Impact of Wheat Grain Selenium Content Variation on Milling and Bread Baking

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ABSTRACT

Selenium (Se) is an essential micronutrient in animals. High levels of Se can accumulate in wheat grain, but it is not clear how high Se affects milling or baking. Low and high Se grain from the same hard red winter wheat cultivar was milled and used for breadbaking studies and Se analysis. Mill stream yields from the low and high Se wheat were comparable, as were flour yields. The amount of total grain Se retained in the flour mill streams was 71.2 and 66.4% for the low and high Se wheat, respectively. Proportionally, Se content in the bran, shorts, and the first reduc-

tion flour stream in high Se wheat was higher by 13–20% compared to the low Se wheat. Flour quality parameters including protein content, ash content, and farinograph traits were similar in low and high Se flours, although high Se flour mill streams exhibited lower farinograph stability. Breadbaking evaluations indicated that high Se had a deleterious effect on loaf volume. There was no evidence of significant Se loss after breadbaking with either low or high Se flour.

Selenium (Se) was identified as an essential animal nutrient in the 1950s (Schwarz and Foltz 1957), and an abundance of research since then has elucidated its role in the prevention of animal diseases such as white muscle disease of ruminants (Combs 2001). A multitude of human health conditions also appear to be affected by selenium status of the individual; these conditions include cardiac, immune, and endocrine function and possibly behavioral and cognitive functions (Ryan-Harshman and Aldoori 2005; Li 2007; Rayman 2008). The Se status of human populations varies greatly, and it is estimated that Se deficiency afflicts as many as 1 billion people globally, particularly in certain regions of the world including parts of China (Combs 2001).

In addition to its role as an essential nutrient, the relationship between improved Se status and reduced cancer risk in humans has been studied extensively (Ryan-Harshman and Aldoori 2005; Gromadzinska et al 2008). While results of these studies can often be contradictory, the efficacy of selenium appears to depend on selenium status of the individual, the chemical form of selenium, and the specific cancer. Nonetheless, there is some evidence that in subjects with low selenium status, more intake of Se may reduce cancer incidence (Duffield-Lillico et al 2002). Thus, increasing the Se intake in human populations may improve health by ensuring that minimum daily levels of the nutrient are provided to protect against the occurrence of Se deficiency-related diseases.

The primary source of Se for humans is through the food chain, with a significant amount of Se derived from plant sources (Finley 2007). Indeed, in the United States, the largest single source of Se in diets is wheat products (Gerrior and Bente 2002). In contrast to animals, Se is not an essential nutrient for plants. However, Se is still absorbed by plants as an analog of the mineral macronutrient sulfur (Ellis and Salt 2003). Thus, plant Se content is largely determined by the amount of Se in the soil on which plants are grown, with other factors also playing a role (Gissel-Nielsen et al 1984). Thus, in regions of the world where soil Se is low, the crops grown on them are low in Se. Similarly, some regions harbor soils that are replete with Se owing to geological history, and crops grown on such highly seleniferous soils can accumulate inordinately high levels of Se (Finley 2007). In the United States, states in the northern Great Plains have regions with high soil Se levels (Kubota et al 1967). Wheat grain Se concentrations >60 μg/g have been reported from fields in South Dakota (Moxon et al 1943).

To achieve the greatest effect, dietary Se supplementation efforts should target crops that are already a major component of human diets. Wheat is just such a crop; it is grown around the world and is a primary staple crop in human diets. Further, wheat exportation and importation is an established global trade activity. Thus, wheat is an ideal crop to employ as a source of dietary Se and has been proposed as a source for large-scale biofortification efforts (Lyons et al 2003). For wheat producers who grow wheat on highly seleniferous soils, an opportunity exists to market wheat with atypically high Se levels ("high Se wheat") as a value-added trait. However, indepth analyses of the effect of high grain Se levels on wheat quality are not available. Such information is important because end use quality is an extremely important feature of wheat. This study sought to compare a range of quality attributes of a hard red winter wheat cultivar with typical ("low Se") versus high Se levels, and to compare the relative retention of Se in products made with these wheats.

MATERIALS AND METHODS

Grain Source and Physical Characteristics

Grain of the hard red winter wheat (Triticum aestivum L.) cultivar Nekota released by the University of Nebraska (Haley et al 1996) was used for this study. The grain was sourced from two locations in South Dakota. The low Se Nekota grain was obtained from the South Dakota Certified Seed Foundation and had been harvested from a field in Brookings, SD, located in the east central region of the state. The high Se Nekota grain was obtained from a ranch in central South Dakota, from a field known to produce high Se wheat due to intrinsic soil properties. Physical characteristics of grain including kernel test weight (lb/bu), size distribution, 1,000 kernel weight, hardness, ash content (14% mb), and protein content (12% mb) were measured according to AACC Approved Methods.

