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# Wheat (*Triticum aestivum L.*) Bran in Bread Making: A Critical Review

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**Abstract:** Wheat bran, a by-product of the industrial roller milling of wheat, is increasingly added to food products because of its nutritional profile and physiological effects. Epidemiological data and scientific studies have demonstrated the health benefits of consuming bran-rich or whole-grain food products. However, incorporation of wheat bran in cereal-based products negatively affects their production process. Furthermore, the organoleptic quality of the obtained products is mostly perceived as inferior to that of products based on refined wheat flour. This review summarizes the current knowledge on the impact of wheat bran on bread making, provides a comprehensive overview of the bran properties possibly involved, and discusses different strategies that have been evaluated up till now to counteract the detrimental effects of wheat bran on bread making.

**Keywords:** bran, bread, dough, wheat

## Introduction

Wheat (*Triticum aestivum* L.) is an extensively cultivated food crop and forms an important staple food in many countries. The wheat kernel or caryopsis can be roughly divided into 3 parts: the endosperm, the germ, and multiple histological outer layers (that is, outer and inner pericarp, seed coat, and nucellar epidermis), commonly denominated as botanical bran (Delcour and Hoseney 2010). During conventional wheat roller milling, a large part of the endosperm is separated from the germ and the bran through consecutive grinding, sieving, and purifying steps. The endosperm is further ground to wheat flour following different degrees of refinement and is traditionally used for bread making. The bran, together with the aleurone layer and remnants of starchy endosperm and germ, end up in a range of milling by-products, which are recovered at different stages in the mill. In contrast to refined flour, these bran-rich products are typically used for animal feed. However, wheat bran has a rich nutritional profile and shows beneficial physiological effects, making consumption of bran-rich food products more interesting from a health perspective than products based on refined flour. Because consumers become more aware of its benefits, wheat bran is increasingly added to mostly cereal-based food products (bread, cookies, breakfast cereals, pasta, snacks, cakes, and more).

Depending on the mill, botanical wheat bran ends up in by-products such as coarse bran (or regular bran), coarse weatings (or fine bran), fine weatings (or middlings or shorts), and low-grade flour (or red dog). These bran-rich streams are roughly distinguishable based on 2 characteristic features: particle size and endosperm content. Coarse bran, as suggested by its name, is made up of coarse bran particles and has a low endosperm content due to a relatively efficient removal of endosperm from the outer kernel layers early in the milling process. Bran-containing side streams recovered further down the milling process typically consist of finer bran particles and contain relatively more endosperm. In this respect, low-grade flour typically consists of the finest bran particles with dimensions that are close to flour particles (Delcour and Hoseney 2010). Dependent on the mill, these side streams may be offered separately or as specific mixtures. Pollard, for instance, is a mixture of all of the bran-rich streams together with germ.

On top of the variety in wheat bran products, bran itself is a complex biological material which is characterized by a specific histological structure and a diverse chemical composition, as well as physical properties, of the constituting tissues (Shetlar and others 1947) (Figure 1). Broadly, on a total bran basis, regular wheat bran is comprised of about 6% to 23% pericarp (epidermis, hypodermis, cross, and tube cells), 6% to 30% seed coat and nucellar epidermis, 33% to 52% aleurone layer, and 9% to 35% starchy endosperm (Zhang and Moore 1997; Antoine and others 2003, 2004; Hemery and others 2009b; Barron 2011). With regard to its overall chemical composition (Table 1), regular wheat bran mainly consists of nonstarch carbohydrates with 17% to 33% arabinoxylan (Bergmans and others 1996; Bataillon and others 1998; Maes and Delcour 2001), 9% to 14% cellulose (Shetlar and others 1947; Bataillon and others 1998), 3% to 4% fructan (Haskå and others 2008; Verspreet and others 2015), and 1% to 3% mixed-linkage $\beta$-D-glucan (Maes and Delcour 2001, 2002; Nordlund and others 2012) as major components. Besides nonstarch carbohydrates, commercial wheat bran also contains high levels of starch (6% to 30%), due to attachment of residual endosperm to the bran or

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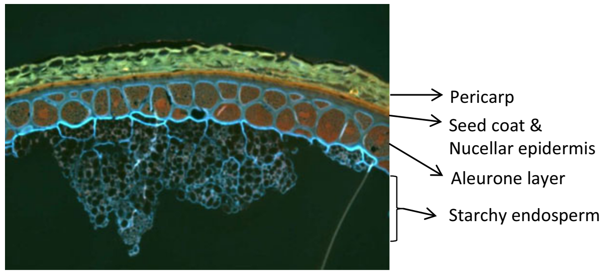

Figure 1–Cross-section of wheat bran (as produced from conventional milling) stained with Acid Fuchsin and Calcofluor. Acid Fuchsin stains proteins red and Calcofluor stains $\beta$-glucan blue in epifluorescent light (excitation 400 to 410 nm, emission >455 nm). The pericarp is not stained, but can be detected due to autofluorescence. The different tissues are appointed on the figure.

Table 1–Chemical composition (shown as ranges of percentages) and arabinose-xylose (A/X)-ratio of regular bran, pericarp, and aleurone (Antoine and others 2003; Parker and others 2005; Haskå and others 2008; Hemery and others 2009a; Barron 2011; Brouns and others 2012; Nordlund and others 2012).[a]

| | Regular bran | Pericarp | Aleurone |
|---|---|---|---|
| Arabinoxylan | [17–33] | [42–46] | [20–46] |
| A/X-ratio | [0.46–0.51] | [1.06–1.15] | [0.36–0.39] |
| Cellulose | [9–14] | [22–40] | [1–3] |
| Fructan | [3–4] | n.a. | [5][b] |
| $\beta$-D-glucan | [1–3] | [3–9] | [5–16] |
| Starch | [6–30] | [0–6] | [0–11] |
| Proteins | [14–26] | [6–10] | [21–30] |
| Lipids | [3–4] | [0–1] | [4–9] |
| Ash | [5–7] | [2–7] | [7–12] |

[a] Terminology of "regular bran," "pericarp," and "aleurone" as defined by the authors.
[b] Only one measurement.

inadequate removal of loose endosperm fragments, protein (14% to 26%), lipids (3% to 4%), lignin (3% to 10%), minerals (5% to 7%), phytic acid (4.5% to 5.5%), phenolic acids (0.4% to 0.8%), and other minor constituents (Shetlar and others 1947; Lolas and others 1976; Bergmans and others 1996; Zhang and Moore 1997; Bataillon and others 1998; Maes and Delcour 2001, 2002; Hemery and others 2009b). These constituents are not homogeneously distributed over the bran structure since the different histological layers have their own specific composition (Table 1).

The daily consumption of bran-enriched products, as mentioned earlier, implies some nutritional as well as physiological benefits. The aleurone layer, for instance, is known to be particularly rich in nutrients. An extended listing of these nutrients and their possible health-related effects has been described by Brouns and others (2012), and includes, *inter alia*, essential amino acids, such as lysine and tryptophan, vitamins, such as thiamin and niacin, antioxidants, such as ferulic acid and alkylresorcinols, and minerals, such as phosphorus and iron. The bioavailability of the latter nutrients is often questioned, as wheat bran and, more specific, the aleurone layer contains considerable levels of phytic acid, which strongly chelates minerals and, hence, reduces their bioavailability. These chelating properties of phytic acid can, however, be counteracted by colonic fermentation which reduces the intestinal pH and disintegrates the formed chelates (Schlemmer and others 2009). Next, besides its nutritional profile, attention should be paid regarding the high levels of dietary fiber present in the bran, to which some generally accepted physiological effects have been attributed. Indeed, it has been recognized by the European Food Safety Authority (EFSA) that the consumption of wheat bran and wheat bran fiber induces an increase in fecal bulk as well as a reduction in intestinal transit time which are considered to be beneficial physiological effects [EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) 2010]. Moreover, the Academy of Nutrition and Dietetics (formerly known as the American Dietetic Association) stated that the intake of 14 g dietary fiber, per 1000 kcal consumed, can decrease the risk of obesity, cardiovascular diseases, and type 2 diabetes, and that associations exist between the consumption of dietary fiber and a decreased risk of colon diverticulosis as well as constipation (Slavin 2008). Furthermore, epidemiological studies clearly demonstrated the health benefits of consuming whole-grain foods such as reduced risk of gastro-intestinal cancers (Chan and others 2007; Hamer and others 2008; Schatzkin and others 2008; Anson and others 2011), type 2 diabetes (de Munter and others 2007), cardiovascular disease (Jacobs and Gallaher 2004), and obesity (Anderson and others 1994; Fardet 2010; Sivam and others 2010; Kumar and others 2011).

