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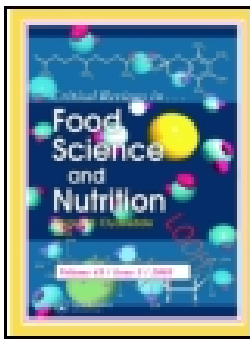
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**Innovative processing techniques for altering the physicochemical properties of wholegrain brown rice (*Oryza sativa* L.) – opportunities for enhancing food quality and health attributes**

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**Running title:** Novel processing methods and brown rice

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## **Abstract**

Rice is a globally important staple consumed by billions of people, and recently there has been considerable interest in promoting the consumption of wholegrain brown rice (WBR) due to its obvious advantages over polished rice in metabolically protective activities. This work highlights the effects of innovative processing technologies on the quality and functional properties of WBR in comparison with traditional approaches; and it is aimed at establishing a quantitative and/or qualitative link between physicochemical changes and high-efficient processing methods. Compared with thermal treatments, applications of innovative nonthermal techniques, such as high hydrostatic pressure (HHP), pulsed electric fields (PEF), ultrasound and cold plasma, are not limited to modifying physicochemical properties of WBR grains, since improvements in nutritional and functional components as well as a reduction in anti-nutritional factors can also be achieved through inducing related biochemical transformation. Much information about processing methods and parameters which influence WBR quality changes has been obtained, but simultaneously achieving the product stabilization and functionality of processed WBR grains requires a comprehensive evaluation of all the quality changes induced by different processing procedures as well as quantitative insights into the relationship between the changes and processing variables.

**Keywords:** Emerging nonthermal technologies; high hydrostatic pressure; low-pressure plasma; GABA; glycemic index; cereal germination

## 1. Introduction

Rice is a staple food for more than three billion people and is mainly consumed as polished white rice (PWR) (Park, Lee, & Choi, 2017). The making of PWR at an industrial scale has a relatively long history with stable and well-established processing procedures. However, PWR manufacturing is still in progress, such as seen by the recent exploitation of commercially available convenience rice (Yu et al., 2017a) and health rice with a reduced glycemic load (Boers, Seijen ten Hoorn, & Mela, 2015), as well as the introduction of novel profiling techniques for evaluating PWR quality (Pramudya & Seo, 2018). In the cases of PWR production and consumption, the examined factors are mainly confined to the design and optimization of traditional post-harvest processing stages, including milling, parboiling, quick-cooking, drying, and storage (Atungulu & Pan, 2014; Boers et al., 2015; Yu et al., 2017a).

PWR consumption provides over 75% of daily calorie intake from rice particularly in Asian countries (Park et al., 2017). However, recent meta-analyses and cohort studies have observed significant positive relationship between PWR intake and the incidence of type 2 diabetes mellitus (Hu et al., 2012; Nanri et al., 2010), partially explaining the largest number of people with diabetes in China and India, where PWR is the primary source for dietary glycemic load. Over the past few years, the increasing attention has been paid to the substitution of PWR with wholegrain brown rice (WBR) for regulation and control of diabetes, since accumulated results have confirmed the better glycemic control of WBR compared with equivalent PWR (Lee et al., 2016; Nakayama et al., 2017; Wedick et al., 2015; Zhang et al., 2010). Importantly, the dietary substitution process did not substantially improve other metabolic risk factors (Zhang et al., 2011). The anti-diabetic effects derived from WBR are mainly attributed to reduced glycemic response of its available carbohydrate (Zhang & Hamaker, 2017), as well as potential regulation of other minor bioactive compounds such as

gamma-aminobutyric acid (GABA) (Adamu et al., 2016; Tian et al., 2011),  $\gamma$ -oryzanol (Kozuka et al., 2013), and magnesium (Schulze et al., 2007). In addition, apart from anti-diabetic effects, there are many other health benefits which can be obtained by consuming WBR grains, such as antioxidant, anticancer, antihyperlipidemic, antihypertensive, and anticardiovascular effects (Roohinejad et al., 2009a; Roohinejad et al., 2010; Jensen et al., 2004; Lee et al., 2016; Liu et al., 2015; Okarter & Liu, 2010; Wu et al., 2013b).

The therapeutic activities from WBR are mainly attributed to its higher levels of bioactive phytochemicals and nutrients than PWR, including  $\gamma$ -oryzanol, GABA, dietary fibers, phenolics, phytosterols, tocotrienols, carotenoids, polyunsaturated fatty acids (omega-3/omega-6), minerals, anthocyanins, proanthocyanidins and vitamin B, among other minor phytonutrients (Cho & Lim, 2016; Park et al., 2017). Generally, these nutritional substances act synergistically and have protective effects against chronic degenerative diseases (**Fig. 1**). For example, bioactive compounds present in WBR with antidiabetic effects include GABA, dietary fibers,  $\gamma$ -oryzanol, and resistant starch (RS) fraction. GABA is a non-protein amino acid and shares various physiological activities in mammals, such as functioning as neurotransmitter in the central nervous system, regulating blood pressure and heart rate, accelerating brain protein synthesis and relieving pain and anxiety (Diana, Quílez, & Rafecas, 2014; Gan et al., 2017; Poojary et al., 2017). Oral supplements with GABA significantly reduced the glycemic response and improved glucose tolerance in rat model, attributing to its enhanced secretion of insulin from the pancreas (Adeghate & Ponery, 2002; Tian et al., 2011). Similarly,  $\gamma$ -oryzanol, a mixture of ferulic acid esters, could also act as a strong secretagogue by directly affecting pancreatic  $\beta$ -cells to promote glucose-stimulated insulin secretion (Kozuka et al., 2013). As for dietary fiber, clinic trials have established the metabolic link between the high dietary fiber intake and the reduced diabetes incidence (Chandalia et al., 2000; Schulze et al., 2004). The positive effects of dietary fiber on diabetes

are considered to be closely related to its viscous and/or gel-forming properties as well as the non-digestibility in the upper gut, which thus decrease postprandial glucose response and slow gastric emptying (Weickert & Pfeiffer, 2008).

Based on the above-mentioned nutritional values and bioactivities, the technology developments and improvements aimed at producing high-quality WBR products have gained attention within the last decade (**Table 1**). Several reviews have focused on the molecular structure and occurrence of phytochemicals existing in WBR grains or its processing (by)-products (Cho & Lim, 2016; Goufo & Trindade, 2014). In addition, others have examined the relationship between selected phytonutrients and potential health outcomes (Park et al., 2017; Perez-Tenero, de Sotomayor, & Herrera, 2017; Wu et al., 2013b). Although a considerable amount of research has been investigated on WBR grains processing, to the best of our knowledge, there is no any formal systematical review to summarize the effects of different conventional and innovative processing strategies on the quality characteristics and health benefits of WBR samples. Thus, the aim of the present review is to highlight the recent advances in the improvement of quality and functional attributes of WBR products through integrating various processing and pretreatment techniques. Major changes in physicochemical properties, especially those involved in consumers preference, and their association with process parameters as well as the underlying mechanisms are described and discussed. Furthermore, the respective advantages, limitations, and challenges are also compared.

## **2. Challenges for WBR production**

To increase wholegrain consumption, the food industry has been endeavoring to enhance the proportion of WBR consumed in existing food products, such as breads (Cornejo et al., 2015; Islam, Ud-Din, & Haque, 2012), noodles (Baek & Lee, 2014; Chung, Cho, & Lim, 2012; Wu



et al., 2017), pastas (Marti, Seetharaman, & Pagani, 2010), snacks (Chung, Cho, & Lim, 2014; Suhem et al., 2015; Suhem et al., 2013b), ethnic puddings and pastries (Gharibzahedi, 2018), and beverages (Koyama & Kitamura, 2014; Mok, 2006). However, although the incorporation of wholegrain foods into the diet is encouraged in newly issued dietary guidelines (Jones et al., 2017), the amount of WBR consumption remains relatively low because of its limited acceptability as a staple food or a regular part in the diet. The limiting factors are mainly relevant to the histochemical structure of WBR and distribution pattern of bioactive components across the grains.

## 2.1 Wholegrain characteristics and its impact on consumer acceptability

Rice cultivars can be classified into two different subspecies: *indica* and *japonica*. According to the histochemical difference (**Fig. 2a**), the technical terms in relation to rice milling include paddy rice, WBR and PWR (Nambi, Manickavasagan, & Shahir, 2017). Paddy rice refers to the intact grain after threshing, which is composed of husk, caryopsis coat (bran), endosperm and embryo. Structurally, bran consists of pericarp, seed coat and nucellar cells (De Datta, 1981). For WBR production (**Fig. 2b**), the husk including palea and lemma is completely removed, whereas the bran is partially milled; the obtained WBR grain is composed of bran layers (6-7% of its total weight), embryo (2-3%), and endosperm (about 90%) (Chen, Siebenmorgen, & Griffin, 1998). WBR grain is considered as a wholegrain food because of the co-existence of endosperm to pericarp, seed coat and germ, where many physiologically important components are principally localized (Okarter & Liu, 2010). It is considered that the synergies of the three kinds of anatomical fractions (bran layer, germ, and endosperm) lead to greater health-promoting effects than any single component (Schaffer-Lequart et al., 2017). Nevertheless, the presence of bran and germ is closely linked to the following intrinsic and extrinsic factors responsible for the limited acceptability of

WBR among consumers. These include: (i) the hard texture perception and chewing palatability due to high fiber content (Gujral & Kumar, 2003); (ii) the poor water absorption characteristics and prolonged cooking time (Bello, Tolaba, & Suarez, 2004; Thakur & Gupta, 2006); (iii) the greater susceptibility to oxidation and off-flavor formation compared with PWR (Goffman & Bergman, 2003; Lanning & Siebenmorgen, 2011); (iv) the presence of anti-nutritional factors (e.g. phytic acid and tannin) (Wang et al., 2011); (v) habitual preference for refined products, as well as the unfamiliarity with health benefits of WBR among average consumers.