Milling Procedures

Forty pounds of cleaned grain sample was tempered to 16.0% mb, conditioned for 16 hr, and milled in a Bühler laboratory...
Selenium Analysis

The concentration of Se was determined in the individual flour mill streams, bran, and shorts fractions, PF, and in bread loaves baked with each individual flour mill stream and PF. Before analysis, samples of each flour mill stream and PF were dried, while samples of bread loaves were freeze-dried. Samples (=0.5 g) were weighed out into a 100-mL beaker, and batches of samples were prepared with three blanks and three standards (NBS 1567a Wheat Flour, National Bureau of Standards, Gaithersburg, MD). Sample preparation entailed addition of 10 mL of 40% (w/v) magnesium nitrate (Alfa Aesar, Ward Hill, MA), 10 mL of 16M nitric acid, and 2 mL of 6M hydrochloric acid (J.T. Baker, Phillipsburg, NJ). Samples were then covered with watch glasses, placed on a hot plate, and refluxed on a low heat setting for 24 hr. After 24 hr, watch glasses were removed and samples were heated to dryness. Samples were then placed in a muffle oven at 490°C for 14 hr, and subsequently dissolved in 10 mL of 12M hydrochloric acid and diluted to 25 mL with deionized water for analysis. The selenium content was determined by atomic absorption spectrometry and flow-injection atomic spectrometry (model 5100PC, Perkin Elmer, Wellesley, MA) with a hydride generator (HGAAS). Results are expressed on a dry weight basis as μg/g. Results for mill streams are means from triplicate analysis of each sample, while those for bread are means from evaluations of two replicate loaves.

RESULTS

Milling of High and Low Selenium Wheat

Whole grain physical characteristics of the low and high Se wheat grain were comparable with test weight, 1,000 kernel weight, whole kernel protein, and the percent of small kernels in the grain sample, all differing by <2% between the low and high Se grain samples (data not shown). However, there was a higher fraction of large kernels in the low Se wheat grain than in the high Se grain sample (71 vs. 62%).

Comparison of flour yields revealed that the relative yield for each mill stream was comparable between the high and low Se grain samples (Fig. 1). The flour mill streams comprising the largest and smallest proportions of both the low Se and high Se milled wheat were R1 (43.7%) and B3 (2.4%). The bran and shorts fractions combined represented 22.2 and 22.7% of milled product from the low Se and high Se wheat, respectively. Thus, the total flour yields (total milled product minus bran and shorts) for the low Se and high Se wheat grain were virtually identical (77.8 vs. 77.2%).

Selenium Content of Whole Grain and Mill Streams

Milling yielded six flour mill streams from each grain sample, as well as bran and shorts fractions that together represented essentially 100% recovery of the starting grain mass. Initial Se concentrations in the original grain samples were estimated from the weighted mean of the Se concentrations in each of the eight mill streams (Table I). Using this method, the low Se grain contained 0.53 μg/g Se, while the high Se wheat grain contained 7.56 μg/g Se. This method of whole grain Se estimation was deemed preferable to whole grain analysis because the latter method may vary due to the small number of seeds included in an individual analysis and accompanying heterogeneity in Se concentration among seeds, whereas the mill streams represent homogeneous samples for the entire 40 lb of grain samples used for the experiments.

The concentration of Se varied between flour mill streams for both the low and high Se wheat. In the low Se wheat, Se concentrations were 0.36–1.40 μg/g. The Se concentration of the comparable flour mill streams from the high Se wheat were 5.86–10.29 μg/g.