Fortification of cereal-based products with wheat bran is basically performed in 2 ways. Flour is either supplemented with bran or bran is mixed together with its germ and flour complements in their naturally occurring proportions in the kernel, resulting in "whole-grain" flour. "Whole-grain" flour, as defined by AACC International and the consortium of the EU HEALTHGRAIN project, is the meal that consists of the intact, ground, cracked, or flaked cereals, whose principal anatomical components—the starchy endosperm, germ, and bran—are present in the same relative proportions as they exist in the intact kernel (AACC International 1999; van der Kamp and others 2014). Either way, the presence of wheat bran in cereal-based food products leads to technological disadvantages and inferior end-product quality compared to refined flour-based processes and products, such as a decrease in bread loaf volume, textural changes, and visual modifications (Figure 2). Since bread is an important staple food in many countries, considerable efforts have been invested in optimizing the quality of bran-rich bread, which has proven to be a challenging task. The challenges of bran-related research begin with the existing diversity of mill-derived bran products and the different histological layers bran is composed of. Indeed, the variety in these bran-containing products should be taken into account when studying the effects of wheat bran in bread making as each of these fractions may have its specific properties and impact on bread making. Fine weatings and low-grade flour, for instance, are shown to be intrinsically more deleterious to bread volume than coarse bran and coarse weatings (Hemdane and others 2015), whereas the pericarp is reported to have a more negative effect on the bread making potential than the more inner layers (Gan and others 1992).

To improve the technological functionality of bran and the sensory aspects which are typically associated with wheat bran incorporation in bread making, different approaches have been explored over the past few years. A first approach to counteract the deleterious effect of bran in bread making involves the use of flour with high protein content, addition of water, processing adjustments, and addition of bread improvers, such as surfactants, enzymes, and commercial gluten. These improvers strengthen the gluten-starch matrix and improve fermentation stability, which ultimately results in increased gas retention and dough expansion. As a result, bread volume as well as color characteristics are significantly improved (Shogren and others 1981; Moder and others 1984; Gan and others 1989; Lai and others 1989b; Sidhu and others 1999; Sanz Penella and others 2008). Alternatively, cultivar selection,

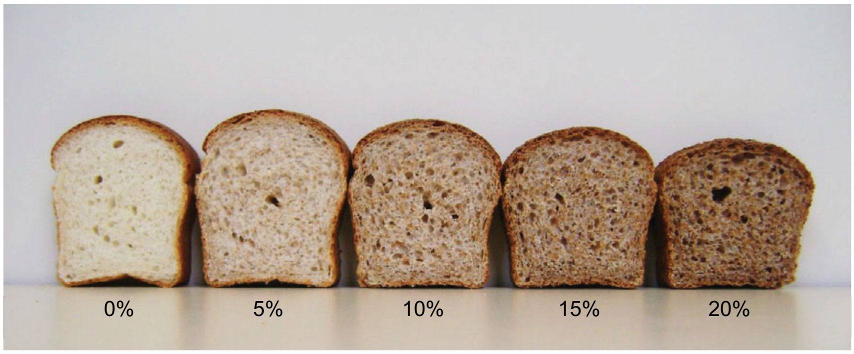

**Figure 2–Effect of different levels of bran addition on loaf volume: bread loaf without bran addition and bread loaves where 5%, 10%, 15%, and 20% flour was replaced by bran. The level of flour replacement is shown under the bread loaves. No bread improvers were added to the breads.**

breeding programs, fractionation techniques, such as pearling and electroseparation, and various bran treatments, constitute some of the approaches to improve the technological quality of the bran itself.

Although the above approaches have proven successful in some cases, mitigation of the organoleptic-related effects of bran derived from any given wheat variety remains difficult. In essence, there is a lack of insight into the mechanism(s) responsible for bran's deleterious effect on cereal-based processes and products. This hampers the development of strategies directed at their improvement. Therefore, this review aims at providing a comprehensive overview on the state of the art of wheat bran functionality in bread making. This may provide a useful background, especially for further research aimed at uncovering the underlying mechanism of bran's deleterious effect. The deleterious impact of wheat bran in bread making is discussed first, followed by an overview of the different bran properties that might be involved in its functionality. Next, strategies that have been used to modify wheat bran will be discussed. Unlike studies which evaluate the use of bread improvers to improve bran-enriched food products, the approach of performing and evaluating bran modifications provides a valuable means to gain insight into bran functionality by linking the changes in bran properties to the impact of the modified bran in bread making.

## Wheat Bran in Bread Making

Numerous studies have demonstrated the deleterious impact of wheat bran on bread making and bread quality in terms of the functional as well as the sensory properties of bran (Pomeranz and others 1977; Shogren and others 1981; Galliard 1986b; Lai and others 1989a,b; Özboy and Köksel 1997; Zhang and Moore 1997, 1999; de Kock and others 1999; Campbell and others 2008; Seyer and Gelinas 2009). Overall, addition of bran to flour results in unwanted effects on dough properties, bread loaf volume, color, texture, and taste. These adverse effects increase with higher levels of wheat flour substitution by bran (Zhang and Moore 1999) (Figure 2). Schmiele and others (2012) observed a decrease in specific volume from 4.4 $cm^3/g$ down to 1.8 $cm^3/g$ when replacing wheat flour with wheat bran up to 40%. Campbell and others (2008) observed a similar trend when adding wheat bran up to 15%. Concomitant with the decrease in loaf volume, Schmiele and others (2012) reported a significant increase in crumb firmness and hardness when wheat bran or whole-grain flour was added in higher concentrations, which could be linked to the lower specific volume of the loaves. However, the effect of bran addition on crumb texture cannot be only due to the loaf volume decrease. In flat breads, for instance, where loaf volume is of minor importance, crumb texture quality also decreased by incorporation of wheat bran, and a darker crumb color can be noticed (Majzoobi and others 2013).

The detrimental effect of bran addition on bread making has been attributed to a number of factors, starting with the dilution of gluten proteins (Pomeranz and others 1977; Moder and others 1984). With bran additions below 7%, Pomeranz and others (1977) reported that the extent of decrease in volume matched the decrease expected from the dilution of gluten proteins by bran. However, above that level, a volume decrease higher than the decrease expected based on dilution of gluten was noticed. This and similar observations suggest that the reduction in loaf volume cannot only be due to gluten dilution (Pomeranz and others 1977; Galliard 1986b; Lai and others 1989b). Various hypotheses concerning the cause of this additional negative effect have been suggested in the past. In general, these hypotheses can be summarized in categories which attribute the additional effect to either physical, chemical, or biochemical properties of bran. Ultimately, the joint occurrence of these properties probably determines the overall functionality of wheat bran in bread making as illustrated in the gear diagram (Figure 3).

However, it is important to keep in mind that, since wheat bran functionality in bread making is assessed based on dough and/or bread characteristics, the wheat flour used to make bran-rich meal will also affect these characteristics and, hence, ultimately also the assessment of wheat bran's functionality (Figure 3). Indeed, it is evident that certain characteristics, such as the gluten quality of the flour that is used in bran-rich meal, determine the bread making quality of the composite meal. Therefore, it can also be expected that flour quality affects the extent to which the factors that determine bran functionality have an impact on bread-making. In this perspective, the residual endosperm attached to bran might also affect the overall bread making performance. Indeed, it has been reported that the outer endosperm, the subaleurone, can contain considerably high levels of gluten proteins compared to the inner endosperm (up to 50% proteins; Kent 1966). These gluten proteins have, moreover, a qualitatively different composition than the gluten present in the inner endosperm, containing proportionally more low-molecular-weight glutenin subunits and $\omega$- and $\alpha$-gliadins than high-molecular-weight glutenin subunits and $\gamma$-gliadins (Tosi and others 2011). Seyer and Gélinas (2009), however, could not predict the baking potential of wheat cultivars in whole-grain bread based on the data obtained with their white

