CHANGES IN WHEAT GRAIN DURING STORAGE AND EFFECTS OF STORAGE TEMPERATURE

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Abstract
Ten winter wheat, five each of 1BL. IRS and non-1BL. IRS types, were selected from a range of varieties grown at three different sites in Northern Ireland, to investigate the effects of storage and storage temperature on the in vitro viscosity (IW) of whole and ground grain. Wheat was stored as whole grain and also in milled form for a period of 24 weeks. Milled wheat was stored (pre-milled and stored, PM&S) at room temperature, whereas whole grain was stored at room temperature (RT, 20-25 °C), refrigerated (FR, 0-5 °C) and frozen (−20 °C). In vitro viscosity of pre-milled and stored wheat increased with time at two sites. Refrigerated whole grain showed the greatest reduction in IVV over the storage period, followed by grain at room temperature. Responses to storage were related to growth site.

Key words: wheat, grain, storage, storage temperature.

INTRODUCTION

Despite the popularity of wheat as a source of energy and main ingredient of poultry diets in the Romania, there exists ample evidence, which suggests that in some cases the expected levels of apparent metabolisable energy (AME) were not attained. Research indicated that arabinoxylans in rye and mixed-linked P-glucans in barley were responsible for the growth depression in poultry fed on these cereals. Annison obtained a negative correlation ($r = -0.91, P<0.001$) between AME values and the total non-starch polysaccharides (NSP) removed from wheat in the first two extractions, and water-soluble NSP of wheat was deemed to possess anti-nutritive activity. Austin et al. found that in vitro viscosity (IVV) was negatively related to soluble ($r = 0.61$) and total ($r = 0.82$) arabinoxylan. It was suggested that this relationship was a consequence of the 6 months storage effect on the wheat, brought about by the enzymes present in wheat.

The presence of endogenous amylases and proteases in wheat has been well documented and more recently evidence for the presence of arabinoxylase-nase, endogenous wheat arabinoxylan degrading enzymic extracts and also the presence of a pento-sanase inhibitor and a wheat xylanase inhibitor has emerged. Endogenous glycanases present in variable quantities and absent in some cases were thought to undergo activation during storage and hasten the breakdown of soluble NSP. Preliminary studies in this laboratory have indicated that stored milled wheat (at RT)
increased in IW values, whereas a growth site dependent increase or decrease was observed for grain stored at room temperature.

The amount of published literature on the subject is limited and the evidence conflicting. The present study was therefore carried out to investigate the effect of storage on the IVV of milled wheat at room temperature and whole grain stored at room temperature, under refrigeration and frozen over a period of 24 weeks.

MATERIALS AND METHODS

Wheat samples. Winter wheat grown by private farmers, harvested in 2010 were used in the study described. Ten varieties were each grown at three sites, Dropia (C), Alex (D) and Crisana (L) in Bihor county. Seedbeds were given a basal dressing of phosphorus and potassium, followed by three top dressings of nitrogen during March, April and May. Management also included the use of fungicides, growth regulator, insecticides and herbicides during the growing season. Sowing was carried out between the months of September and November and wheat harvested during August the following year. Grain was collected in bags and warm air (40 °C) dried to approximately 12% moisture.

Sample preparation. Two portions (approximately 1-5 kg) of each wheat were sub-sampled and placed in a plastic container and sealed with tape. One of these containers was stored in the laboratory at room temperature (RT, 20-25 °C) while the other was stored in a refrigerator (FR, 0-5 °C). At the same time, 200 g of each wheat was sub-sampled separately, cleaned of any extraneous matter such as straw, stones, etc. and then milled through a hammer mill fitted with a 0-75 mm screen and stored at room temperature in a screw top polypropylene container. This sample was measured for IW soon after milling and this result is referred to as 'initial IW and the sample itself will be referred to as pre-milled and stored (PM&S). In vitro viscosity measurements on the PM&S samples were carried out subsequently at 4 week intervals along with the stored grain samples. The process of sub-sampling (200 g), cleaning and milling (immediately prior to IW determination) was carried out on each of the stored (RT and FR) 1-5 kg samples at intervals of every 4 weeks for a period of 24 weeks. At the start of the experiment, 200 g of each wheat, was also stored frozen (—20 °C). This was thawed out at the end of the 24 week period and tested for IW.