## **2.2 Distribution characteristics of nutritional components**

The extent of bran layer removal is defined as the degree of milling (DOM), closely associated with an overall nutritional value of WBR grain. In general, DOM for WBR grain production tends to span a wide range from 0 to 10%. The reduction in lipids, proteins, antioxidants, dietary fiber, vitamins and minerals increases with the DOM value. For this reason, the DOM is often limited to below 2% (Nambi et al., 2017).

Quantitative information related to the effects of DOM on nutritional and physicochemical characteristics of WBR is summarized in **Fig. 3**. The loss of protein, minerals, free phenolics and flavonoids during the milling process might be reached up to 28.6%, 84.7%, 65.1% and 72.3%, respectively (Lamberts et al., 2007; Liu et al., 2015). Among essential minerals, the milling process resulted in 25–84% of iron loss depending on rice genotypes (Prom-u-thai et al., 2007). In the case of pigmented rice, the milling process from paddy grain to PWR was reported to remove more than 90% of free phenolics from black and red rice grains (Paiva et al., 2016). These quantitative results have clearly confirmed that a substantial proportion of nutrients exist in the fractions of outer bran and rice germ.

On the other hand, however, as DOM increases, a softer oral palatability and improved cooking index can be achieved, as outer rice bran (RB) possesses a chewy texture and compacted structure which hinders the penetration of water into the interior layers of rice caryopsis. Thus, a negative relationship between sensory characteristics and nutritional values of WBR can be concluded. Furthermore, although the outer bran fraction and germ have higher contents of bioactive components, the co-existence of oxidative enzymes including lipoxygenase and peroxidase, and the resultant off-flavor, as well as anti-nutritional factors such as phytic acid and tanning can largely compromise the desirability to consumers. In spite of their antioxidant and anticancer properties, phytate and tanning compounds are considered to be anti-nutritional factors as a whole (Coulibaly, Kouakou, & Chen, 2011; Torkamani, Razavi, & Gharibzahedi, 2014).

Overall, all these distinct characteristics intrinsically require the application of innovative processing strategies in WBR production for improving the balance between sensory characteristics and nutritional values. On one hand, overcoming those obstacles involved in storability and textural properties is the major prerequisites for promoting WBR consumption. On the other hand, minimizing the loss of bioactive components during processing, particularly those heat-sensitive substances, is another great concern for the selection of optimum processing procedures and parameters.

#### **4. Multiple strategies to improve WBR quality and functionality**

Over the past few years, there have been several studies conducted to minimize the limitations of WBR using biological approaches and innovative processing technologies and their combined patterns (**Fig. 4**). Some of the advantages and limitations of these techniques are summarized in **Table 1**.

## 4.1 Bioprocess-based approaches

Bioprocessing of WBR grains includes germination, fermentation and the use of hydrolytic enzymes at various processing stages.

### 4.1.1 Germination

Germination is a complex physiological process that is traditionally used as an economical method for the preparation of sprouted seeds, such as soybean, mungbean sprouts, etc. There is a great interest in developing cereal germination mainly due to the enhancement in nutritional and functional properties during the process and resultant health outcomes (Roohinejad et al., 2011; Roohinejad et al., 2009b; Hübner & Arendt, 2013; Hahm, Park, & Martin Lo, 2009; Huang, Cai, & Xu, 2014). The differences between germinated and non-germinated grains have been widely studied in terms of physicochemical and sensorial properties of WBR-derived products (**Table 1**). Short-period germination is capable of softening texture, improving palatability, and shortening cooking time as well as reducing anti-nutritional factors (Jiamyangyuen & Ooraikul, 2008; Liang et al., 2008; Sirisoontaralak et al., 2015; Watchararparpaiboon, Laohakunjit, & Kerdchoechuen, 2010).

During germination, significant changes in the chemical composition of WBR grains can be observed, as summarized in **Table 2**. Although the final contents of chemical substances in WBR may be influenced by rice genotypes and germination conditions (e.g., temperature, time, pH, and culture solution composition), there are still some general patterns found for the variation of macro- and micro-molecular nutrients within the process. For instance, the starch content was reported to decrease by the increased hydrolytic activities of the enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, resulting in the accumulation of simple sugars (Saman, Vázquez, & Pandiella, 2008; Sibian, Saxena, & Riar, 2017). Regarding protein, it shows an overall increasing tendency as a result of the synthesis of new proteins and enzymes mainly

in the GBR sprouts (Kim et al., 2012; Moongngarm & Saetung, 2010; Sibian et al., 2017). Moreover, anti-nutritional compounds in WBR, such as phytic acid and oxalate, can be greatly reduced due to the activation of related hydrolases across the entire germination process (Bartnik & Szafrńska, 1987; Liang et al., 2008; Nissar et al., 2017). Biochemical and metabolic activities during germination process result in the significant accumulation of bioactive components, such as dietary fibers, GABA, folate, vitamins,  $\gamma$ -oryzanol, flavonoids and phenolic acids (**Table 2**). All these changes within the grains, associated with macro- and micro-nutrients, are regulated by various metabolic pathways and also interacted with each other (Pramai et al., 2017; Wang et al., 2016). Biochemical activities of specific pathways may vary at different germination periods and result in the fluctuation of some metabolites during the process. As in the case of the transformation of phenolic compounds, soluble fractions of phenolic acids were synthesized at initial stage of germination while insoluble fractions were mainly produced at later stage of germination, which was due to the difference in the activities of key enzymes at different germination periods, such as phenylalanine ammonia-lyase and cell wall peroxidase (Cho & Lim, 2018).

Although the effects of germination process on the physicochemical and nutritional properties of GBR have been extensively reported, the molecular mechanisms of action are still not very clear. For example, to explain the reasons for the increased *in vitro* starch digestibility derived from germination, changes in molecular and crystalline structures have been described by microstructural characterization approaches (You et al., 2016). The authors concluded that the increased susceptibility of starch to the enzymatic attack was related to the pores and pins formed on the surface of starch granules, reduced crystallinity and increased short-chains amylopectin. However, regarding the distribution of starch molecular weight, the results were controversial. You et al. (2016) observed a reduced average molecular weight attributing to the decomposition of some amylopectin molecules during the germination

process; and interestingly, they also found a slightly higher average branch chain length of amylopectin after germination. In contrast, according to Wu et al. (2013a) and Li et al. (2017), there were no significant differences observed in the molecular weight between raw and germinated grains starch, including both weight-average molecular weight (Mw) and number-average molecular weight (Mn).

It should be noted that a long-period soaking process during the germination may cause the loss of micronutrients (e.g. minerals, soluble antioxidants, and aroma-active compounds) due to the leaching out into the aqueous phase. Furthermore, the microbiological spoilage during steeping stage is one of the biggest challenges for sprouts production, because bacteria, molds and yeasts can proliferate quickly owing to favorable temperature and nutritional conditions. For overcoming these problems, thermal sterilization such as hot-water (90 °C/90 s) and dry-heat treatments (50 °C/17 h) were suggested to inactivate microbial growth (Bari et al., 2009; Bari et al., 2011). Nevertheless, the seed germination performance could be compromised greatly.

Several studies reported the feasibility of using electrolyzed water (EW) as a culture solution in GBR production instead of tap water (**Table 1**). Generally, EW is produced via electrolyzing dilute salt solutions or hypochlorous acid. EW has been authorized as an antimicrobial agent in Japan since 2002, due to its confirmed biological safety and environmental friendliness at effectively bactericidal levels (Huang et al., 2008). Disinfection effectiveness originating from EW treatments was closely related to its high oxidation-reduction potential and the presence of free chlorine, causing the unbalanced metabolic fluxes and energy production as well as the damage of cell wall/membranes of microorganisms (Huang et al., 2008; Liao, Chen, & Xiao, 2007; Osafune, Ehara, & Ito, 2006; Wang, Xia, & Li, 2017a, 2017b). Interestingly, apart from efficiently inhibiting microbial proliferation, the use of EW as a washing and soaking agent had the capability to promote

WBR sprouting and GABA accumulation (Li et al., 2015; Liu et al., 2013; Rui et al., 2011), indicating that metabolic activities and biochemical pathways of plant cells can be affected by EW during the germination. However, the specific mechanisms relating physicochemical attributes to the variation of metabolic pathways and biochemical reactions for EW-mediated germination process need to be further clarified.

#### 4.1.2 Fermentation

WBR fermentation is used as a supplementary technique to utilize substantial bioactive components in the grains by producing WBR-based foods, such as beverages, porridges, vinegar and bread (**Table 1**). Fermenting strains include yeast, lactic acid bacteria (LAB), *Monascus sp.* and *Aspergillus oryzae*. Powerful metabolic activities and enzymatic transformation derived from microorganisms during WBR fermentation process help to enhance the bioavailability of micronutrients as well as to reduce the anti-nutritional factors. According to Ilowefah et al. (2015), inoculating the yeast to WBR flour exerted significant positive effects on functional properties when fermented at a moderate acidity (pH 5-6), which achieved the increase of protein, insoluble fiber, soluble fiber, minerals, phenolics, total antioxidants, RS, and vitamins B and E as well as the reduction of phytate. Simultaneously, physicochemical characteristics of WBR flour, including foaming capacity, foaming stability, oil holding capacity, gelatinization temperatures, enthalpy, and whiteness can be largely influenced by fermentation. These changes could be regulated by modifying fermentation process parameters, such as temperature, time and yeast inoculum concentration.