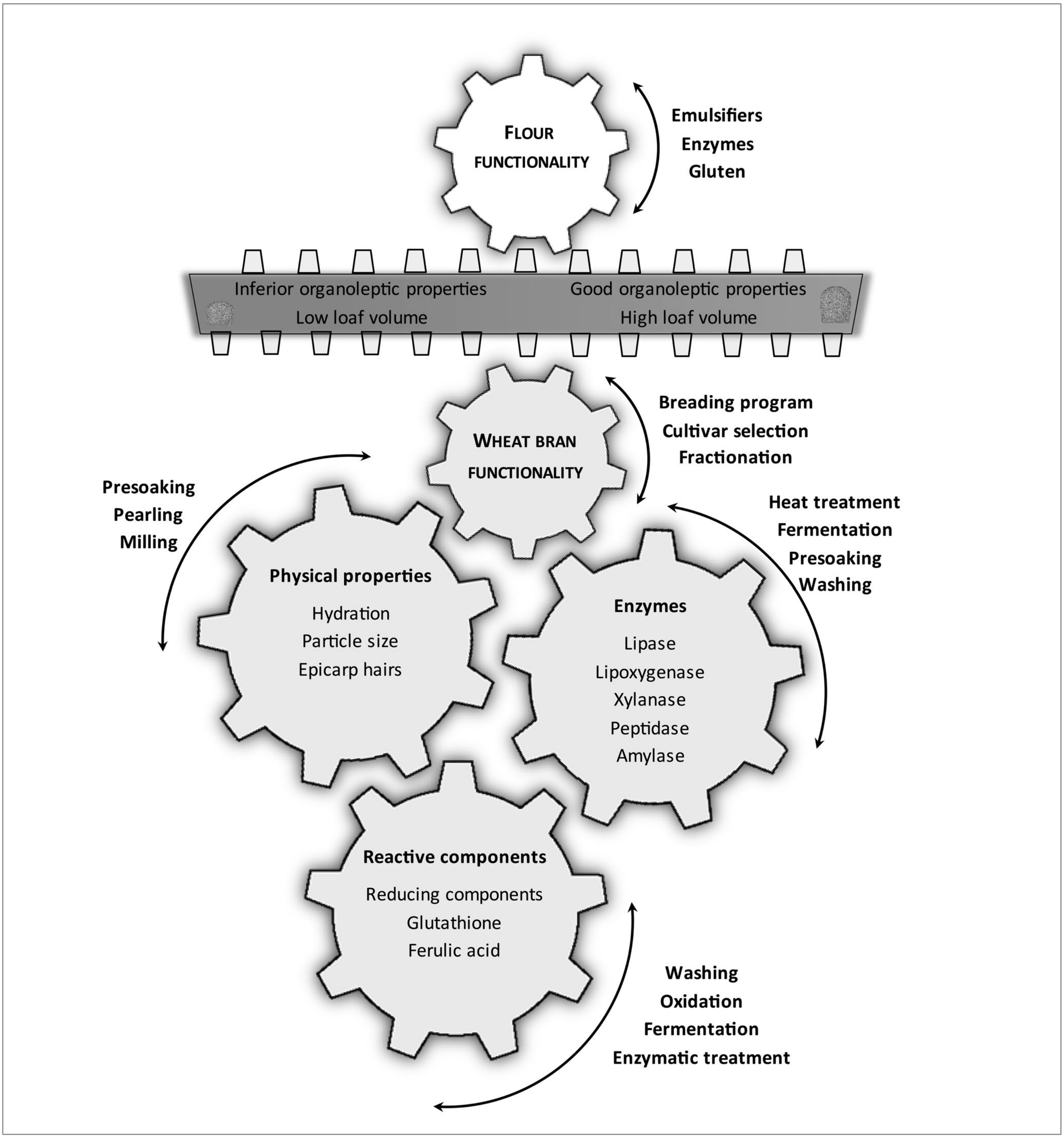

**Figure 3–Gear diagram representing the different categories of wheat bran properties which are believed to govern wheat bran functionality in bread making besides gluten dilution together with different approaches which aim at modifying and evaluating the relevance of certain bran characteristics. Note that bran samples originating from different wheat cultivars are most likely represented by a unique gear diagram in which the different gears have their specific importance toward the final wheat bran functionality.**

wheat flour. In this case, the effect of the differences in wheat bran functionality on bread quality parameters probably dominated the effects of the variation in the wheat flour.

Furthermore, it should be noted that the relevance of properties which govern bran functionality and their combined effect on bread making is dependent on the wheat cultivar the bran originates from. This is evidenced by the fact that many authors report different effects of bran on bread making properties upon the use of different cultivars (Moder and others 1984; Finney and others 1985; Özboy and Köksel 1997; Nelles and others 1998; de Kock and others 1999; Zhang and Moore 1999; Greffeuille and others 2006; Seyer and Gelinas 2009). These dissimilar effects on bread making properties of bran samples derived from different cultivars have been correlated with differences in parameters, such as hydration behavior (Nelles and others 1998), mechanical properties (Greffeuille and others 2006), and particle size (Seyer and Gelinas 2009). Thus, in relation to Figure 3, a bran sample originating from a specific cultivar could be represented by a unique gear diagram in which the different functional parameters, represented by the different gears, have their specific importance toward the final bread making potential.

In the following part of this review, the physical, chemical, and biochemical properties of bran and their potential relevance toward bread making will be discussed in detail, followed by an overview of bran treatments that have been used to validate or dismiss hypotheses about their relevance.

## Physical properties of bran

**Interaction with water.** A first remarkable wheat bran characteristic is its ability to absorb considerable amounts of water. In a recent publication, Jacobs and others (2015) reported that water-binding mechanisms on macro-, micro-, and nanoscale, and on a molecular level allow bran to retain water either weakly or strongly. Water retention by bran on a macroscale is ascribed to filling of void spaces in between bran particles, which arise from random stacking of bran particles. On a microscale, the pericarp cells, which are empty (Stone 2006), and spacing in between tissue layers provide sites for water retention. With regard to water-binding on a nanoscale, capillary mechanisms are involved. Nanopores in bran can be found, for instance, in cell wall matrices as they are known to consist of nanoporous structures (Chesson and others 1997). In addition, cellulose present within this matrix also contains nanopores as a result of its specific structure (Topgaard and Söderman 2001). Finally, bran is rich in polysaccharides which can bind water on a molecular level through formation of hydrogen bridges (Chaplin 2003). These mechanisms contribute to water uptake by bran in the case of unconstrained hydration. Alternatively, when bran is exposed to an external stress, only the water strongly bound in nanopores or through hydrogen bonds will govern water retention. This mechanism explains the various hydration phenomena which can be observed as a function of wheat bran particle size (Figure 4). In the case of exposure to an external stress, wheat bran particle size does not affect water retention, due to the fact that the water-binding capacity ascribed to nanopores or through hydrogen bonds is not significantly affected within an average particle size range of 80 to 1600 $\mu$m. During unconstrained hydration, bran with a large average particle size will be able to retain more water compared to fine bran, due to its larger potential to bind water in its intact micropores and as a result of more pronounced stacking phenomena (Jacobs and others, 2015).

With regard to bread making, however, hydration of bran occurs during mixing where bran is exposed to kneading forces and hygroscopic-like forces exerted by various flour constituents. Therefore, stacking phenomena and micropores do not contribute to hydration, since water bound through these mechanisms is relatively weakly bound and released in the presence of these external forces. This view is consistent with the fact that no changes in Farinograph absorption are observed as a function of average particle size (Auffret and others 1994; Zhang and Moore 1999; Jacobs and others, unpublished data).

The most obvious effect of bran's particular hydration properties in bread making is the significant increase in meal water absorption (Zhang and Moore 1997). This phenomenon is observed in Farinograph (Zhang and Moore 1997; Sanz Penella and others 2008) as well as in Mixograph (Pomeranz and others 1977) analyses. The strong tendency of bran to absorb water might result in competition for water between bran and other key flour components like starch and gluten (Roozendaal and others 2012). Various researchers indeed correlate the effects of wheat bran on bread making to this hydration behavior. Some authors ascribe the typical longer dough development time in bran-rich dough to slower water uptake kinetics of bran compared to flour constituents (Sanz Penella and others 2008; Schmiele and others 2012). Lai and others (1989b) ascribe the detrimental effects of bran on bread making to bran-water interactions based on the observation that the bread loaf volumes increased when adding 2% extra water compared to the level of water indicated by Mixograph absorption. It should be noted that different aspects of the hydration behavior of bran may be of relevance throughout bread making given the dynamic conditions of the process. For instance, during kneading, bran can be envisaged to absorb only strongly bound water due to the presence of an external stress. When this external stress disappears at the end of mixing, bran might tend to bind water, which could not be bound during mixing. In comparison, Dreese and Hoseney (1982) and Rogers and Hoseney (1982) also partially ascribe bran's detrimental effect to its dynamic hydration behavior by suggesting that the excess water absorbed in bran-rich dough is available for starch gelatinization during baking which would lower the starch gelatinization temperature and ultimately decrease the final loaf volume. This is a plausible hypothesis since Roozendaal and others (2012) pointed out that bran releases absorbed water during heating. Furthermore, Li and others (2012) share the opinion that arabinoxylan networks can be formed in whole-grain flour, causing water migration from the gluten network to the arabinoxylan network and, hence, result in inferior baking quality. However, no clear scientific evidence is available to confirm or dismiss these hypotheses. Therefore, it is difficult to estimate the exact relevance of bran's hydration behavior toward bread making functionality.

