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Density separation as a strategy to reduce the enzyme load of preharvest sprouted wheat and enhance its bread making quality

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ABSTRACT

As preharvest sprouting of wheat impairs its use in food applications, postharvest solutions for this problem are required. Due to the high kernel to kernel variability in enzyme activity in a batch of sprouted wheat, the potential of eliminating severely sprouted kernels based on density differences in NaCl solutions was evaluated. Compared to higher density kernels, lower density kernels displayed higher α-amylase, endoxylanase, and peptidase activities as well as signs of (incipient) protein, β-glucan and arabinoxylan breakdown. By discarding lower density kernels of mildly and severely sprouted wheat batches (11% and 16%, respectively), density separation increased flour FN of the batch from 280 to 345 s and from 135 to 170 s and increased RVA viscosity. This in turn improved dough handling, bread crumb texture and crust color. These data indicate that density separation is a powerful technique to increase the quality of a batch of sprouted wheat.

1. Introduction

Preharvest sprouting (PHS) of wheat is defined as the germination of the grain in the ear of the parent plant in the field and impairs both grain yield and quality. For example, flour derived from preharvest sprouted wheat results in dough that is sticky, less elastic, and difficult to handle. These doughs give rise to bread with a sticky crumb, poor sliceability and a darker crust color (Hwang & Bushuk, 1973; Ibrahim & D'Appolonia, 1979; Kozmin, 1933). As the quality of bakery products made from sprouted wheat is generally unacceptable to producers and consumers, sprouted grains are often downgraded to feed grain, resulting in considerable economic losses.

Previous efforts to minimize the occurrence of PHS mostly focused on breeding wheat varieties to increase seed dormancy at maturation (Gao et al., 2013). Nevertheless, these breeding techniques have their limits as long-lasting dormancy requires prolonged seed storage for new crop generation before satisfactory seedling emergence (Rodríguez, Barrero, Corbineau, Gubler, & Benech-Arnold, 2015). Moreover, as weather conditions are unpredictable, PHS will always occur to some extent. As a consequence, the development of postharvest remedies to upgrade the quality of sprouted wheat grains remains of interest. Olaerts and colleagues (2016a, 2016b) previously confirmed that PHS in the field mainly reduces the techno-functional performance of wheat during processing through a vast increase in enzyme activities while the intrinsic starch and gluten properties in flour are not affected. Hence, the quality of flour from sprouted wheat may be enhanced by reducing the enzyme load using well-selected postharvest technologies.

Due to the heterogeneity of ambient conditions in the field and due to differences in genetic properties, ear morphology, water availability, and stage of maturity of the kernels, the extent of sprouting may vary notably among the kernels in the field and even within the ear (Gao et al., 2013; Gosling, Butler, Black, & Chapman, 1981; King & Richards, 1984). This non-uniform extent of germination causes a heterogeneous distribution of enzyme activity in a population of sprouted kernels with only a minority of the kernels being severely sprouted (Olaerts, De Bondt, & Courtin, 2017). Physical separation and removal of the most sprouted kernels would, hence, provide the possibility to enhance the quality of the flour obtained from the remaining, less sprouted kernels. In theory, this separation can be based on density differences. Indeed, light microscopy analysis of sprouted grains suggested that within a batch of sprouted kernels, severely sprouted grains have a lower density than the mildly sprouted ones (Olaerts et al., 2017). Irreversible swelling of kernels upon imbibition and desiccation in the field together with germination-induced (carbohydrate) respiration affects both kernel volume and weight, and hence the kernel density (Bettge & Pomeranz, 1993).

Abbreviations: AU, α-amylase activity units; avDP, average degree of polymerization; AX, arabinoxylan; dm, dry matter; EU, endoxylanase activity units; FN, falling number; PHS, preharvest sprouting; RVA, rapid visco analyzer; SDS, sodium dodecyl sulfate; SDSEP, amount of proteins extractable in sodium dodecyl sulfate; SE-HPLC, size exclusion high performance liquid chromatography; WEAX, water extractable arabinoxylan; WUAX, water unextractable arabinoxylan

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The fact that grain density changes in function of sprouting stage opens perspectives for the segregation of sound and sprouted grains. Specific gravity tables are routinely used in the milling industry as cleaning devices for removing light foreign material from seeds before the actual milling process. Research indicated that these tables can also be used to exclude low Falling number (FN) grains and to recover grain fractions of improved quality from sprouted wheat (Bettge & Pomeranz, 1993; Hook, Salmon, Greenwell, & Evers, 1988; Tkachuk, Dexter, & Tipples, 1990, 1991). The slight difference in kernel density coupled with an increased roughness of the outer coating of sprout damaged kernels enables an effective separation (Bettge & Pomeranz, 1993). This way, severely sprouted kernels with high α-amylase activity end up in the low density fractions, while kernels with high FN value and improved test weight can be recovered in the high density fractions (Hook et al., 1988; Tkachuk et al., 1991). The removal of sprouted, shrunken, and broken kernels in the lightest fractions resulted in greater flour yields and brighter flour color for the remaining wheat (Hook et al., 1988; Tkachuk et al., 1991). However, the technique was less promising in enhancing bread loaf volume or crumb texture when flour made from the high density kernels was used (Hook et al., 1988; Tkachuk et al., 1991) as the starting material was of poor bread making quality or damaged to a too large extent by PHS (FN values were even under detection limit of the Hagberg test). Improving the quality of sprouted wheat by air separation was only successful when large amount of kernels (> 50%) were discarded (Bettge & Pomeranz, 1993). Besides these studies from the late 1980s and early 1990s, to the best of the authors' knowledge, no studies looked into the improvement of the quality of end products made with sprouted wheat following the use of gravity tables.