In addition, the formation of organic acids and volatile flavoring compounds as well as the decomposition of polysaccharide into simple sugars by microbial strains and hydrolytic enzymes can endow the final products with pleasant taste presenting slightly acid and sweet

(Li, Dai, & Lu, 2007). Based on the quantification results by liquid chromatography/tandem mass spectrometry (Ogawa et al., 2017), it has been confirmed that esterase activities from *A. oryzae* enhanced the liberation of bound phenolic acids and significantly increased hydroxybenzoic acids. This process allows the improvement of food functionality as free forms of phenolic compounds are inclined to represent higher bioactivities than the corresponding polysaccharide-bound forms (Subba Rao & Muralikrishna, 2002). Increasing evidence of *in vitro* and *in vivo* researches has suggested that the fermented WBR products have multiple preventive and therapeutic effects against carcinogenesis, oxidative stress, inflammation and glucose homeostasis disorders (Chung, Rico, & Kang, 2014; Kuno et al., 2015; Onuma et al., 2015). Despite a promising tool to produce WBR-based products with desirable organoleptic, nutritional and functional attributes, fermentation process involves in obvious transformation in the form of food matrix and in most cases, the WBR flour rather than integral grains is used as the fermenting substrate for optimum metabolic activity of the inoculated microbes, which may limit the application of fermentation in WBR processing.

#### 4.1.3 Enzyme-based approaches

Enzymatic action occurs for the specific transformation of targeted molecules, and this feature is currently utilized to engineer food structure for obtaining novel physical and functional attributes (Zeeb, McClements, & Weiss, 2017). One of major advantages of enzymatically treating WBR grains is to achieve better textural and cooking attributes by degrading bran layers using cellulase enzymes or related complex enzymes. According to Das, Banerjee and Bal (2008a), applying xylanase and cellulase to WBR grains before cooking resulted in the easier diffusion of water into the kernel, thus reducing cooking time. More importantly, major nutrients including phenolic compounds, minerals, thiamine, niacin, and pyridoxine were largely maintained after enzymatic treatments. In this sense, the substitution



of mechanical milling with enzymatic polishing was proposed for the production of rice products (Das et al., 2008b). To reduce abrasive perception from bran layer, protease pretreatments were also introduced to soften WBR-based bread through decreasing the hardness and chewiness (Renzetti & Arendt, 2009). Interestingly, apart from shortening the soaking time, enzymatic pretreatments with xylanase and cellulase before germination were reported to improve germination yields and GABA levels (Jia et al., 2016).

Combining mechanical treatments with the use of enzymes was proposed to maximize the advantages of enzymatically processed WBR. Zhang et al. (2015a) evaluated the applicability of ultrasonic treatment followed by the cellulase solution soaking, who observed more obvious improvement of cooking and sensory attributes such as textural quality and taste value compared with purely using enzymatic processing. This was attributed to the cracks formed on the surface of impervious compact fibrous layer after ultrasonic treatments, which favored the penetration of enzymes into the interior and accelerated enzymatic degradation of RB. In the case of extrusion, it was found that the addition of thermostable  $\alpha$ -amylase during the extrusion of WBR flour greatly increased the retention of phenolics and free flavonoids (Zeng et al., 2017; Zeng et al., 2018), which became more prominent when the enzyme levels were increased. However, the specific mechanisms about the relationship between high reducing sugar contents and the retention of antioxidant compounds were not clarified.

## **4.2 Innovative nonthermal processing techniques**

### *4.2.1 High hydrostatic pressure*

High hydrostatic pressure (HHP), also named high pressure processing (HPP), is regarded as the most successfully commercialized innovative nonthermal approach (Misra et al., 2017a). This technique employs water as the medium to transmit pressure (generally in the range of 100-600 MPa, at an industrial level) throughout food system aiming to produce

organoleptically high-quality, microbiologically safe and nutritionally intact products. Application of high pressure (HP) inactivates microorganism and deteriorates enzymes but it has a minimal impact on low-molecular-weight compounds such as vitamins, flavoring molecules and antioxidants (Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015; Barba, Esteve, & Frígola, 2012; Barba et al., 2017a; Huang et al., 2017; Oey et al., 2008). Recent research focuses regarding the use of high pressure are more put on the exploitation and development of health foods, based on its capability to promote the bioaccessibility/bioavailability of bioactive components, to reduce food additives and salt and also to inhibit the formation of contaminants (Barba et al., 2017b; Barba et al., 2015b; Pottier, Villamonte, & de Lamballerie, 2017).

The rice ingestion may cause allergic disorders including asthma and dermatitis, mainly associated with the allergenic properties of albumin and globulin (Estrada-Girón, Swanson, & Barbosa-Cánovas, 2005). The application of HHP in rice products was initially driven by its potential to reduce allergenicity relying on the HP-induced partial destruction in cell integrality and protein structure (Kato et al., 2000; Zhang et al., 2015b). After pressurization at 100-400 MPa for 30 min, the significant solubilization of allergenic proteins in rice was observed, resulting in the release of the allergens into the solution (Kato et al., 2000). By combining HHP treatments and protease, the release process of rice allergens was promoted, resulting in their complete elimination from grains.

Recent applications of HP technique in rice have been concentrated on its ability to achieve quick cooking and good texture (Boluda-Aguilar et al., 2013), and these HP-induced changes in rice qualities are urgently demanded for WBR grains. The feasibility of using HP treatment combined with various processing parameters and patterns, in relation to their effects on sensory and nutritional characteristics of final processed products have been investigated (**Table 1**).

It has been well-known that HP treatments can accelerate water uptake kinetics by enhancing the hydration of cereal biopolymers such as starch and protein (Knorr, Heinz, & Buckow, 2006). Pre-soaking is generally used to soften the grain and to facilitate the starch gelatinization during the cooking process, particularly for the rice with poor water absorption. In this line, Ahromrit, Ledward, and Niranjana (2006) investigated HP-induced water uptake characteristics of glutinous rice (polished form), concluding that the equilibrium moisture content (EMC) and water uptake rates increased with pressure intensities (100-600 MPa) and temperature (20-70 °C). Regarding WBR grains, however, the values for EMC fluctuated within the applied pressures of 100-500 MPa, which was related to the histochemical difference and the formed cracks at the RB surface (Yu et al., 2015).

Yu et al. (2015) reported that the HP-mediated pre-soaking contributed to shortening about 60% of the cooking time of WBR grains; and more importantly, the palatability was largely improved due to the damaged structure of pericarp and aleurone layer under pressures. These changes in cooking and textural attributes were greatly dependent on the applied pressure intensities (holding times and pressures) and processing patterns. For example, the more obvious structural damage of RB under two-cycle HP processing led to a lighter color and a softer texture for cooked WBR grains, compared with the one-cycle mode based on same processing duration (Yu et al., 2017b).

Given the high nutritional value of germinated cereals, HP processing has been applied to enhance the utilization efficiency of nutrients in GBR grains, particularly bioactive micronutrients. Through an *in vitro* digestion model, it was revealed that HP treatments (100-500 MPa/10 min) greatly improved the *in vitro* bioaccessibility of micronutrients including GABA, indispensable amino acids, and antioxidant compounds as well as the starch digestibility, whereas bioaccessible minerals varied depending on the pressures and element species (Xia et al., 2017b; Xia et al., 2017c). For enhancing biochemical changes

under HP, WBR grains were germinated and immediately kept under HP conditions for 24-48 h (Kim et al., 2015a, 2017; Kim et al., 2015b). The pressures were limited within moderate ranges varying from 10 to 100 MPa, mainly used to maintain the enzyme activities and to induce partial cell decompartmentalization for the easier accessibility of enzymes to the targeted substrates in GBR matrix. Thus, the substantial bioconversion reactions responsible for the accumulation of bioactive compounds, including polyphenols, GABA, arabinoxylans, flavonolignans, and phenolic acids and vitamin E, was significantly promoted at post-germination stages, depending on the applied pressures.

It should be noted that the above-mentioned researches were mainly focused on the changes after immediate HP treatments. However, recent results suggested that the direct HP processing on WBR grains could cause some deleterious outcomes, including the acceleration of lipid hydrolysis and oxidation kinetics as well as the presence of browning compounds during the prolonged storage (Xia & Li, 2018b; Xia et al., 2017d). To avoid these negative effects, HP pretreatments prior to the germination could be a potential strategy for improving functional and quality properties of WBR grains, because through this combined process the increased GABA content, the reduced lipid hydrolysis and oxidation, and the stabilized color development during storage process were simultaneously achieved (Xia & Li, 2018a; Xia, Wang, & Li, 2018). These observations could be associated with the modifications of metabolic pathways during malting due to HP stress effects. Particularly, when plants like WBR grains are stressed by HP, some plant secondary metabolites may be accumulated through adaptive response during their germination, similar to what happened under other abiotic stress factors (e.g., salt, drought and anoxia) (Barba et al., 2017c). In this sense, biological implications together with their unique biochemical and molecular effects behind HP can be exploited for the modulation of food functionality and quality characteristics.

#### 4.2.2 Pulsed electric fields (PEF)

As a newly employed nonthermal technology, PEF is applied to various foodstuffs through transient electronic pulses ( $\mu\text{s} \sim \text{ms}$ ) produced from two high-voltage electrodes, with an electric field strength varying from 0.1 to 80 kV/cm (Oey et al., 2016; Barba et al., 2015a). One of the major applications of PEF processing is inactivation of a wide range of microorganisms and quality-related enzymes with minimum effect on sensory and nutritional properties (Barba et al., 2018; Barba et al., 2015a; Silva et al., 2016). Qian et al. (2014) have examined the contribution of different PEF parameters to lipase inactivation in WBR samples, finding that the inhibitory effect was positively related to the frequency (200-1000 Hz), pulse width (3-15  $\mu\text{s}$ ), residence time (1-9 s), and voltage (3-15 V), while it was negatively influenced by the material thickness (5-25 mm). Moreover, voltage was found to be the main factor influencing microbial inactivation followed by pulse width and frequency (Gabrić et al., 2018).