**Physical hindrance and disruption effect.** Besides the fact that dough development and quality is affected by bran due to dilution of gluten proteins, some additional negative effects seem to play a role. Bran might impede proper gluten development by physically preventing proper contact between flour particles. This hypothesis, together with the relatively slow water uptake of bran, can explain the fact that higher dough development times are reported in Farinograph analyses when flour is replaced by higher levels of bran (Sanz Penella and others 2008). In Mixograph analyses by Pomeranz and others (1977), this phenomenon was observed to a much lesser extent.

A second hypothesis relates to the suggestion that incorporation of bran particles in to the gas cell walls of the dough matrix disturb this structure. The presence of bran particles in dough might force gas cells to expand in a particular dimension (Gan and others 1992) or could even pierce gas cells, leading to coalescence or disproportionation and, hence, result in diminished gas retention in the dough, low bread volume, and dense crumb texture of whole-grain bread. Bran epicarp hairs are suggested to play a predominant role here (Gan and others 1989), and removing them through a pearling process prior to milling might reduce this effect to a certain extent (Gan and others 1989, 1992).

However, since pearling is a heterogeneous process that mainly removes the rounded surfaces of the wheat kernel instead of well-defined single histological layers (De Brier and others 2015), the

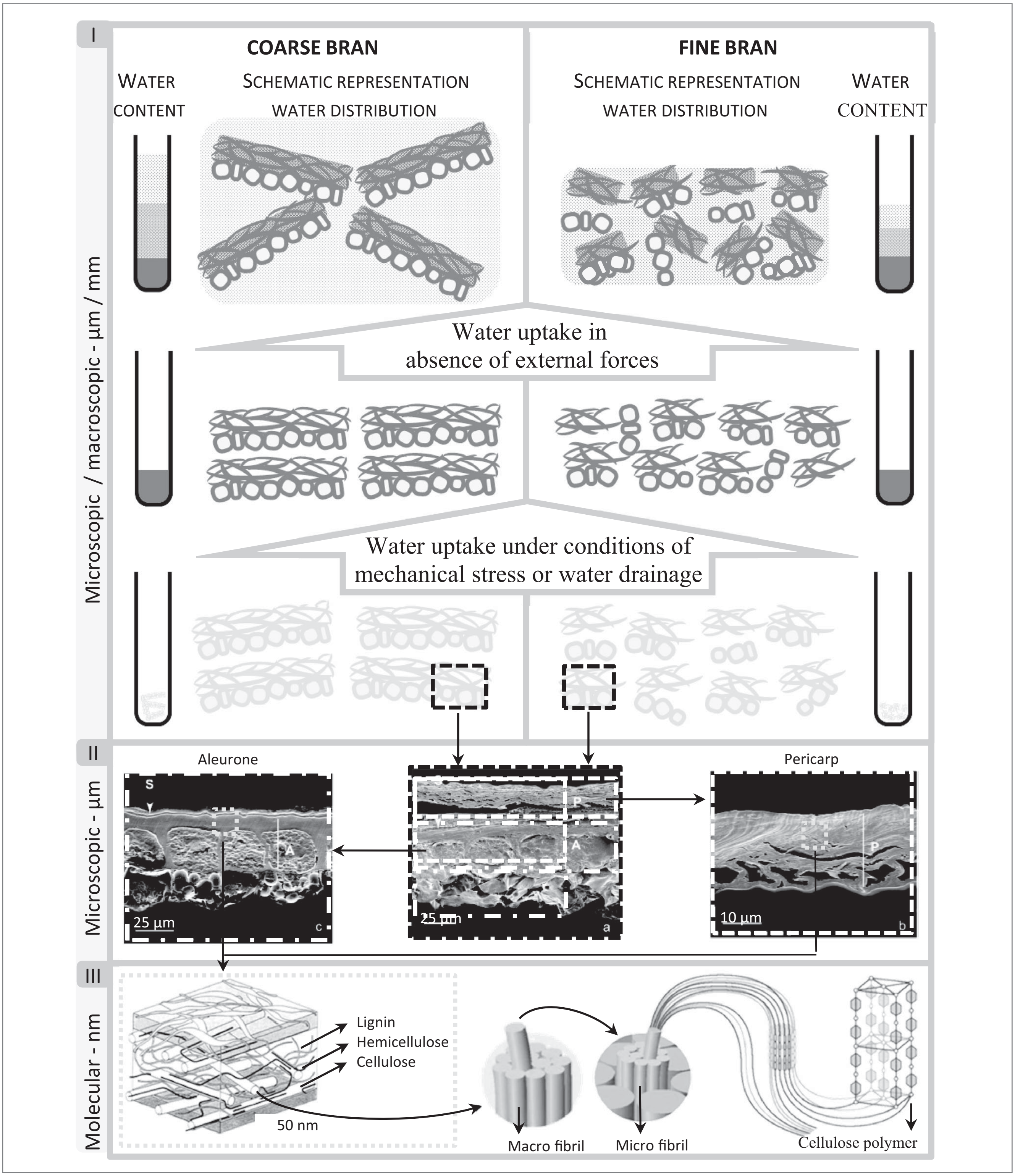

Figure 4–(I) Water bound or retained by coarse (left) and fine (right) wheat bran (⬜) under different experimental conditions at different scales; strongly bound water within bran matrix by nanopores or through hydrogen bonds (■), water retained in micropores of bran matrix (▩), stacking water (▢). (II) Complex bran structure; filled aleurone cells and hollow pericarp matrix constitute the greater part the bran structure. (III) Characteristic cell wall matrix and detail of the supporting cellulose fibril matrix (Esau 1960; Fosket 1994). Reproduced from Jacobs and others (2015).

positive effects observed with pearling might also be attributed to the concomitant removal of some of the outer histological bran layers which might even be more detrimental than the epicarp hairs.

Whether or not the epicarp hairs exert a significant role, microscopic analyses of bran-rich dough (Gan and others 1992) and bread (Pomeranz and others 1977) suggest that the bran particles somehow do cause a physical disruption of the gluten starch matrix. Different opinions exist though about the specific stage of bread making during which this disruption effect would be most pronounced. It could present itself during mixing when the gluten network is formed or during the later stages of fermentation (Campbell and others 2008) and early stages of baking when the gluten network is stretched thin (Gan and others 1989; Campbell and others 2008). Campbell and others (2008) concluded that wheat bran has its major effects on the aerated structure during baking, rather than earlier during the bread making process as they found a poor correlation between the effect of wheat bran on expansion during fermentation and loaf volume. The disruptive effect could also occur during the dough stage based on the fact that many authors report a decrease in dough strength and extensibility (Chen and others 1988; Rao and Rao 1991; Zhang and Moore 1997; Sanz Penella and others 2008; Gómez and others 2011; Schmiele and others 2012). These inferior rheological properties of bran-enriched dough may be ascribed to a weakened gluten network due a disruptive effect of the embedded bran particles. However, it is just as well possible that these effects are merely the result of gluten dilution.

The exact effect of bran addition on dough properties is, however, not clear-cut as even beneficial effects on dough strength may be noticed when coarse wheat bran is added, depending on the wheat cultivar. Such a strengthening effect was reported by Özboy and Köksel (1997) using coarse bran from a soft white winter wheat cultivar added to flour from the same cultivar. According to the authors, addition of the latter bran increased the dough resistance to overmixing and to extension as measured with the Farinograph and Extensigraph, respectively. When the latter coarse bran was added to a strong flour from a hard red winter wheat cultivar, a similar increase in dough strength was observed for bran addition levels of 5% and 10% (Özboy and Köksel 1997). At the end, bread properties were, nevertheless, still negatively affected when the bran was incorporated.