An alternative way to separate wheat grains according to density is the use of density baths in which kernels sink or float when their density is significantly higher or lower than that of the solution, respectively. Munck (1989) showed that a strongly sprouted wheat sample (FN = 61 s) can be divided in three fractions by density separation in sodium nitrate solutions of different densities. A light fraction (11%, FN = 61), a medium fraction (69%, FN = 93) and a heavy fraction (20%, FN = 402) were obtained. Nevertheless, no additional information on the fractionation of wheat in density baths is available in literature. Knowledge on the properties of lower and higher density kernels obtained through density separation is lacking, as well as the potential of this technique to enhance the flour and bread quality of preharvest sprouted wheat.

Therefore, the aim of this study was to assess the use of density baths as a tool to separate sound wheat kernels from sprout damaged wheat and to upgrade the quality of sprouted wheat. NaCl solutions are used as an alternative, food grade fractionation medium in the density bath. Both a mildly sprouted (wheat FN = 205 s) and a severely sprouted wheat batch (wheat FN = 100 s) are used to test the separating power of NaCl density baths. In the case of severely sprouted wheat, also the combination of the density separation and a pearling treatment of the higher density kernels is performed to further reduce the enzyme activity. The removal of the outer layers of sprouted wheat by pearling is known to have beneficial effects on the flour obtained from these kernels (Hareland, 2003; Henry, Martin, & Blakeney, 1987). It remains to be seen if the wet separation technique holds potential to be implemented in an industrial setting. While it could be combined with conditioning of wheat grains before conventional roller milling and the use of a salt solution during tempering also holds the benefit of reducing the microbial load (Sabillón, Stratton, Rose, Flores, & Bianchini, 2016), the legal and technological feasibility of the technique will need to be evaluated.

2. Materials and methods

2.1. Materials

Winter wheat (*Triticum aestivum*) cultivar Sahara was cultivated in Belgium and harvested as described in Olaerts et al. (2016a). A sound wheat sample of Sahara was harvested around harvest maturity (July 30). By delaying harvest time under rainy weather conditions, PHS occurred in the ear in the field. This way, a mildly sprouted sample (harvested 9 days after harvest maturity, wheat FN = 205 s) and a severely sprouted sample (harvested 19 days after harvest maturity, wheat FN = 100 s) were obtained. Classification of wheat samples into mildly, moderately, or severely sprouted wheat is based on the FN measured on whole meal. Wheat with whole meal FN values above 250 s, between 200 and 250 s, between 150 and 200 s, or below 150 s is classified as sound, mildly sprouted, moderately sprouted, or severely sprouted wheat, respectively (Skerritt & Heywood, 2000). Azurine-cross-linked arabinoxylan (AX) and amylose tablets were purchased from Megazyme (Bray, Ireland). All chemicals, solvents and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade unless specified otherwise.

2.2. Density separation and pearling of wheat kernels prior to milling

Sprouted wheat kernels were separated based on density differences in NaCl solutions. Three NaCl solutions with different densities were used: either 1.15 g/cm^3 (250 g NaCl/L), 1.18 g/cm^3 (300 g NaCl/L), or 1.21 g/cm^3 (350 g NaCl/L). Upon transferring the kernels (100 g) into the NaCl solution and gentle manual stirring, segregation of the kernel population into subpopulations of kernels with lower (floating) and higher (sunken) density (as compared to the NaCl solution used) occurred immediately. Subsequently, the respective kernel fractions were collected, rinsed, dabbed with paper tissue and dried to the air. This way, the kernels were exposed to the salt solution for only a couple of minutes. The separation procedure was performed at least in triplicate. Lower and higher density kernels of the severely sprouted wheat batch were ground separately into whole meal using the Cyclotec 1093 sample mill (FOSS, Höganäs, Sweden).

To obtain sufficient material for milling, five batches of 400 g each of the mildly and severely sprouted wheat batches were separated in a salt solution with a density of 1.18 g/cm^3. Higher and lower density kernels were separately collected as described above. Subsequently, higher density kernels were conditioned to 16.5% moisture and milled into flour with a Bühler MLU-202 laboratory mill (Bühler AG, Uzwil, Switzerland). For the severely sprouted wheat batch, the effect of pearling the higher density kernels was also tested. The outer kernel layers (10% w/w) were removed by pearling for 28 s using a Satake batch debranner, type TM05 (Satake, Bredbury, UK). Subsequently, higher density and pearled kernels were conditioned to 14.5% moisture and milled into flour with the Bühler laboratory mill.

2.3. (Bio)chemical analyses

Moisture content was determined according to the AACC International method 44–15.02 (AACC International). Protein levels (N × 5.7) were determined using an adaptation of the AOAC official method 990–03 (AOAC, 1995) and an automated Dumas protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The Netherlands). The FN was measured in triplicate according to AACC International method 56–81.03 (AACC International) in an FN 1500 System (Perten Instruments, Hägersten, Sweden) and with a sample size of 7.00 g (14% moisture basis) in 25 mL deionized water. α-Amylase, free β-amylase, endoxylanase, and peptidase activities of whole meal and flour were determined and expressed in AU/g dry matter (dm), BU/g dm, EU/g dm, or PU/g dm, respectively, as described before (Olaerts et al., 2016a). All analyses were performed in triplicate and values were