safety Authority
LDL	Low-density lipoprotein
P	Phosphorus
Fe	Iron
Zn	Zinc
Ca	Calcium
Mn	Manganese
Mg	Magnesium
Cu	Copper
IP6	Inositol ~~hexphosphate~~
IP5	Inositol pentaphosphate
IP4	Inositol tetraphosphate
IP3	Inositol triphosphate
FAO	Food and Agriculture Organization
RDA	Recommended dietary allowance
GABA	γ-Aminobutyric acid
CVD	Cardiovascular disease
HDL	High-density lipoprotein
LMW	Low molecular weight

CFU	Colony-forming unit
NA	Not available

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