## Reactive components in bran

Besides specific physical properties, wheat bran is also believed to have a certain chemical reactivity which might determine its functionality. This chemical reactivity relates to specific components that are present in wheat bran. To start with, the presence of ferulic acid on arabinoxylan polymers present in bran (Zhou and others 2004) is believed to be relevant for bran's impact on bread making (Noort and others 2010). Ferulic acid might enable arabinoxylan polymers to form a network which might cause a detrimental effect through interfering with proper gluten agglomeration. Furthermore, it has also been suggested that arabinoxylan interferes with gluten proteins, mainly by covalent binding of ferulic acid with tyrosine residues in gluten proteins, affecting gluten network formation (Wang and others 2004; Piber and Koehler 2005). This was substantiated by the observation that addition of ferulic acid could prevent this oxidative cross-linking during gluten formation (Wang and others 2002, 2003). Arabinoxylan gels, formed through oxidative gelation of ferulic acid, could furthermore cause water migration from the gluten network to the arabinoxylan gels and hence result in inferior baking quality (Li and others 2012). It should be noted, however, that the relevance of these mechanisms may strongly depend on the accessibility of ferulic acid. In coarse bran, ferulic acid will be mainly embedded in the bran matrix and may therefore display minimal reactivity. Upon milling, these mechanisms may become more relevance as ferulic acid will become increasingly accessible.

Second, glutathione is believed to be present in wheat bran and involved in its deleterious effect (Noort and others 2010). Based on the work of Every and others (2006a) wheat bran is estimated to hold approximately 1 $\mu$mol glutathione per gram of bran, about 30 times the level that is found in white flour. Given the potential of glutathione to weaken the gluten network through disulfide interchanges (Joye and others 2009), the presence of glutathione might indeed be involved in bran's detrimental effect on bread making.

Also phytate in bran is believed to have the potential to react with gluten proteins and cause a negative effect (Cheryan and Rackis 1980; Hídvégi and Lásztity 2003; Noort and others 2010). So far, no hypothesis on the mode of action has been suggested.

In summary, it can be stated that wheat bran contains at least some reactive components, which have the potential to disturb the bread making process. However, little scientific evidence is available that actually substantiates the various hypotheses that have been proposed. Hence, it is difficult to accurately assess the relevance of these reactive components toward wheat bran functionality.

## Bran-related enzymes

A final aspect of bran which may determine its functionality is the pool of enzymes associated with it. Wheat contains various enzymes of multiple enzyme classes of which the majority is concentrated in the bran fraction of wheat grain (Poutanen 1997; Bonnin and others 1998; Rani and others 2001; Every and others 2006b). Table 2 illustrates this specific distribution for various hydrolases and oxidoreductases and an isomerase based on enzyme activities reported for specific milling products. While the majority of enzymes is concentrated within bran, some enzyme activities in coarse bran are similar or even lower compared to those of flour (Table 2). In addition, the data also point out that enzyme activities may vary among bran-rich milling products, such as coarse bran, shorts, and pollard, which can be ascribed to the fact that these milling by-products contain different amounts of bran, germ, and endosperm. This variety in enzyme-loading among milling by-products should be taken into account when studying the effects of nonstabilized bran on bread making, since these bran-related enzymes have the potential to interact with specific wheat flour constituents and, in doing so, affect the bread making process (Wootton and Shams-Ud-Din 1986; Noort and others 2010).

Similar to what is observed with sprouted wheat (Meredith and Pomeranz 1985), an excess of $\alpha$-amylase activity can cause starch degradation during dough mixing and fermentation to the extent that dough becomes sticky and unmanageable. This typically results in breads that are generally unacceptable to consumers (Chamberlain and others 1981). The work of Lunn and others (2001) at least indicates that bran-related $\alpha$-amylases may affect bread making as they observed that addition of pericarp-associated $\alpha$-amylases caused an exponential decrease in Hagberg falling number.

Endoxylanases with a specificity for water-unextractable arabinoxylan can have a positive effect on bread making through conversion of arabinoxylan into enzyme-solubilized arabinoxylan that increases the viscosity of the dough aqueous phase and, hence,

**Table 2–Relative comparison of enzyme activities reported for flour and different bran products (Galliard 1986a; Bonnin and others 1998; Rani and others 2001; Dornez and others 2006a; Every and others 2006b; Jerkovic and others 2010; Kaprelyants and others 2013). For each study, the absolute values for enzyme activities were expressed relative to the activity measured in sound flour which was first normalized. Based on the obtained intervals, a common interval was then composed for each enzyme.**

| Class | Enzyme | Flour | Coarse bran | Shorts | Pollard | Germ |
|---|---|---|---|---|---|---|
| Hydrolases | $\alpha$-amylase | 1–2 | 1–15 | 1–6 | 3–5 | 1–15 |
| | $\beta$-amylase | 1–9 | 2–3 | n.a. | 5–6 | 2–5 |
| | Peptidase | 1 | 0–5 | 0–5 | n.a. | n.a. |
| | Endoxylanase | 1–3 | 7–32 | 4 –15 | n.a. | 5 |
| | Xylosidase | 1 | 5–15 | 5– 8 | n.a. | n.a. |
| Oxidoreductases | Lipoxygenase | 0–4 | 3–12 | 3 | 8–20 | 35–50 |
| | Peroxidase | 0–1 | 1–4 | 3 | 1–3 | 6 |
| | Ascorbate oxidase | 1–2 | 3–4 | n.a. | n.a. | n.a. |
| | Dehydroascorbate reductase | 1 | 2–4 | n.a. | 2–10 | 3–7 |
| | Polyphenol oxidase | 0–3 | 3–9 | 12 | 4–16 | 3–5 |
| | Superoxide dismutase | 1 | 4 | 3 | n.a. | n.a. |
| Isomerases | Protein disulfide isomerase | 1 | 10–16 | n.a. | 16–18 | 18–19 |

stabilizes the dough foam structure. At high endoxylanase concentrations, though, this positive effect is overruled by the fact that extensive degradation of water-unextractable arabinoxylan leads to a decrease in the water-holding capacity of dough, which again results in sticky dough (Courtin and Delcour 2002). The conversion of water-extractable arabinoxylan or enzyme-solubilized arabinoxylan to lower-molecular-weight arabinoxylan can also destabilize the dough foam structure (Courtin and Delcour 2002). Literature data indicate that the levels of bran-associated endoxylanases may increase dough stickiness, as well as bread volume (Dornez and others 2008).

With regard to peptidases, endopeptidases are most relevant for the functionality in bread making as these enzymes can cleave proteins of the gluten network and, hence, weaken it (Linko and others 1997; Goesaert and others 2005). Also, in this case, this negative effect occurs only if the proteolytic activity exceeds a certain threshold. Indeed, a limited action of these enzymes may be desirable as it can result in positive effects, such as reduced mixing time, proper gluten strength, dough consistency, desirable bread texture, and improved flavor (Mathewson 2000). To the best of our knowledge, whether or not the actual levels of wheat bran-associated peptidases may affect bread making has not been studied yet.

Next, bran lipases may release fatty acids from grain lipids, which are mainly triacylglycerols, starting from the moment of storage of whole-grain flour under ambient conditions (Galliard 1986a). Lipolysis can lead to a negative impact on bread making by increasing the overall level of free fatty acids on the one hand and by decreasing the overall level of triacylglycerols (Tait and Galliard 1988). Alternatively, the resulting mono- and diacylglycerols may exert a beneficial effect with regard to crumb properties (Pareyt and others 2011). While the effects of bran-related lipase during whole-meal flour storage are relatively well known, a possible additional role during bread making has only been hypothesized, though not studied extensively (de Kock and others 1999). Following lipolysis, lipoxygenase may oxidize polyunsaturated fatty acids and improve dough and bread characteristics on one hand (Bahal and others 2013) but may undermine the improving action of ascorbic acid due to competition for oxygen and cause rancidity through formation of fatty acid hydroperoxides on the other hand (Galliard 1986a; Galliard and Gallagher 1988). In practice, the importance of lipoxygenase should not be overestimated since this oxidoreductase requires molecular oxygen as substrate. Therefore, its activity in dough systems might be limited to the 10 to 15 min after which oxygen in dough is consumed (Joye and others 2012).

Next, peroxidases may reinforce the gluten network through catalysis of the formation of cross-links between thiol groups as well as the formation of dityrosine dimers (Jerkovic and others 2010; Manu and Prasada Rao 2011). Every and others (2006b), however, found that the role of peroxidase toward final loaf volume and crumb structure is rather minimal. This could also be expected from the fact that the enzyme requires oxygen which is consumed early in the bread making process (Joye and others 2012).

Protein disulfide isomerase may positively influence bread making as it catalyzes the oxidation of protein thiols to disulfide bonds in the presence of ascorbic acid, thereby producing larger glutenin polymers which are associated with good bread quality (Every and others 2003). The reported levels of protein disulfide isomerase activity measured in bran greatly exceed the minimum activity required to observe a positive effect on bread making. However, the presence of ascorbic acid is essential as only a poor correlation was observed between protein disulfide isomerase content and bread making performance in the absence of ascorbic acid (Every and others 2006b).