PEF is also used as a non-conventional extraction tool for recovering functional components from plant materials since electronic pulses can improve membrane permeabilization by modifying membrane microstructure, thus enhancing the mass transfer rate (Roohinejad et al., 2014a, Roohinejad et al., 2014b; Puértolas & Barba, 2016; Puértolas, Koubaa, & Barba, 2016; Zhu et al., 2016). As expected, when applied to WBR samples, PEF-assisted solvent extraction increased the yields of many bioactive components including polyphenols, phenolic acids,  $\gamma$ -oryzanol tocopherols as well as saturated and unsaturated fatty acids, and therefore corresponded to higher antioxidant activity and cytotoxic activity against colorectal cancer cells compared with the extract obtained from PEF-untreated samples (Quagliariello et al., 2016). Considering that the by-product of WBR and PWR production is a source containing considerable amounts of phytochemicals, the integration of PEF pretreatment into the solvent extraction of RB could be a promising strategy to improve the

recovery rates of bioactive components. To our knowledge, only few studies examined the applicability of PEF for WBR processing, and little information about the changes in sensory attributes and biological effects of WBR grains has been reported, particularly in terms of PEF-derived electrochemical effects and the possible metabolic response after electric stimulus for GBR (Dymek et al., 2012; Misra et al., 2017b).

#### *4.2.3 Cold plasma*

Cold plasma technology has drawn considerable attention from food sectors in the last decade owing to its large potential for the safe and sustainable food production (Bourke et al., 2018; Misra et al., 2017a; Misra et al., 2017b). Plasma refers to an ionized gas mixing positive and negative ions, photons, electrons and molecules, overall representing nearly no net electrical charge; and the generation of plasma involves in the ionization of neutral gas under different electromagnetic fields with sufficient power (Misra et al., 2016). Cold plasma is initially required to generate at the low-pressure condition, and recent advances in plasma source manufacturing techniques allow the use of plasma technique under atmospheric pressure, which is rather important for wide application in food processing (Misra et al., 2016; Segat et al., 2016). Generally, relying on the plethora of chemically active species produced during gas ionization, plasma treatment shows the capability to induce chemical modification to food components and to inactivate deteriorative enzymes and microorganisms (Bourke et al., 2018; Misra et al., 2016). Based on this fact, the practical applicability of cold plasma in WBR processing has recently been explored by several studies.

After plasma treatments (1-3 kV/1.2 mA/30 min), morphological observation showed the presence of etching patterns on the surface of WBR bran, causing easy water absorption and reduction in cooking time and hardness (Chen, 2014; Chen, Chen, & Chang, 2012). These results have recently been confirmed by Thirumdas et al. (2016), using low-pressure plasma

technique. Even though the plasma-processed samples were subjected to a three-month storage, the processing effects on cooking properties (cooking time and hardness) were still significant (Chen et al., 2015a). This kind of stability in plasma-induced physicochemical modification is extremely meaningful because the processed samples must be subjected to a storage process before consumption. Therefore, a further study was performed to examine plasma effects on WBR grains during the storage (Chen et al., 2015a), showing the decrease in lipid hydrolysis and oxidation in plasma exposed samples owing to the inhibitory effects on the activities of the enzymes such as lipase and lipoxygenase. In addition, a significant reduction in  $\alpha$ -amylase activity was also observed in plasma-treated samples during storage. The plasma-induced inactivation effects on enzyme activities were mainly attributed to the spatial changes of enzyme structures and chemical modification on individual amino acids by ionized gas, in relation to enzyme varieties, plasma treatment parameters (e.g., voltages, frequency, and exposure mode) and types of plasma sources (Misra et al., 2016).

Inactivation effects can be enhanced by enlarging plasma power and extending treatment time, but the possibility of compromised sensory characteristics derived from increased plasma exposure should be considered. For example, WBR cereal bars were subjected to the radio-frequency power of 40 W for 25 min to completely inhibit the growth of *A. flavus* during storage (Suhem et al., 2013a). However, by applying these processing conditions, the odor like cooking rice was produced and thus reduced consumer's acceptability. To maintain an acceptable odor and inhibit the microorganisms efficiently, the exposure time of cold atmospheric plasma was reduced to 20 min (Suhem et al., 2013a).

On the other hand, plasma-induced biological effects were also used to improve quality characteristics and functionality of WBR products. Chen et al. (2016) investigated the effects of low-pressure plasma pretreatments (1-3 kV/10 min) prior to the WBR germination on seed vigor and functional components. It was found that plasma shock contributed to the

significant increase in germination rates, seedling length, GABA, total phenolic compounds, total antioxidant capacity and  $\alpha$ -amylase activity in GBR samples, presenting more prominent enhancement with increased plasma voltages in the range of 1-3 kV. When the plasma voltage was kept at 80 kV, the germination rates of wheat remained unchanged for 5-min treatment, whereas extending the processing time to 20 min greatly decreased the seed sprouting capability, regardless of exposure mode (Los et al., 2018). These results demonstrated that mild plasma treatments could be used as a successful strategy for enhancing the functionality of germinated products.

#### *4.2.4 Ultrasound*

Ultrasound is a well-established technique and its novel applications in food industry related to the analysis, extraction, quality control, and processing have been persistently exploited in the last two decades (Awad et al., 2012; McClements, 1995; Zhu et al., 2016). In the ultrasonication process, ultrasonic waves are capable to create an alternating compression and expansion in a liquid medium in the form of cavitation bubbles, thus resulting in a series of physicochemical effects (Roselló-Soto et al., 2015; Zinoviadou et al., 2015). According to the power density, ultrasound waves can be grouped into low-intensity (LIU) and high-intensity types (HIU). The HIU power is generally in the range of 10-1000 W/cm<sup>2</sup>, which can induce obvious structural disruption and chemical reactions, so that HIU treatments may promote lipid hydrolysis and oxidation during the storage process (McClements, 1995; Xia et al., 2017d). So far, the ultrasonic application in WBR processing mainly focused on the use of LIU, the power of which is typically below 1 W/cm<sup>2</sup> and considered as a non-destructive. However, due to its limited effects on surface structure of RB, the cooking attributes of WBR grains may not be obviously affected by LIU, as observed by Cui et al. (2010), who reported the decrease of cooking time from 39.6 to 37 min for the samples ultrasonically treated at



25 °C. To enhance ultrasonic effects, Park and Han (2016) added a soaking process (2-8 h) prior to ultrasound treatments (400 kHz/25-50 °C/30-60 min), which greatly promoted water uptake ratio during processing. After ultrasound treatments (50 °C/60 min), the fully hydrated samples caused the reduction of cooking time and softening of WBR texture, which showed similar hardness to that of cooked milled rice. Nevertheless, a long-period soaking could result in the loss of micronutrients, particularly water-soluble vitamins such as thiamin, riboflavin and niacin.

Ultrasound application for biotechnological purposes has been extensively reported as an effective tool for regulating and controlling bioprocesses, including fermentation, enzymatic transformation, seed germination and plant growth (Hashemi et al., 2018; Mota et al., 2017; Ojha et al., 2017; Teixeira da Silva & Dobránszki, 2014). Ultrasonic effects on the course of these bioprocesses in mild conditions are closely associated with the improved mass transfer, activated enzyme activities as well as modified cell permeability and cellular metabolism. Particularly, ultrasonic vibration on seeds has shown the capability to improve seedling vigor and physiological features of grown plants (Chen et al., 2013; Wang et al., 2012). In this sense, ultrasound has recently been used for producing germinated foods with improved edibility and nutritional quality. As for WBR, application of ultrasounds accelerated the germination rates and sprouts length compared with the untreated GBR samples and simultaneously enhanced the starch hydrolysis due to the amylase activation (Ding et al., 2018a). Moreover, due to the abiotic stress, pretreatments of WBR with ultrasound waves resulted in GABA accumulation during the sprout growth (Ding et al., 2018a). Metabolomics analysis suggested that many metabolites in GABA-shunt pathway responsible for regulating GABA formation were largely modified as a response to ultrasound stress (Ding et al., 2018b).

#### 4.2.5 Irradiation

Food irradiation is mainly used for sanitary purposes relying on its ability to inactivate living cells of microbes and insects, as is the case for the disinfestation of vegetables, cereals, herbs, spices and fruits; and it also inhibits sprout growth of garlic and potato (Kume et al., 2009). Although the safety of irradiated foods has been confirmed by many international expert groups, misinformation and concern about their safety among consumers are still the limiting factors to implement this technique in the food industry (Farkas & Mohácsi-Farkas, 2011). Generally, gamma irradiation caused no obvious change in the contents of starch, amylose, protein and lipid after immediate treatments (Sung, 2005; Wu et al., 2004). However, the microstructure of biological macromolecules and physicochemical attributes, such as color parameters, texture, viscosity, and antioxidants, can be influenced by gamma irradiation, particularly after prolonged storage. Several literatures have reported the effects of irradiation treatments on phenolics content and antioxidant activity of WBR grains (Shao et al., 2013; Sultan, Wani, & Masoodi 2018; Zhu et al., 2010). It was found that gamma irradiation could affect covalent bonds between bound phenolics and sugars, thus leading to an overall increase in phenolic contents and antioxidant capability with increased irradiation doses. However, high dose of gamma irradiation may induce browning phenomenon and off-flavor, thus reducing consumer acceptability. The color changes induced by gamma irradiation could be attributed partially to the formation of dark spots in starch granules, although irradiation treatments also favored the inhibition of starch aging during storage (Chen et al., 2015b). On the other hand, the reduction in irradiation dose can compromise the efficiency of disinfection and stabilization. Therefore, process parameters for gamma irradiation treatments of WBR should be optimized to achieve extension of products shelf life matched with good sensory features.

Ultraviolet (UV) radiation is also used as a nonthermal treatment for improving products stability (Gharibzahedi et al., 2018). Jongyingcharoen and Cheevitsopon (2016) investigated the effect of UV treatments (254 nm wavelength/30-60 min) on the microbial inhibition of cooked GBR grains. Although UV delayed the growth of aerobic bacteria, yeast and mold during storage, the samples treated for 30 min and 60 min have showed the total colony counts exceeding the microbiological criteria at day 2 and 4 of storage, respectively. UV had no influence on color and hardness of cooked GBR samples but caused the degradation of GABA. These results suggested that single UV irradiation was hard to achieve the significant improvement of WBR storability and quality.