<table>
<thead>
<tr>
<th>Mill Streamb</th>
<th>Low Se (μg/g)</th>
<th>High Se (μg/g)</th>
<th>% Total Seed Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.53 ± 0.04</td>
<td>7.41 ± 0.30</td>
<td>9.0</td>
</tr>
<tr>
<td>B2</td>
<td>0.69 ± 0.08</td>
<td>8.87 ± 0.36</td>
<td>10.6</td>
</tr>
<tr>
<td>B3</td>
<td>1.40 ± 0.10</td>
<td>10.29 ± 0.55</td>
<td>6.4</td>
</tr>
<tr>
<td>R1</td>
<td>0.36 ± 0.04</td>
<td>5.95 ± 0.42</td>
<td>29.4</td>
</tr>
<tr>
<td>R2</td>
<td>0.47 ± 0.03</td>
<td>5.86 ± 0.39</td>
<td>10.7</td>
</tr>
<tr>
<td>R3</td>
<td>0.84 ± 0.11</td>
<td>6.31 ± 0.55</td>
<td>5.4</td>
</tr>
<tr>
<td>Bran</td>
<td>0.69 ± 0.03</td>
<td>11.81 ± 0.39</td>
<td>20.1</td>
</tr>
<tr>
<td>Shorts</td>
<td>0.64 ± 0.04</td>
<td>9.82 ± 0.28</td>
<td>8.4</td>
</tr>
</tbody>
</table>

a Values ± standard error from triplicate Se analysis of individual mill streams.
b1, first break; B2, second break; B3, third break; R1, first reduction; R2, second reduction; R3, third reduction.
µg/g (Table I). The mean Se concentration of the high Se flour, obtained from analysis of the PF, was >12-fold higher than that of the low Se flour (6.68 vs. 0.54 µg/g). Similar trends in the relative distribution of Se in the six flour mill streams were observed in both low and high Se wheat samples. However, a few notable differences in proportional Se content between pairs of flour mill streams were observed. In particular, this was most pronounced for B3 and R3, where the low Se mill streams had approximately twice as much of the overall seed Se as the comparable mill streams from the high Se wheat (Table I).

The proportion of grain Se in the bran and shorts fractions differed in low and high Se milled samples. For the low Se wheat, the bran and shorts exhibited Se concentrations (0.69 and 0.64 µg/g, respectively) near the mean Se concentration across the six flour mill streams (0.71 µg/g). In contrast, the Se concentration of the high Se bran and shorts (11.81 and 9.82 µg/g, respectively) was elevated relative to mean flour Se (7.45 µg/g) (Table I).

The proportion of total grain Se that ended up in the flour was 71.2% for the low Se wheat and 66.4% for the high Se wheat, while that ending up in the bran and shorts was 28.8 and 33.4% for low Se and high Se wheat, respectively.

**Flour Quality**

Flour quality parameters are dependent upon the efficiency of the milling operation. Quality parameters of individual flour mill streams and PF are shown in Table II. The PF ash content was 0.475 and 0.461% for the high and low Se wheat, respectively, a 3% difference. Likewise, PF protein content between the high Se (11.0%) and low Se wheat (11.3%) was essentially the same. Cumulative ash curves were also similar (Fig. 2). The variation in flour protein and ash content between the mill streams carries over to differences in farinograph properties (Table II), where ash as well as protein contents are associated with farinograph water absorption. The higher protein content of both low and high Se break flour mill streams translates to higher peak times, greater dough stability, and lower mechanical tolerance index than that ending up in the bran and shorts when paired with flour Se flour (Fig. 3). Farinograph peak times were not significantly different between individual low and high Se mill streams (paired t-test P = 0.11), with PF peak times being the same. While farinograph stabilities were not significantly different between low and high Se mill streams (paired t-test P = 0.067), they were uniformly lower than the low Se values (Table II).

**Baking Quality**

Each flour mill stream and PF from the high and low Se wheat grain was used for breadmaking studies. Bread loaves baked with flour from mill streams B1, B2, and B3 were of higher volume than bread baked with flour from mill streams R1, R2, and R3, for both the low and high Se flour (Fig. 3). These results are largely concordant with differences for flour quality traits between break and reduction mill streams. Despite the highest flour ash content, B3 loaf volume was not eroded. This was consistent across both high and low Se samples, with B3 bread loaves exhibiting the second highest loaf volumes among mill streams in both cases.

Comparison of low and high Se loaves indicated that the volumes of the high Se wheat loaves were frequently lower than those from the low Se flour (Fig. 3). The loaf volume of the high Se loaves was 10% less, on average, than loaves baked with low Se flour. A paired t-test of loaf volumes baked from the individual flour mill streams indicated that the difference in loaf volumes of high Se bread was significantly lower than that of the comparable low Se loaves (P < 0.005).