Finally, wheat bran-associated polyphenol oxidases are involved in the darkening of wheat products due to their oxidative effect on endogenous phenols. The actual levels of polyphenol oxidases found in wheat bran may cause loss of both organoleptic quality and nutritional value of whole-meal products (Soysal and Söylemez 2004). With regard to loaf volume and crumb structure, Every and others (2006b) saw no clear correlation. Again, these oxidases use molecular oxygen so that their activity in dough is rather limited in time (Joye and others 2012).

It is clear that various and complex biochemical processes can occur during bread making in the presence of bran-associated enzymes. While specifically selected amylases, xylanases, peptidases, and lipases can have positive effects at low concentrations, in general, they will display detrimental effects, especially when they are uncontrolled and present in high concentrations. Oxidoreductases, such as lipoxygenase and peroxidases, in contrast, are more likely to result in positive effects on the gluten network and hence bread volume. However, they can give rise to inferior taste and color characteristics. Further research is required to validate or dismisses the actual relevance of these wheat bran related enzymes during bread making since limited work on this topic has been performed so far.

At last, it is important to bear in mind the following aspects with regard to enzymes and bran. The levels of enzymes present in bran depend on parameters, such as type of milling product, cultivar type, and physiological stage of the kernel (Every and others 2002; Dornez and others 2006c). Upon germination of wheat kernels, for instance, enzyme synthesis and activity in the bran will increase significantly. It is well known that the

$\alpha$-amylase activity of sprouted wheat drastically increases in the aleurone, embryo-scutellum, and adjacent endosperm (Akazawa and Miyata 1982; Henry and others 1987). Furthermore, whereas most enzymes are endogenous to wheat, some bran-associated enzymes are of exogenous origin. Endoxylanases found in or on wheat bran consist of a large majority of microbial endoxylanases and a minority of endogenous endoxylanases (Dornez and others 2006b). The activity of such wheat-associated microbial enzymes during bread making might be limited, however, due to the presence of inhibitors (Dornez and others 2008). Furthermore, bran-associated enzymes in general may not display optimal activity during bread making due to poor substrate accessibility in dough and nonoptimal temperature and pH. Hence, the exact overall enzymic activity of wheat bran and its resulting impact on the bread making process might vary significantly amongst different bran samples, and is difficult to predict.

## Wheat Bran Treatments

Bran-rich breads are usually made starting from strong wheat cultivars. Additionally, to improve the volume, wheat gluten and/or bread improvers such as enzyme mixtures, emulsifiers, and ascorbic acid can be added to the recipe. In relation to the gear diagram (Figure 3), these countermeasures to bran's negative impact basically result in a boost of the flour functionality. Despite the possibility to effectively improve bread properties, this approach offers limited possibilities to study the mechanisms through which bran affects bread making. A more direct approach to do so is to introduce variation in wheat bran functionality itself and evaluate its impact on bread properties. This may be achieved by comparing the bread making performance of multiple bran samples which may for instance originate from wheat breeding programs, various wheat cultivars, or different bran fractions. Following this approach, it may still be difficult to pinpoint the relevance of distinct bran characteristics given the inevitable constraint that each sample may display differences in overall composition, physical properties, reactive components, and enzymic load. In an attempt to modify bran properties more specifically, researchers have evaluated various bran treatments. Most of these treatments are mechanical, (hydro)thermal, chemical, or enzymatic in nature or involve prefermentation of bran. As illustrated in the gear diagram (Figure 3), implementation of those treatments can result in changes in bran physical properties, chemical composition, and/or enzymatic load, which can lead to modified functionality. Therefore, the use of bran modifications constitutes an interesting approach to assess the role of specific bran properties and their working mechanisms. Nevertheless, since wheat bran treatments generally result in modification of more than just one bran property, a comprehensive analysis of changes that occur in both bran and bread properties is required to allow for a correct interpretation of the observed phenomena. This challenge may explain the frequently reoccurring contradictions with regard to the impact of specific bran treatments on bread making performance. In the following part, common bran treatments will be discussed together with the hypotheses regarding bran functionality that were derived.

### Particle size reduction of bran

Particle size reduction is by far the most investigated treatment of wheat bran and several studies exist in which the impact of this treatment on dough and bread properties was reported. Prior to the discussion of this treatment, it has to be pointed out that the effect of particle size has been studied using samples with different particle sizes which have been obtained by means of particle size reduction on the one hand or by using bran-rich milling streams with different particle sizes on the other hand. By reducing the particle size of coarse bran, both coarse and ground particles will have a similar overall chemical composition. This is not the case when studying the effect of particle size using different bran-rich milling streams, which are known to have different compositions (Pomeranz and others 1977; Haskå and others 2008; Hemdane and others 2015). Likewise, a similar reasoning can be made for enzyme activities (Table 2). Hence, following the latter approach, interpretation of results might be difficult, as changes in bread properties due to differences in bran particle size and differences in the bran's chemical composition are hard to differentiate. Therefore, this paragraph will mainly focus on studies where the approach of particle size reduction was followed. Looking at the impact of particle size reduction on dough properties, Zhang and Moore (1997) found that the water absorption capacity of bran-rich dough, measured with the Farinograph, does not depend on bran particle size. This observation is in agreement with the hydration mechanisms described in our previous study (Jacobs and others, 2015). That is, hydration of wheat bran in the presence of an external stress is governed only by water retention in nanopores and water-binding through hydrogen bonds. Since these hydration mechanisms are not affected by particle sizes in between roughly 100 to 1700 $\mu$m, no change in dough water absorption occurs. Nevertheless, several authors found that fine bran is characterized by a higher Farinograph water absorption than coarse bran, which was ascribed to a higher specific surface that is exposed to hydroxyl groups (Sanz Penella and others 2008; Noort and others 2010; Cai and others 2013). Dough mixing time and mixing stability as measured by the Farinograph decrease when using fine bran particles instead of coarse particles. Sanz Penella and others (2008) suggested that the prolonged development time observed with large particles could be ascribed to the fact that coarse bran needs more time to absorb water than fine bran. The negative effect on dough stability is attributed to the fact that for a same bran substitution level, fine bran has more particles than coarse bran, which would result in a more severe disruption of the gluten network due to an increased flour-bran contact for small particles (Zhang and Moore 1997; Sanz Penella and others 2008). Dough mixing behavior as measured by the Mixograph does not change significantly with decreasing average particle size (Cai and others 2013).

The impact of wheat bran particle size on bread loaf volume remains unclear. Several studies have demonstrated that ground bran has a more detrimental effect on bread volume compared to large bran particles (Galliard and Gallagher 1988; Özboy and Köksel 1997; de Kock and others 1999; Campbell and others 2008; Noort and others 2010). However, it has also been reported that addition of finely ground wheat bran to flour produces bread with higher loaf volume and better crumb characteristics as compared to coarse bran (Shetlar and Lyman 1944; Pomeranz and others 1977; Moder and others 1984; Lai and others 1989b). Zhang and Moore (1999) reported that bread made with medium-sized bran (415 $\mu$m) had higher specific volume than breads made with coarse (609 $\mu$m) and fine (278 $\mu$m) bran, irrespective to their substitution levels, indicating that an optimum bran particle size may exist for the production of bran-rich bread. Coda and others (2014) supported the concept of an optimum particle size as they observed the highest specific loaf volume when wheat bran with average particle size of 160 $\mu$m was added compared to other bran particle sizes (750, 400, and 50 $\mu$m). Finally, in other studies it was concluded that bran particle size did not exert any significant effect on bread volume, at least for certain wheat types (Galliard and Gallagher 1988; Özboy and Köksel 1997; Sanz Penella and others 2012; Cai and others 2013). Cai and others (2013) only noticed smaller bread volumes with ground hard white wheat bran compared to the coarse bran. When hard red wheat was ground, no significant differences in bread volume were observed compared to bread containing coarse bran (Cai and others 2013). Also, Sanz Penella and others (2012) did not notice significant differences in volumes between coarse and ground bran. Nevertheless, bread crumb was found to be firmer upon addition of ground bran.

The impact of reducing the particle size of wheat bran on bread making might thus be due to changed hydration properties, but also due to an increased specific surface of bran and an increased accessibility of cell components. Reactive components, as mentioned earlier, are believed to have a considerable impact on bran functionality in bread. The relevance of some reactive, bran-related compounds is supported by de Kock and others (1999), who found that the negative effect caused by bran particle size reduction could be counter-acted by decreasing the bran's reactivity with a heat treatment. Moreover, Noort and others (2010) suggested that particle size reduction resulted in a higher accessibility of arabinoxylan chains, leading to more ferulic acid and gluten protein interactions and causing adverse effects on the functionality of the gluten network. The authors also suggested that reactive components like conjugated ferulic acid monomers or glutathione might be liberated due to cell breakage (Noort and others 2010).