#### **4.3 Heating pretreatments and drying techniques**

For WBR production, traditional heating treatment mainly refers to parboiling process, which is designed to improve textural properties and storage stability (Fig. 2). For the same purpose, parboiling was also employed for GBR samples (Cheevitsopon & Noomhorm, 2015; Liu et al., 2017). Though trials have been made to optimize parboiling procedure and conditions for improving quality and functional characteristics of WBR products (Hu et al., 2017; Kwofie & Ngadi, 2017; Patindol, Newton, & Wang, 2008b), the general parboiling process includes three essential steps: soaking at 60-80 °C for a duration ranging from several hours to days; steaming (100-120 °C/0-30 min); and drying which may continue up to several days at 25-40 °C. Therefore, this method remains to be considered as a time-consuming, laborious and energy intensive. In recent years, for the purposes of extending shelf life and enlarging products availability, new types of heating and drying techniques have been applied to WBR samples, including infrared (Ding et al., 2015), high-temperature fluidization techniques (Jaisut et al., 2009), fluidized bed drying (Cheevitsopon & Noomhorm, 2011), microwave irradiation (Zhong et al., 2013), extrusion (Zhang et al., 2018), and puffing (Mir et al., 2016).

High temperature heating significantly inactivates deteriorative enzymes and stabilizes WBR grains during the storage, but also causes some negative effects such as degradation of bioactive components (e.g., GABA, free phenolics, flavonoids, vitamin) and browning (Chalermchaiwat et al., 2015; Mir et al., 2016; Zhang et al., 2018). For example, compared with cold extrusion, hot extrusion has wider applications in the food industry, but it is a thermomechanical process where food materials are subject to high temperature, shear forces and pressures for a short time, thus causing the loss of thermally sensitive bioactive components (Alam et al., 2016; Kumar et al., 2017). On the other hand, the impact of heating and drying techniques on WBR is based on their effects on definite commonly-used indices such as texture, lipid degradation, taste, cooking properties, and major functional components. For evaluating the overall processing effects of these newly-tailored techniques, not only single-response studies, but also multiple-response studies which rely on both targeted and non-targeted approaches should be integrated to give comprehensive information relating to the food quality modification (Grauwet et al., 2014).

## **5. Concluding remarks**

Overall, increasing strategies for improving sensory, nutritional and functional properties, as well as storage stability of WBR products have been developed and optimized in the last decade, due to the importance of WBR consumption to health and longevity. Among the techniques employed, nonthermal innovative processing approaches including HHP, cold plasma, PEF and ultrasound can shorten cooking time and soften texture because of the changes in water uptake characteristics and bran structure. Compared with traditional heating and drying techniques, one of the remarkable advantages for WBR processing via these nonthermal procedures is to maintain and improve the bioactivities of WBR samples. Possible mechanisms linked to the increase in bioactive compounds using nonthermal

processing could be attributed to the process-induced modification of the following factors: (i) enzyme activities, (ii) the intracellular microenvironment (pH, metal distribution, molecular interaction, etc.) and microstructure, (iii) metabolic pathways during bioprocess, and (iv) the extractability. The immediate increase in the content of bioactive components after treatments could be the result of their enhanced extractability. However, contribution of factors (i) and (iv) should be specially considered while examining the variation in targeted molecules during processing, preservation and storage. Furthermore, application of nonthermal techniques as shock pretreatments for GBR germination can also induce a series of biological effects which has the potential to modulate the quality and functionality of final products. Since only few investigations have been conducted on nonthermal approaches mediated germination, the parameters related to both germination process and pretreatment techniques should be matched and optimized for maximizing product quality and functionality, and novel combination patterns should be explored, such as the combination of electrochemical stimulus and WBR germination. To better understand the mechanisms behind the physicochemical properties and biological responses derived from innovative techniques, omics approaches including metabolomics and proteomics should be introduced.

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The authors have declared that there is no conflict of interest.

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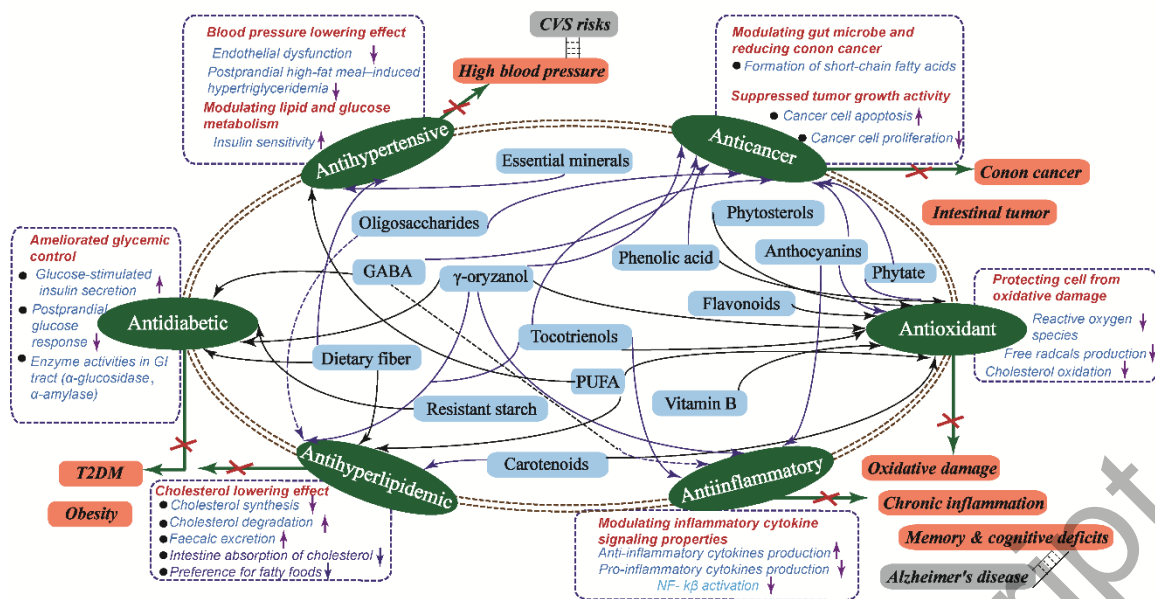


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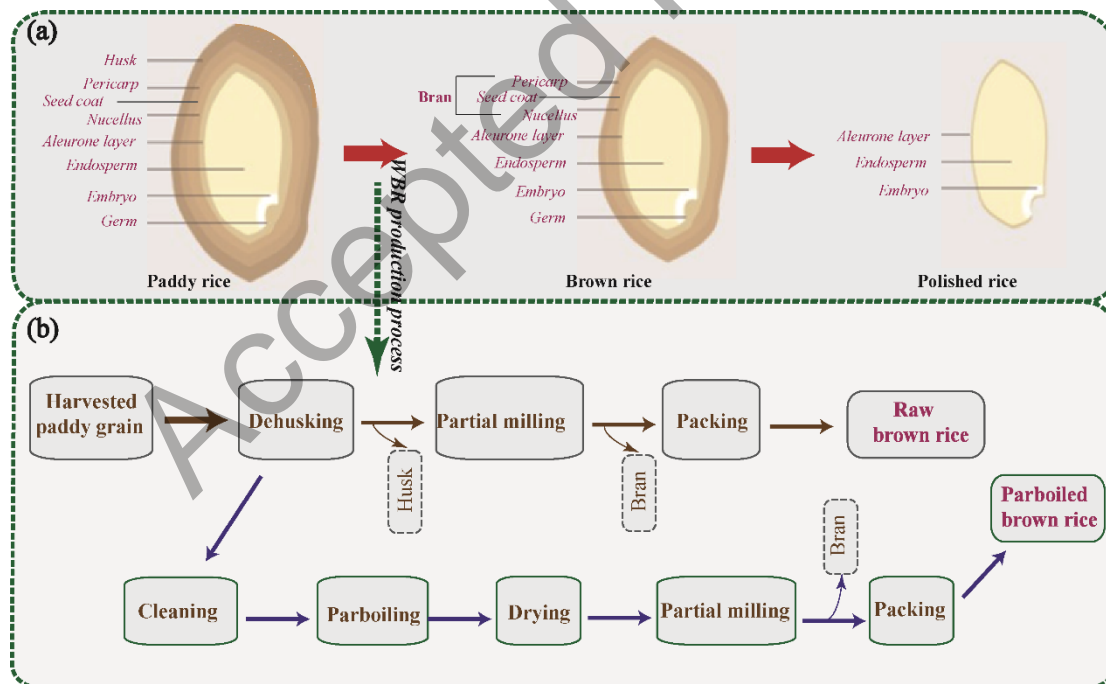
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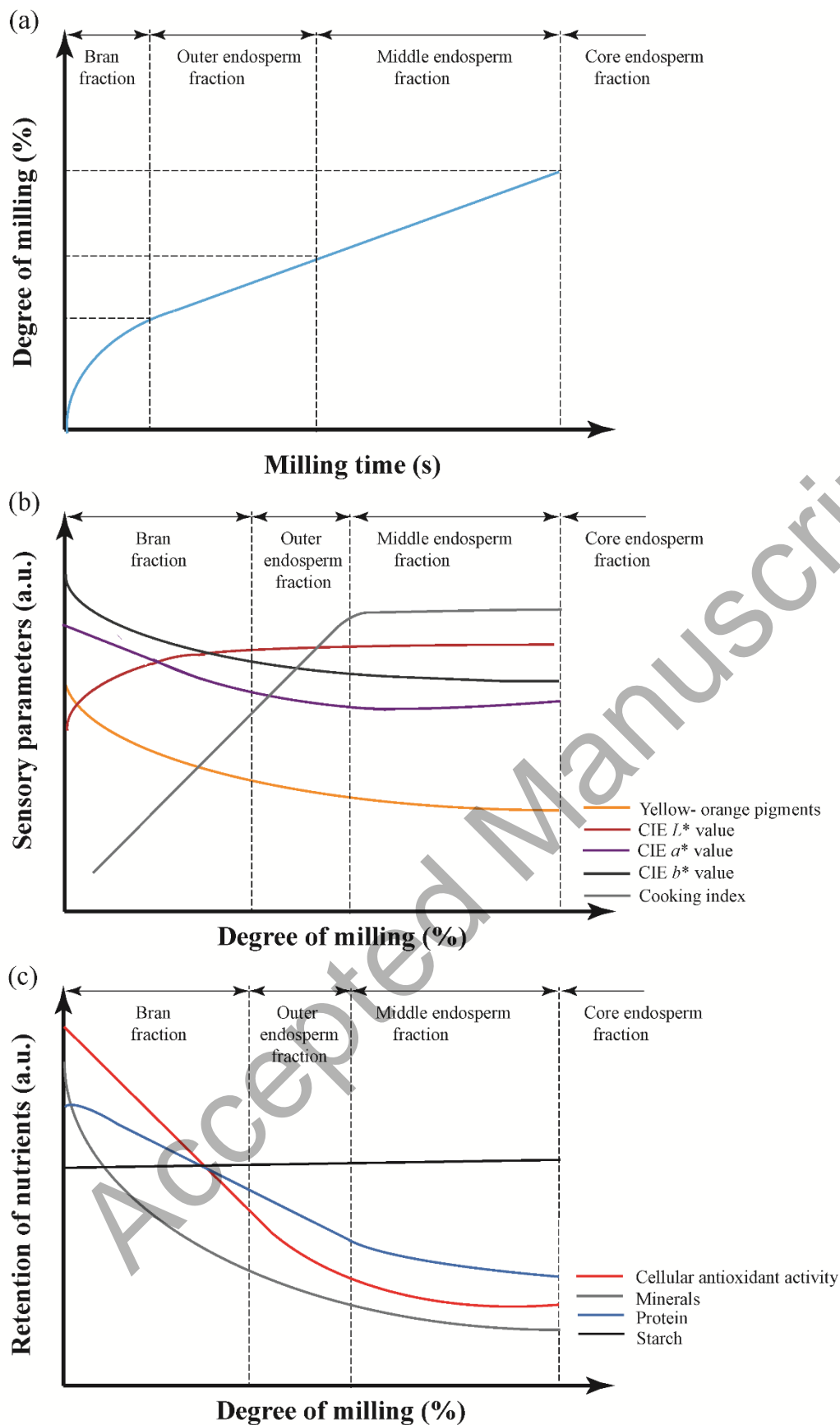
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**Figure 1.** Health benefits and related bioactive components of wholegrain brown rice (WBR). The synergistic action of multiple bioactive components in rice bran (RB) and germ largely contributes to the protective metabolic activities and show the capability to modulate metabolic pathway related to the development of chronic degenerative diseases. Abbreviations: GABA (gamma-aminobutyric acid); PUFA (polyunsaturated fatty acids); CVS (cardiovascular); T2DM (type 2 diabetes mellitus); NF- $\kappa$ B (nuclear factor kappa beta); GI (gastrointestinal).