**Selenium Retention in Baked Bread**

To assess retention of Se in bread made from low Se versus high Se wheat flour, sections of individual bread loaves were lyophilized, ground to a powder, and then used for Se analysis. The bread loaf Se concentrations were then compared to concentrations of the flours used to make bread. Selenium concentrations in loaves did not differ significantly from concentration in the starting flours when compared by a paired t-test, in either low or high Se samples (Fig. 4).
To ensure that Se from components mixed with the bread dough before the baking evaluations was not confounding bread loaf Se results, Se concentrations were determined for each of these components. Results indicated that a small amount of Se (≈2 μg, data not shown) was introduced to each loaf from both yeast and milk solids. However, this amount is minor relative to the amount of Se contributed to each loaf from the flour, which was between 36 μg and 140 μg in the low Se loaves and between 470 μg and 1,029 μg in the high Se loaves, depending on the flour mill stream. Thus, the maximum Se loss from flour during breadmaking that could have been masked by the introduction of Se from nonflour sources was 5.6% in the mill stream with the lowest Se concentration. For the flours with higher Se concentrations, the potential masking effect ranged from <5% to as low as 0.2%.

DISCUSSION

Wheat enrichment with Se has been touted as a strategy to improve the health of human populations at risk of Se deficiency (Lyons et al. 2003). Direct comparative assessment of milling and bread baking quality and the relative retention in low Se versus high Se wheat products have not been reported previously. Our study provides insight into possible deleterious effects of high Se on wheat end use properties that should be taken into account during wheat processing.

Our use of the term “low Se” and high Se within the context of this study are relative. The Se concentration in the grain sample designated low Se was elevated relative to values reported elsewhere (Gissel-Nielsen et al. 1984; Adams et al. 2002; Lyons et al. 2005), but in the range of the overall mean Se concentration of U.S. wheats, including hard red winter wheat (Lorenz 1978; Hahn et al. 1981). The term “high Se” reflects an atypically high Se level obtained by growth on seleniferous soils, rather than a particular concentration threshold.

It is of particular importance to control for genotypic variation in quality evaluations because of significant differences in physical and quality characters between cultivars (Finney et al. 1987). Thus, we used solely high and low Se grain from a single hard red winter wheat cultivar (Nekota) for experimentation. In contrast, by necessity, the high and low Se Nekota grain for this study came from different locales in South Dakota where soils differ dramatically in Se content and result in dramatically different grain Se content. Thus, background environmental effects could have confounded our results. Interestingly however, our evaluation of a set of grain physical characteristics indicated that the elevated Se content in the high Se wheat had no apparent deleterious impact on them when compared to the low Se wheat containing 14-fold less Se.

The primary use of hard red winter wheat is for breadmaking. Flour yield from milling is an important quality character because it is a direct measure of the recovery of the most valuable component of the grain used in most food processing applications. Our milling comparison of the low and high Se grain indicated that they milled in a virtually identical manner, with flour yields differing by <0.8%. Thus, high grain Se concentrations did not compromise the recovery of flour.

Many flour quality parameters (flour ash, flour protein, farinograph dough strength traits) were similar in the low and high Se flour, suggesting that high grain Se does not broadly affect wheat milling quality when directly compared to low Se wheat of the same cultivar. One notable exception is farinograph stability. The difference between low and high Se wheat for this parameter approached biological significance and, importantly, all six high Se mill streams and patent flour exhibited lower stability than the low Se counterpart. This result suggests that high Se in flour may be detrimental to this particular parameter, which in turn could

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**Fig. 3.** Volumes of bread loaves baked from low and high Se Nekota flour mill streams. Means of two loaves ± standard error in bars. B1, First break flour; B2, second break flour; B3, third break flour; R1, first reduction flour; R2, second reduction flour; R3, third reduction flour; PF, patent flour.

**Fig. 4.** Comparison of Se concentrations in different flour mill streams and bread baked with each mill stream. A, Low Se flour and bread; B, high Se flour and bread. B1, first break flour; B2, second break flour; B3, third break flour; R1, first reduction flour; R2, second reduction flour; R3, third reduction flour; PF, patent flour. Bars represent standard errors for bread volume; standard errors for triplicate Se determinations on each flour mill stream are presented in Table II.
contribute to lower bread loaf volumes in the high Se samples. Evaluation of multiple wheat cultivars with contrasting low and high Se grain content will shed more light on this potential link between high Se and reduced dough stability.