Bringing the above observations together, it is still not clear what the real impact of bran particle size reduction on bread making is. Possible explanations for discordant results might reside in the use of wheat cultivars with different properties or different particle size reduction techniques. Different approaches of bread making might also influence results. Possible explanations for the lack of insight in this matter might reside in the fact that particle size reduction may result in a concurrent modification of multiple wheat bran characteristics, such as hydration properties, accessibility of reactive components, and physical dimensions. The phenomena observed in bread making upon particle size reduction may therefore be a resultant effect of the interplay of various mechanisms related to specific bran characteristics. Uncovering these mechanisms is therefore a challenging task. This inherent complication of interrelations between bran properties may as well account for the contradictions in literature since the extent to which multiple modifications occur as well as their relevance in bread making may vary as function of the specific wheat cultivars, milling technique, and bread making procedure. Regarding the differences in bread making procedures, de Kock and others (1999), for instance, mixed dough at a high speed, followed by a fermentation step for 70 min and a baking step for 30 min at 230 °C. Under these experimental conditions, the authors noticed a lower bread volume with smaller bran particles. In contrast, Moder and others (1984) applied a straight-dough procedure with a longer fermentation time and several proofing steps and reported higher bread volumes with smaller bran particles. More recently, Curti and others (2013) made bran-rich breads with a home bread-maker and did not observe any significant differences between the breads with different particle sizes (Curti and others 2013). Based on recent work, these contradictions may be clarified to a certain extent. Following straight-dough bread making trials at different water absorptions and mixing times, it was observed that particle size in fact did not affect the bread making potential of bran when optimal conditions were used for making dough. Whereas the optimal bread making potential was achieved at the same optimal water absorption, particle size reduction reduced the optimal mixing time, and as long as these optimal mixing times were respected, loaf volumes were alike. Neither these optimal mixing times, nor the optimal water absorption could successfully be predicted based on Farinograph or Mixograph analyses. Since baking trials are often performed based on these analyses, contradicting reports may be due to poor estimation of optimal baking conditions (Jacobs and others, unpublished data).

In-depth sensory research about bran-rich bread and the impact of bran particle size reduction is rather limited. Besides its effect on the loaf volume, particle size reduction has been reported to result in a darker crumb color than bread containing coarser particles (Moder and others 1984; Zhang and Moore 1999; Majzoobi and others 2013). Moreover, reducing bran particle size leads to crumb with a more uniform color and a less gritty mouthfeel than breads containing coarse bran, making it more acceptable for consumers (Zhang and Moore 1999). Flavor was, however, not affected by bran particle size (Majzoobi and others 2013). This suggests that the release of negative flavor components through particle size reduction is rather limited.

### (Hydro)thermal treatment of bran

In a number of studies, bran properties were modified by means of thermal and hydrothermal treatments. Caprez and others (1986) studied the effects of boiling, steam-cooking, roasting, and autoclaving wheat bran on its chemical composition and physical properties. Each of these heat treatments significantly affected the physical properties of the bran, and especially the rheology of the bran-rich dough. Water uptake, as measured with the method of Enslin (1933), significantly increased when heat-treating ground wheat bran, except upon autoclaving. Furthermore, steam-cooking, autoclaving, and roasting of bran increased Farinograph water absorption and mixing time and decreased maximum resistance of dough made with it, compared to the regular, ground bran. Upon boiling, Farinograph water absorption decreased and maximum dough resistance was not affected.

Some studies also investigated the effect of (hydro)thermal treatment of bran on bread properties. Regarding bread volume, different observations are reported in the literature, mainly depending on the type of treatment. Dry heat treatment of wheat bran by autoclaving results in increased loaf volumes (de Kock and others 1999). It is plausible that this increased bread volume is caused by the inactivation of readily accessible components, such as small reactive molecules or detrimental enzymes, as it was reported before that bran extract is more and bran residue less deleterious to bread volume than regular bran (Wootton and Shams-Ud-Din 1986). However, de Kock and others (1999) suggested that the deleterious effect of wheat bran on bread volume is not only caused by reactive, bran-related compounds but its physical properties are also involved (de Kock and others 1999).

Upon incorporation of wheat bran soaked in an excess boiling of water for 15 min, improved bread volumes were observed compared to regular bran (Nelles and others 1998), similar to what was observed for autoclaved bran (de Kock and others 1999). Furthermore, Mosharraf and others (2009) showed that overnight soaking of bran in acetate buffer (pH 4.8, 55 °C) and subsequent drying at 37 °C, resulted in improved dough rheological properties compared to dough with regular bran. The mode of action was not unraveled.

Extrusion of wheat bran, finally, leads to solubilization of chemical components, especially of dietary fiber (Ralet and others 1990;

Wang and others 1993). For a mechanical energy input higher than 230 kWh/t, the degree of solubilization was linearly correlated to the specific mechanical energy put in the process by the extruder (Ralet and others 1990). Extruded wheat bran has a higher water absorption than regular bran, which might at least be partly attributed to the presence of pregelatinized starch after extrusion (Ralet and others 1990; Wang and others 1993). Regarding the effect of extruded bran on bread properties, different observations were reported. Wang and others (1993) noticed that bread containing extruded bran had an inferior volume compared to bread containing regular bran. Nevertheless, breads containing medium-speed or high-speed extruded bran did not differ significantly in volume compared to the bread containing regular bran. The organoleptic quality of bread with extruded bran was, moreover, slightly lower, but still acceptable. More recently, Ugarčić-Hardi and others (2009) reported that extruded bran has a less negative impact on dough rheology than the nonextruded product. Gómez and others (2011) also showed that breads prepared with extruded bran and an improver containing ascorbic acid, monoglycerides, diglycerides, lecithin, amylases, and hemicellulases, were higher in volume than breads with regular bran and the improver. This might be due to the hydrolysis of pregelatinized starch present in extruded bran by the amylases from the improver, leading to a higher release of fermentable sugars and thus an increased gas production. When no improver was added, inferior bread volumes were observed if extruded bran was incorporated instead of regular bran, despite an increased fermentation dough height observed for dough containing extruded bran. Bran-related components, hence, seem to counteract the functioning of one or more components present in improvers. From an organoleptic point of view, bread quality did not differ significantly between breads containing regular or extruded bran (Gómez and others 2011).

Similar to the impact of particle size reduction, no consistent view on the impact of (hydro)thermal treatment on wheat bran bread making suitability can be synthesized from the literature at hand. This may be simply demonstrated by the fact that some (hydro)thermal treatments may lead to an increase of loaf volume (de Kock and others 1999) while others do the opposite (Gómez and others 2011) or have no effect at all (Wang and others 1993). These contradicting and largely unexplained observation may once more arise from the changes in various properties of bran which may be induced by (hydro)thermal treatment. For instance, besides the inactivation of heat labile bran components, (hydro)thermal treatments may as well affect bran's hydration behavior as Ralet and others (1990) as well as Wang and others (1993) observed an increased water absorption upon extrusion which was ascribed to the pregelatinization of starch. Therefore, contradicting observations with regard to (hydro)thermal treatments may originate from the occurrence of additional modifications besides their stabilization effect which may not have been taken into account.

## Presoaking of bran

In the past decades, some researchers have investigated the potential of counteracting the detrimental effects of wheat bran by presoaking bran in water. Practically, presoaking of wheat bran has been performed using 2 different approaches. Bran can be soaked in a limited amount of water which is added together with the bran in subsequent bread making trials. Alternatively, bran is presoaked in an excess of water which is either partially or completely removed after presoaking. In the latter case, water-soluble components are partially washed out due to the removal of soaking water. Both approaches have been reported to improve bread quality (Wootton and Shams-Ud-Din 1986).

Lai and others (1989b) reported that presoaking bran in a limited amount of water gives higher loaf volumes compared to bread containing untreated bran, especially if high bran concentrations are added. They suggested that the slow rate of water uptake by coarse bran during mixing is an important cause of its deleterious effect in bread making. Adding bran that is already saturated with water could overcome this deleterious effect. The same authors observed a similar increase in bread volume when shorts were presoaked in a limited amount of water for 1 h prior to addition to flour (Lai and others 1989c). In this case, they ascribed this phenomenon to the activity of endogenous lipoxygenase, which would oxidize components that can be detrimental to loaf volume, such as methoxyhydroquinone and glutathione (Lai and others 1989c). The authors tested this hypothesis by adding lipoxygenase or enzyme-active soy flour (containing lipoxygenase) to a sponge-and-dough system containing glutathione and methoxyhydroquinone. In this model system, bread volume was completely restored compared to bread without added lipoxygenase (Lai and others 1989c).