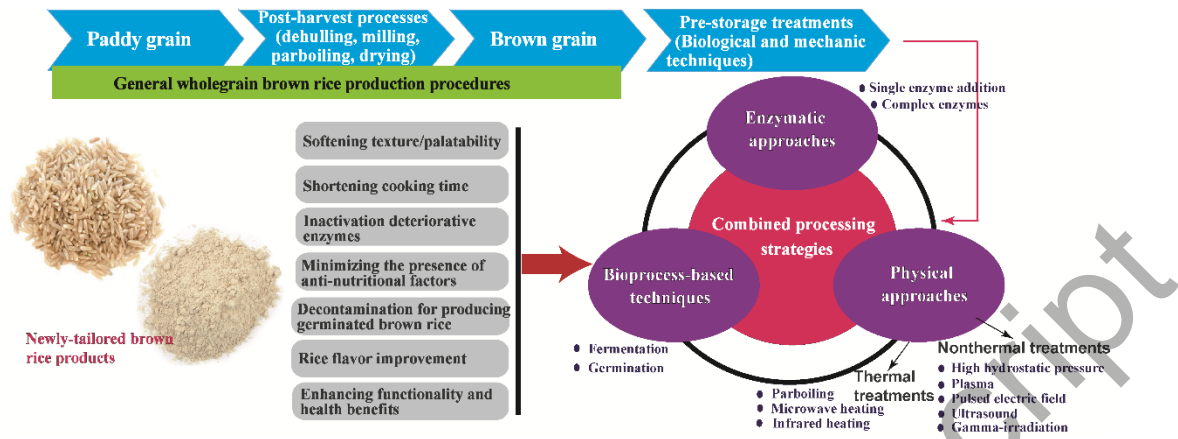


**Figure 2.** Schematic diagram of wholegrain brown rice (WBR) production: histological composition (a) and the production processes for raw and parboiled WBR grains (b)



**Figure 3.** Representative curves showing quantitative relationship between degree of milling (DOM) and physicochemical and nutritional attributes; cooking index (CI) is defined as  $CI =$

volume expansion ratio  $\times$  length expansion ratio  $\times$  water uptake ratio/cooking time, and an increased CI value is desirable



**Figure 4.** Current challenges and related strategies for wholegrain brown rice (WBR)

**Table 1.** A summary of various processing and pretreatment techniques used for wholegrain brown rice (WBR) and their main processing characteristics

Processing approaches		Processing conditions and patterns	Research purposes/innovations	Major findings	The stages examined	References
Bioprocess-based techniques	Germination	Soaking with gaseous treatment at 35 °C	Improving the GABA content by the gaseous treatment	- GABA contents of GBR increased by changing gaseous treatment.	Immediately after soaking	(Komatsuzaki et al., 2007)
		Water soaking with different pH (3-8), temperature (25-45 °C) and soaking time (12-24 h)	Optimizing soaking conditions for the improved nutritional composition	- Germination increased Vitamin B1 and GABA and reduced phytic acid. - Compositional variation depended on the soaking conditions (pH, time, and temperature). - GBR showed a softer texture than WBR grains	Immediately after soaking	(Watchararparpaiboon et al., 2010)
		Soaked at 28-30 °C for 12 h and then germinated within double layers of cotton cloth for 24 h	Comparing the compositional difference between rough rice and brown rice after germination	- Higher total FAA, $\alpha$ -tocopherol, $\gamma$ -oryzanol, thiamine, niacin and pyridoxine were found in germinated rough rice than	Immediately after germination	(Moongnarm & Saetung, 2010)
		Germination: 25 °C/1-5d; in the dark	Evaluating the effects of germination time and rice cultivars on the physicochemical properties of starch and rice flour	- Germination caused significant changes in pasting and rheology of WBR flour but no changes for the isolated starch as well as starch molecular weight. - Protein bodies disappeared under the action of proteolytic enzymes. - Starch granules became rougher and eroded.	Immediately after germination	(Wu et al., 2013a)
		Sterilized by 0.1% NaClO; soaking for 24 h before germination; germination conditions: in darkness, 28/34 °C, 90% humidity, and 48/96 h	Exploring health benefits of sprouts prepared from different Ecuadorian WBR cultivars	- Germination greatly increased GABA, antioxidants and total phenolic content, depending on genotypes, germination time and temperature.	Immediately after germination	(Cáceres et al., 2014)
		Sterilization: 0.1% NaClO for 30 min; germination: 25 °C and 5 d	Examining germination effects on the flavor of cooked WBR	- Germination decreased the characteristic odorants of WBR grains, depending on germination stages, rice cultivars, and compound types.	Immediately after germination	(Wu et al., 2011)
		Soaking in sterilized deionized water for 24 h at 30 °C, then germinated (48 h/30 °C) by spraying water to maintain moisture	As a blending material for noodle-making, so as to utilize nutritional components of WBR and reduce abrasive perception	- The ratio of GBR flour in wheat noodle affected the hardness, tensile strength, cooking loss and water absorption of final product. - Heat-moisture treatment of GBR greatly improved the texture and cooking quality of the composite noodles.	After preparation of GBR-based products	(Chung et al., 2012)



	Anaerobic treatment during germination	Applying anaerobic treatment to enhance bioactive components in GBR	- GABA, antioxidant capability, ferulic, <i>p</i> -coumaric, and sinapic acids significantly increased, whereas the FAA contents varied responding to anaerobic conditions.	Immediately after germination	(Shen et al., 2015)
	Soaked in an AEW: 37 °C, 84 h; the water was replaced every 12 h	Inhibiting the microbial growth during germination by AEW	- AEW showed higher inhibition rates of microbial growth than NaClO solution and alkaline electrolyzed water, with reductions of 5.38 log CFU/g. - GABA was improved by AEW treatment.	Immediately after germination	(Lu et al., 2010)
	Germinated using different electrolyzed water, showing varied pH, oxidation-reduction potential, and available chlorine levels	Investigating the effects of electrolyzed water combined with other treatments on GABA, germination potential and decontamination	- Sprout growth was promoted by AEW, but inhibited by AIEW; AEW achieved higher GABA than AIEW; presoaking and ultrasonic treatments could enhance electrolyzed water derived advantages	Immediately After germination	(Liu et al., 2013); (Li et al., 2015)
Fermentation	Fermented flour with yeasts and LAB	Modifying quality attributes and functionality of WBR by fermentation	- Increased contents of functional components and reduced phytic acid. - Sensory characteristics of WBR products were improved by fermentation.	Immediately After fermentation	(Ilowefah et al., 2015); (Li et al., 2007)
	WBR fermentation using yeast for vinegar production: agitated and static culture	As raw materials for producing vinegars	- Acetic acid fermentation using WBR favored the accumulation of FAA and GABA.	Immediately After fermentation	(Lee et al., 2010)
	WBR was inoculated with 2 % (v/w) of <i>Monascus sp.</i> ; incubation: 15 days/28-32 °C	Solid -state fermentation of <i>Monascus sp.</i> using WBR as substrate	- The higher yellow pigment concentration using WBR as substrate was obtained compared with other substrates	Immediately after fermentation	(Yongsmith et al., 2013)
	Koji fermentation using WBR as substrate by <i>Aspergillus oryzae</i>	Metabolomic profiles of Koji fermentation with WBR as substrate	- WBR Koji showed higher sugars, flavonoid aglycons and antioxidant activities than white rice	Immediately After fermentation	(Lee et al., 2017)
	Enzymatic treatments	WBR pretreatments before cooking using xylanase and cellulase enzymes	Degrading bran layers to improve texture and cooking performance	- Increased water uptake ratio, volume expansion ratio with reduced cooking time over untreated WBR grains	After enzymatic treatments
	Ultrasonic treatments (30 min/40 °C) followed by the addition of cellulase addition	Combining enzyme use and ultrasound	- Significantly lower hardness, cooking time and higher taste value as well as more obviously changed pasting properties in ultrasound-enzyme treated WBR.	After combined treatments	(Zhang et al., 2015a)
	WBR flour was mixed	Combining enzyme	- Enzymatic extrusion contributed to higher retention	After combined	(Zeng et al., 2017);