Previous studies have examined the distribution of Se in milled wheat fractions (Moxon et al. 1943; Ferretti and Levander 1974; Lorenz 1978). However, it is not known whether this distribution is altered between wheat grain high in Se versus grain with more typical Se levels. Our results revealed an increased percentage of seed Se in the bran and shorts fractions from the high Se wheat compared to the low Se wheat, suggesting a difference in distribution of Se in the high versus low Se wheat grain. The potential cause of this difference is not clear. Despite this observation, the majority of the Se (71.5% in the low Se sample and 66.4% in the high Se sample) was retained in the flour of both milled samples, which is in agreement with previous studies (Moxon et al 1943; Ferretti and Levander 1974; Lorenz 1978). Because the majority of the Se in wheat grain is selenomethionine, a Se analog of the amino acid methionine (Wolf and Goldschmidt 2007), and the majority of the seed protein is in the endosperm, which becomes the flour during milling, this is concordant with biological expectations.

Ultimately, controlled breadbaking evaluations provide a final critical quality evaluation for hard red winter wheat flour. Larger loaf volume is desired because it suggests higher quality protein that provides the gluten network required to retain gas. We detected a consistent reduction in loaf volume from the high Se flour mill streams with reductions up to 18%, depending on the mill stream compared, with patent flour loaves differing by 10%. The differences in loaf volume could be due to an environmental effect associated with the wheat being grown in two different locations in South Dakota. But, given that most other grain agronomic and quality traits evaluated are very similar between the samples, this effect appears attributable to the difference in Se content. It is well-established that seed protein composition is a large determinant of breadmaking quality (Worland and Snape 2003). However, because this evaluation was conducted with the same wheat cultivar, the results indicate the possibility that replacement of methionine with higher levels of selenomethionine in seed proteins may alter the properties and lead to an erosion of physiological and functional protein properties necessary for dough development and larger loaf volume in bread. This hypothesis is supported by the previously noted observation that farinograph stability was uniformly lower in all of the high Se flour mill streams.

Various studies have been conducted to examine whether Se is retained in wheat after a variety of processing treatments, with contradictory results. According to Ferretti and Levander (1974), producing shredded wheat cereal did not reduce Se concentrations. Higgs et al (1972) found that boiling wheat cereal did not reduce Se content. Additionally, Se concentrations were not reduced in bread after baking or toasting (Lyons et al 2005). However, other studies have reported measurable losses of Se from wheat products. Heating wheat breakfast cereal at 100°C overnight reduced Se by ≈25% (Higgs et al 1972). A similar effect of high heat on Se loss from wheat flour was reported by Hakansson and Finley (1978). Thus, it appears that the method of processing can affect Se retention in wheat products. A significant proportion of wheat flour Se is selenomethionine (Wolf and Goldschmidt 2007), but the chemical species of Se comprising the rest of the grain Se are not well-characterized. If the chemical forms of Se in the non-selenomethionine fraction in high Se wheat are different than those present in low Se wheat, greater loss from high Se wheat products might occur during baking, for instance from volatilization of particular chemical forms of Se, which occurs in many plant species (Terry et al 1992). This has not, to our knowledge, been tested previously. We found that there were no significant differences in Se concentration between bread and the flour from which it was baked, for either the low or high Se wheat. This indicates that regardless of the chemical forms of Se present in wheat, they are not lost during exposure to thermal conditions encountered during baking; thus, initial flour Se concentration is a good predictor of bread Se concentration over a range of flour Se levels.

CONCLUSIONS

This study provides results of a detailed comparison of the effect of increased Se concentrations on a set of grain physical characteristics and milling and quality parameters of hard red winter wheat grain differing in Se content by 14-fold. Few differences in grain characteristics between high and low Se wheat grains were detected, and milling and flour yields from this grain were comparable. Similarly, flour quality parameters were comparable across low and high Se mill streams, with the exception of a trend toward lower farinograph stability in high Se flour. High Se wheat appears to exhibit modest shifts in the allocation of Se in various tissues of the seed, with Se relatively more abundant in the bran, shorts, and first reduction mill stream than found in the low Se samples. No evidence of Se loss during baking was detected across a wide range of flour Se levels, indicating that starting flour Se levels are a good predictor of final Se levels in bread. High Se wheat appeared to compromise bread loaf volume. However, high Se wheat will be blended with low Se wheat to a desired target Se concentration. For instance, a blend of 5% of the high Se patent flour in this study with flour containing a negligible amount of Se would result in a 0.33 μg/g Se final concentration. Consumption of food products containing just 100 g of this flour would provide ≈60% of the recommended daily allowance (RDA) for Se in adults, and would approach or meet the daily RDA for Se in children, depending on their age.

LITERATURE CITED


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