The hypothesis on activation of endogenous lipoxygenase was supported by Nelles and others (1998). In their set-up, the decrease of potentially oxidizable substances was also partially due to a washout effect, as they presoaked the bran in an excess of water.

In contrast to these observations, no increased or even slightly decreased bread volumes compared to bread containing untreated bran were observed when Chen and others (1988) presoaked wheat bran for 12 h in excess water and replaced 4% and 8% of white flour by the presoaked bran.

In conclusion, a complete understanding of the impact of presoaking and its related effects on wheat bran's bread making performance has not yet been established. The fact that multiple bran properties may be simultaneously modified upon soaking bran may be at least in part held responsible for this. For instance, bran does not only hydrate during presoaking. Bran-related enzymes may also exert a relevant effect depending on the time of presoaking. In addition, presoaking bran in an excess water involves a washout of water-soluble components which will complicate interpretation of results even more.

## Enzymatic treatment of bran

Several studies report that addition of various enzymes, such as $\alpha$-amylases (Sanz Penella and others 2008), phytases (Sanz Penella and others 2008), xylanases (Laurikainen and others 1998), lipoxygenases (Lai and others 1989a), glucose oxidases, and hexose oxidases (Gül and others 2009), can be used to optimize the quality characteristics of dough and bread supplemented with bran. On enzymatic treatment of wheat bran itself and on the structural as well as physicochemical changes induced by these treatments very little work has been carried out.

Incubation of bran with a bacterial, thermostable $\alpha$-amylase results in increased fiber levels compared to untreated bran due to the enzymatic degradation of starch and subsequent washout of starch hydrolysis products (Rasco and others 1991). Substitution of flour with amylase-treated bran yielded breads with inferior loaf volume and crumb quality compared to substitution of the same level of flour with untreated bran (Rasco and others 1991). This observation is most likely related to the fact that breads containing treated bran were higher in botanical bran compared to breads containing regular bran, as the starch was removed from the treated bran. When the bran was further treated with a peptidase, bread properties were even worse (Rasco and others 1991), probably because of insufficient inactivation of the peptidase, causing a weaker gluten network. Indeed, the authors drum-dried the bran after the enzymatic treatment, but did not verify if the enzymes were completely inactivated after the drying process. Finally, enzymatic treatment of rye bran with purified xylanase or $\alpha$-amylase-xylanase mixtures significantly decreases the detrimental effects of bran on bread volume (Laurikainen and others 1998). This effect can be attributed to the release of arabinoxylan from the bran and endosperm cell walls, leading to an increase in water-soluble arabinoxylan content and in the viscosity of the dough aqueous phase. It was previously demonstrated that this improves bread loaf volume (Courtin and Delcour 2002). The mixtures were moreover more effective than pure enzymes, probably due to the presence of $\alpha$-amylase in the mixtures (Laurikainen and others 1998). It is important to note here that the used enzymes were also active during bread making. The positive effect of the enzymatic treatment on bread making could thus be due to the interaction between the enzymes and wheat endosperm components, and their effect on bran.

One inherent problem in this type of studies is that, while bran can easily be preincubated with enzymes prior to addition to flour, it is rather difficult to get rid of the enzyme activity of the added enzymes after incubation. Although this problem can be resolved by heat-inactivation of the enzymes, this will induce additional modifications to bran, adding even more complexity to the system. This makes it difficult to determine whether enzymes modify the bran properties positively, or if observed effects should rather be attributed to the activity of the enzymes in the meal.

### Fermentation of bran

Another means to modify bran is to ferment it prior to its addition to flour. Katina and others (2012) reported that bread containing bran obtained from pearled kernels which has been yeast-fermented for 20 h showed higher specific bread volumes and softer crumbs compared to unfermented bran. The authors suggested that this quality improvement could mainly be attributed to arabinoxylan solubilization due to the fermentation, modifying the structure of bran cell-walls. As yeast was not completely removed after fermentation of the bran, also the presence of more yeast in the dough upon addition of treated bran might add to better bread properties. Also, bran fermentation may even improve bran functionality such that the quality of bran-rich breads even surpasses that of white bread. Coda and others (2014) fermented wheat bran for 8 h with lactic acid bacteria and yeast strains, in combination with hydrolytic enzymes. According to the authors, the activity of *Lactobacillus brevis* was essential to bread volume improvement by fermentation as its activity correlated with a better dough stability and enhanced gas retention.

Nevertheless, in this type of studies, it is difficult to conclude whether these observations were caused by modifications of the bran, as such, or by the presence of microorganisms, and enzymes as copassengers. Indeed, while significant modifications can be induced through fermentation, these can be due either to the modification of the bran or to the incorporation of microorganisms and/or enzymes in dough and bread. This should be kept in mind when interpreting the observations on the use of fermented wheat bran in bread. Indeed, it has been reported that fermentation by yeast is enhanced in the presence of heterofermentative lactic acid bacteria (Gobbetti and others 1995). Besides, the volume of bread made with prefermented bran can also be improved due to the enhanced acidification of the dough pH by the microorganisms. The latter has been suggested to solubilize gluten proteins, through an increased intramolecular electrostatic repulsion, and to enhance the activity of endogenous peptidases, resulting into a modified gluten network (Katina and others 2006). Finally, it should be noted that a combination of prefermentation together with enzymatic treatment not only improves the loaf volume and textural properties but also enhances the shelf-life of the bran-rich bread. The effects are mainly attributed to redistribution of water among starch, gluten, and bran particles during storage (Katina and others 2007).

### Chemical treatment of bran

Finally, researchers tried to modify bran functionality by steeping bran in chemical reagents. Such treatment can modify the hydration properties of bran, as well as specific chemical constituents and enzymatic load, depending on the type of chemical used, the incubation conditions, and whether or not if the bran is coincubated with enzymes.

Studies have been published wherein bran was steeped in $KIO_3$, $H_2O_2$, citric acid, CaO, or ethanol (Lai and others 1989c; Rasco and others 1991). None of these treatments showed improvements in the quality of the bran-rich breads, on the contrary. Therefore, this type of treatment will not be further discussed in this review.

## Conclusion and Perspectives

Incorporation of wheat bran in cereal-based products such as bread leads to significant organoleptic quality losses, such as decreased bread volumes, textural changes, and decreased sensory acceptance. Although the dilution of gluten proteins by incorporation of bran will greatly affect bread quality, specific bran-related properties also seem to play a significant role in the detrimental impact of bran on bread making. These can be either physical properties, such as dynamic water interaction properties, the presence of specific reactive compounds, and bran-associated enzymes. In an attempt to assess the potential role of each of these individual actors in bran's detrimental effect or simply to counteract the detrimental effect of bran, different bran treatments such as particle size reduction, (hydro)thermal treatments, chemical or enzymatic treatments, or bran fermentation have been explored. Despite these efforts, a general consensus on the role of individual bran properties or how to successfully modify bran to counteract its negative effects on bread making is still lacking. The leading cause for this controversy ultimately resides in the fact that little is actually known about the mechanisms behind the effects of wheat bran on bread making. Whereas the use of bran modifications may be the most promising approach to assess the role of specific bran properties and their working mechanisms, such an approach has proven to be and probably will remain challenging as bran treatments in general result in modification of more than just one bran property. Therefore, profound insights on the effects of bran modifications on its properties are at least equally important as mapping its effects in bread making to correctly assess the role of specific bran properties in their detrimental effects. The use of wheat bran treatments thus constitutes a useful tool to investigate the effect of bran in bread making provided that changes in bran and bread properties upon bran treatments are carefully analyzed, linked, and interpreted in the specific experimental set-up of the research. In any case, insights into the relationship between wheat bran properties and their impact on bread making obtained in such manner are required to develop suitable, directed techniques that successfully improve the bread making potential of wheat bran. Finally, besides the need for improved insight on wheat bran functionality with regard to technological and organoleptic aspects of bread making, further research is also needed to investigate the impact of these bran-directed treatments on bran nutritional properties, as a proper balance should exist between improving bread quality, on one hand, and optimally utilizing the nutritional potential of bran nutritional properties, on the other hand.

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## Author Contributions

S. Hemdane and P.J. Jacobs contributed equally to this work.

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