		with $\alpha$ -amylase and then extruded	use and extrusion	of free phenolic acid and total antioxidant activities than traditional extrusion.	treatments	(Zeng et al., 2018)
		WBR grains were pretreated by xylanase and cellulase (pH 5), then germinated: 30 °C/24 h, at 85% humidity	Application of enzymatic treatments in regulating WBR germination process	- Enzymes-pretreated WBR shortened 65% of soaking time, increased germination yields and GABA contents.	Immediately after treatment	(Jia et al., 2016)
Nonthermal processing methods	HHP	HHP treatments: 100-500 MPa/10 min	Enhancing moisture migration by HHP	- High pressure-mediated changes in WBR microstructure resulted in better moisture sorption characteristics.	Immediately after HHP processing	(Yu et al., 2016)
		HHP treatments: 200-600 MPa/0-20 min	Improving cooking properties and rice texture	- Shortening 20 min of the cooking time; - Reduced hardness by 47.8% by presoaking and HHP treatments; - Damaged structures of pericarp and aleurone layer	Immediately after HHP processing	(Yu et al., 2015)
		Soaking (30-50 °C/30 min), followed by one-cycle or two-cycles HHP treatments (100-500 MPa/10 min)	Evaluating effects of HHP processing patterns on WBR quality properties	- Two-cycle HHP achieved softer texture and lighter color than one-cycle model.	Immediately after HHP processing	(Yu et al., 2017b)
		WBR germination at 37 °C, over 2 or 6 days, followed by HHP treatments (10-100 MP/24-48 h)	Enhancing functional components in GBR grains	- The contents of total polyphenol, GABA, arabinoxylan, and $\gamma$ -oryzanol increased. - Phytate decreased significantly.	Immediately after treatment	(Kim et al., 2015a; Kim et al., 2015b)
		WBR grains were germinated at 37 °C for 48 h, followed by HHP processing (10-100 MPa/24 h)	Clarifying the impact of HHP conditions on the vitamin E and phenolic acids in GBR samples	- Total phenolic acid contents increased with pressure, while vitamin E contents varied depending on the pressures	Immediately after treatment	(Kim et al., 2017)
		WBR germination (37 °C/36 h), followed by HHP treatments (100-500 MPa/10 min)	Evaluating the changes in bioaccessibility of major nutrients after WBR germination	- Bioaccessible antioxidants, GABA and free amino acids increased, while minerals <i>in vitro</i> bioaccessibility relied on HHP conditions. - HHP increased starch resistance to enzymatic hydrolysis. - Obvious microstructural change in bran fraction at pressures above 300 MPa was observed by AFM.	Immediately after treatment	(Xia et al., 2017c); (Xia et al., 2017b)
		WBR germination (37 °C/36 h), followed by HHP treatments (100-500 MPa/10 min)	Revealing the effects of HHP treatments on the headspace volatile compounds in GBR	- HHP showed obvious effects on volatile fingerprinting of GBR. - Aldehydes, ketones, and alcohols were increased by high pressure.	Immediately after treatment	(Xia et al., 2017a)
		Direct HHP treatments (150-450 MPa/10 min)	Biochemical changes derived from HHP	- Direct HHP treatments could result in the enhanced lipid hydrolysis and oxidation and browning	Within storage process	(Xia et al., 2017d); (Xia & Li, 2018b)

		treatments within storage process	phenomenon during storage.		
	WBR grains were firstly stressed at 50-350 MPa/ 20 min, then germinated	Applying HHP-induced stress effects to modulate WBR functionality and quality properties	- Stress pressures under 100 MPa greatly increased GABA, <i>in vitro</i> starch digestibility and storability.	Immediately after treatment	(Xia et al., 2018);
Plasma	Low-pressure plasma treatments: 1–3 kV with constant current of 1.2 mA for 30 min	Changes in physicochemical properties of plasma treated WBR grains	- Reduced cooking time and improved texture and palatability of cooked WBR samples; - Modified structure and physicochemical attributes of starch by plasma treatments	Immediately after treatment	(Chen et al., 2012); (Chen, 2014)
	Low-pressure plasma exposure: 1-3 kV/10 min	Evaluating changes in physicochemical properties of plasma-treated WBR grains	- Cooking time and hardness decreased. - The activities of $\alpha$ -amylase and lipoxigenase were inhibited. - Higher lipid stability during storage in plasma-treated WBR samples was found.	Within storage process	(Chen et al., 2015a)
	Atmospheric-pressure cold plasma: voltage, 10 kV; flow rate, 10 L/min; treatment time, 5-25 min; radio frequency, 20-40 W	Inhibiting the growth of microbes in WBR using plasma	- Plasma powered at 40 W for 20 min significantly inhibited the growth of <i>A. flavus</i> .	During storage process	(Suhem et al., 2013a)
	Low-pressure plasma treatments (1-3 kV/10 min) followed by germination process	Using plasma shock to regulate WBR germination	- Plasma-pretreated WBR samples showed the increase in germination performance, GABA levels, antioxidant activity and $\alpha$ -amylase activity after germination.	After plasma-mediated germination	(Chen et al., 2016)
PEF	PEF treatment conditions: pulse width, 3-15 $\mu$ s; frequency, 50-1000 Hz; and voltage, 0-15 kV	To achieve the lipase inactivation and obtain optimum processing parameters	- The contribution of PEF processing parameters to inactivate lipase activity was voltage > frequency and pulse width > time.	Immediately after treatment	(Qian et al., 2014)
Ultrasound	Ultrasonic treatments: 16 kHz, 2000 W, 30 min; and treatment temperature: 25, 40, and 55 °C	To achieve quick cooking by ultrasonically treating WBR grains	- The reduction in cooking time was limited. - Physicochemical properties of rice flour including viscosities, crystallinity and thermal behavior were modified.	Immediately after treatments	(Cui et al., 2010)
	A soaking process (2-8 h), followed by ultrasonic treatments: 25 °C/30 min, or 50 °C/60 min; 400 kHz, and 185 W	To improve textural properties and examine physicochemical changes of isolated starch from WBR	- Soaking followed by ultrasound promoted water absorption and texture softening of WBR. - Pasting and thermal properties and crystalline structure of starch were changed.	Immediately after treatment	(Park & Han, 2016)

		ultrasound treatment (25 kHz, 16 W/L, 5 min); treatment temperature: 23-24 °C; then germinated for 36 h at 28 °C	To evaluate the effects of ultrasound combined with germination on starch	- Ultrasound pretreatment promoted the starch hydrolysis during germination, the sprouts growth speed, and improved cooking properties due to ultrasound induced structural changes in bran	Immediately after germination	(Ding et al., 2018a)
	Low-power microwave	WBR was germinated under millimeter wave conditions (60/70 GHz)	To improve WBR germination process	- Germination under millimeter irradiation showed higher germination rates, antioxidant activities, but reduced GABA contents	Immediately after germination	(Seo et al., 2016)
	Gamma irradiation	Irradiation source: <sup>60</sup> Co γ irradiator; dose: 0~10 kGy; and dose rate: 2 kGy/min	To evaluate gamma irradiation effects on WBR quality	- Gamma irradiation caused the slight browning, the reduction in hardness, amylose, crystallinity, viscosity and onset temperature ( $T_0$ ).	Immediately after germination	(Mir et al., 2015)
		Irradiation source: <sup>60</sup> Co γ irradiator; dose: 0~2 kGy; and dose rate: 5 kGy/min	To examine physicochemical changes of irradiated WBR during storage	- Gamma irradiation significantly reduced the lipid hydrolysis during storage, maintained the shape of starch granules and caused cracks among starch.	Within storage process	(Chen et al., 2015b);
	UV irradiation	Irradiated by a lamp with 254 nm wavelength; exposure time: 30/60 min	To assess changes of quality and storability in UV-treated samples	- UV postponed the microbial growth during storage and maintained color changes and hardness, but reduced GABA contents.	Within storage process	(Jongyingcharoen & Cheevitsopon, 2016)
Heating pre-treatments and drying techniques	Parboiling	Soaking: 65 °C for 2 h; steaming: 20 min, 100 °C, 0 kPa or 20 min, 120 °C, 98 kPa; drying: at 25 °C and 40% relative humidity until 12% moisture content	To examine the changes in functional properties of WBR samples treated with different parboiling conditions	- Parboiling decreased WBR whiteness, increased lipid contents and hardness, modified pasting and thermal properties of WBR flour, relying on rice cultivars.	Immediately after treatment	(Patindol, Newton, & Wang, 2008a)
		WBR was germinated (48 h/30 °C), followed by immediate parboiling: steaming for 2-15 min	To study effects of parboiling conditions on phytochemicals profiles and distribution for GBR	- Gallic acid, bound protocatechuic acid, bound phenolics, total flavonoid and proanthocyanidin decreased with increasing parboiling time.	Immediately after treatment	(Hu et al., 2017)
	Puffing	Preheating in water at 80 °C for 6 h; puffing at 220 °C for 45–60 s	Evaluating puffing effects on functional components in WBR grains	- Browning phenomenon, highly porous structure and changed diffraction pattern were observed during puffing process	Immediately after treatment	(Mir et al., 2016)
	Ohmic heating	WBR flour was suspended in water and heated by ohmic heating; alternating	Investigating thermal properties of ohmically heated WBR flour	- Ohmic heating caused the variation of thermal characteristics of WBR flour, different from traditional heating.	Immediately after treatment	(An & King, 2007)

		currents: 1-90 Hz; electric field strengths: 20-70 V/cm				
Microwave heating	Irradiation conditions: ~2450 MHz/15 kw	Applying microwave to increase stabilization of WBR samples	- Microwave significantly inactivated the activities of lipase and lipoxidase and thus reduced lipid hydrolysis during storage. - Microwave destroyed vitamins.	Within storage process	(Zhong et al., 2013)	
Infrared radiation	Infrared heating conditions: target temperature, 60 °C; radiation intensity, 4685 W/m <sup>2</sup> ;	Applying infrared heating to enhance storage stability of WBR grains	- Infrared tempering treatments caused more effective inactivation of lipase and lower FFA formation compared with hot-air drying.	Within storage process	(Ding et al., 2015)	
Extrusion	GBR was milled to flour; extrusion equipment, a co-rotating twin-screw extruder; feed rate, 3.6 kg/h; barrel temperature profiles: 35, 45, 55, 95, 125, 140 and 130 °C for section 1-7 and 120 °C for the die plate	Examining the influence of extrusion processing on functional components of GBR	- Extrusion process reduced GABA levels, ferric reducing antioxidant power, DPPH radical-scavenging activities, and total phenolic contents.	Immediately after treatment	(Chalermchaiwat et al., 2015)	
High-temperature fluidization	Drying conditions: at 130 and 150 °C and tempered for 30 ~ 120 min	Applying high-temperature treatment to improve WBR texture and flavor	- High-temperature fluidization greatly inhibited FFA formation, lowered glycemic index, and accelerated WBR aging related to cooking and eating properties	Within storage process	(Jaisut et al., 2009)	

**Notes:** 1. Rough rice means brown rice with the extra husk; 2. WBR, wholegrain brown rice; GBR, germinated brown rice; GABA, gamma-aminobutyric acid; FAA, free amino acids; NaClO, sodium hypochlorite; AEW, acidic electrolyzed water; AIEW alkane electrolyzed water; LAB, lactic acid bacterial; HHP, high hydrostatic pressure; PEF, pulsed electric field; UV, ultraviolet; FFA, free fatty acid; AFM, atomic force microscopy; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

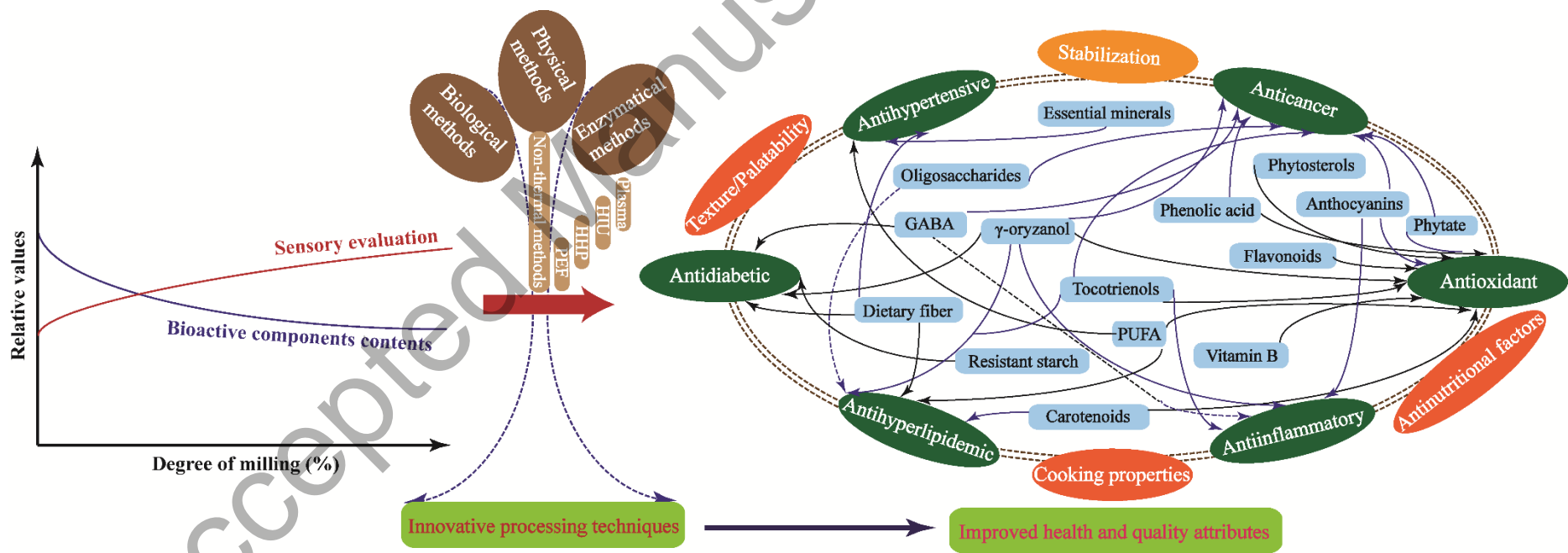
**Table 2.** Major changes in chemical composition of wholegrain brown rice after germination treatments

Chemical substance		Contents changes	Possible response mechanisms and/or health effects	Reference
Major chemical components	Starch	Decreased	- Amylose and amylopectin decreased, accompanying with an increase of reducing sugars - Starch was hydrolyzed into oligosaccharides by enzymes including $\alpha$ -amylase, $\beta$ -amylase, debranching enzyme and $\alpha$ -glucosidase. - Starch granules became eroded, showing higher <i>in vitro</i> digestibility.	(Xu et al., 2012); (Li et al., 2017);
	Non-starch polysaccharides	Decreased	- Polysaccharides Degradation into simpler compounds (e.g., glucose, fructose and oligosaccharides) under the action of hydrolytic enzymes.	(Sibian et al., 2017)
	DF	Increased	- The increased DF could be related to the transformation of soluble and insoluble DF fractions (e.g., inulin, and $\beta$ -glucans, cellulose, hemicelluloses, and RS) as well as the formation of new cell walls during germination. - GBR with high DF contributes to reducing the postprandial glucose response and regulating blood lipids.	(Hübner & Arendt, 2013); (Sibian et al., 2017)
	Protein	Total protein increased	- Although protein hydrolysis occurs during germination, protein content can be increased due to the net synthesis of protein and enzymes in sprouts	(Moongngarm & Saetung, 2010); (Sibian et al., 2017)
	Lipid	Increased	- The formation of lipid is derived from the biosynthesis of new compounds within the germination process.	(Kim et al., 2012); (Sibian et al., 2017)
Minor bioactive components	GABA	Increased after germination; total content fluctuate during germination	- GABA is formed by the decarboxylation of L-glutamic acid under the catalysis of GAD, and this process is influenced by GAD activity, glutamate and physiological states of plant cell	(Zhang et al., 2014)
	Phenolics	Increased	- Germination increased free and bound phenolics, as well as the contribution of free forms to total phenolics. - The activation of phenylpropanoid pathway and the release of cell wall-bound phenolics by hydrolysis account for the increased antioxidant activities.	(Cáceres et al., 2014); (Ti et al., 2014); (Tian, Nakamura, & Kayahara, 2004)
	Folic acid	Increased	- Folate deficiencies are related to megaloblastic anemia, birth defects, the risk of cardiovascular disease and cancer.	(Sibian et al., 2017)
	Vitamin E	Increased	- Physiological metabolic materials (e.g., tocopherol and tocotrienol) for the WBR seedling growth are synthesized.	(Kim et al., 2015b)
	Vitamin B	Total contents were increased	- The content changes are related to vitamin B biosynthesis due to an increase of enzyme activities during the germination process.	(Moongngarm & Saetung, 2010); (Kim et al., 2015b)
	$\gamma$ -Oryzanol	Increased	- $\gamma$ -Oryzanol is formed via ester bonds between ferulic acid and triterpenes; the increase of free ferulic acid could promote the formation of $\gamma$ -oryzanol, which is mainly locates in GBR sprouts	(Miller & Engel, 2006); (Kim et al., 2012)
	Phytic acid	Decreased	- The degradation of phytate is catalyzed by phytase, the activity of which increased during germination	(Moongngarm & Saetung, 2010); (Kim et al., 2012)
	Anthocyanin and proanthocyanidin	Decreased	- Attributing to the leaching out of water soluble fractions during soaking; - The bound forms with cell wall and proteins can be hydrolyzed, showing higher diffusion rates	(Sutharut & Sudarat, 2012)

	Total flavonoid	Increased	- Transformation of free and bound forms resulted in the fluctuation of flavonoid contents; the percentage contribution of bound flavonoids to total flavonoid maintained constant after the germination.	(Ti et al., 2014)
	Total antioxidants	Increased	- The increase in total antioxidants is related to the formation of new compounds presenting antioxidative effects and the hydrolysis of bound antioxidants into free forms	(Esa et al., 2013); (Ti et al., 2014); (Xia et al., 2017c)

**Notes:** 1. DF, Dietary fibers; RS, resistant starch; GBR, germinated brown rice; GAD, glutamate decarboxylase; WBR, wholegrain brown rice; GABA, gamma-aminobutyric acid.

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Graphical abstract (300 × 300 DPI)