Measurement of in vitro viscosity. The in vitro viscosity assay was carried out according to the method of Bedford and Classen with minor modifications relating to the speed of centrifugation and temperature control of the viscometer cup (described below). Duplicate 1-2 g of milled wheat
were weighed into polypropylene tubes with screw caps and 1.8 mL of pepsin solution (2000 U/mL dissolved in 0.1 M hydrochloric acid) was added to the sample. The wheat-enzyme mixture was vortex mixed vigorously and samples placed in a controlled water bath at 40 °C for 45 min. During this period samples were vortex mixed at intervals of 10 min. Then, 0.6 mL of pancreatin, activity 8x USP (8 mg/mL prepared in 1 M sodium hydrogen carbonate), was added to the tubes, vortex mixed and incubated for a further 60 min at 40 °C. Samples were vortex mixed at regular intervals as before, then removed and placed on ice to cool, transferred to 2 mL micro centrifuge tubes and centrifuged at 13400g for 8 min. An aliquot of 0.5 mL of the supernatant was used for the measurement of viscosity. The tubes were stored on ice while awaiting measurement. Viscosity measurements were conducted using Brookfield LVDV-II + cone and plate viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA 02172, USA) fitted with a CP-40 spindle. The viscometer cup was maintained at 20 °C by circulating water through the surrounding jacket.

In vitro viscosity measurements were carried out in batches of 36 plus 2 (quality control) samples. Each batch consisted of 2 (varieties) X 3 (sites) X 3 (storage types i.e. PM&S, RT, FR) X 2 (replicates). The entire set of samples, was analyzed in 5 days. Varieties were analyzed in the same order every four weeks, so that the time interval remained constant throughout the storage period.

Analysis of variance on the results was carried out using Genstat 5, release 3.

Table 1

<table>
<thead>
<tr>
<th>Weeks</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>164</td>
<td>149</td>
<td>136</td>
<td>124</td>
<td>134</td>
<td>130</td>
<td>131</td>
<td>14.2</td>
</tr>
<tr>
<td>Frozen</td>
<td>186</td>
<td>180</td>
<td>173</td>
<td>149</td>
<td>171</td>
<td>162</td>
<td>170</td>
<td>174</td>
</tr>
</tbody>
</table>

However, the responses to storage were dissimilar, with the PM&S-IVV at C being similar to or lower than initial IW in contrast to the situation at D and L. The C samples showed the greatest reductions in IW for RT, FR and frozen samples (approximately 20, 26 and 21% respectively compared with initial IW). Smaller decreases occurred with D samples and there was no consistent effect of storage at RT or in FR for the L samples which had the lowest initial IW (13 – 5 mPa.s).

Table 1 illustrates the significant interaction \( P=0.08 \) between the wheat types and the method of storage and storage temperature. As expected from previous study samples had lower IW but the difference in mean initial values was small (2 – 2 mPa.s). However,
the difference between initial and FR-IW was more pronounced in the samples (6 - 9 m Pa.s) than in the samples (2-7mPa.s), whereas PM&S, RT, and frozen samples showed a similar pattern for both.

The interaction between wheat types and storage time \((P= 0-016)\) is presented in Table II. Data used for this were the mean of RT and FR-IW values of stored grain, analyzed at 4-weekly intervals. The samples do not appear to have reduced in IW over the 24-week period with the notable exception of the value of 14-9 mPa.s obtained at 12 weeks. The lowest value (12-4 mPa.s) for the first samples group was noted for the same time period, possibly due to a problem with the viscometer. In contrast, the other samples group showed a definite trend for lowered IW (about 20%), which appears to have stabilized after 8 weeks storage.

Table 2 shows the interaction between wheat varieties (whole grain) and storage temperature. Both samples wheat groups (mean values across the three sites) showed similar and consistent reductions in IW values for both types of storage, with FR storage resulting in lower IW than RT storage. Of the 10 wheat studied, seven showed significant \((P < 0-001)\) differences between the initial IW and FR-IW and, of these, types (AT and AL) showed significant differences between initial, RT and FR-IW values.

<table>
<thead>
<tr>
<th>Variety code</th>
<th>Initial</th>
<th>IW mPa.s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>AA</td>
<td>8-8</td>
<td>8-0</td>
</tr>
<tr>
<td>AB</td>
<td>11-5</td>
<td>9-5</td>
</tr>
<tr>
<td>AP</td>
<td>17-2</td>
<td>15-3</td>
</tr>
<tr>
<td>AT</td>
<td>21-7</td>
<td>19-0</td>
</tr>
<tr>
<td>AL</td>
<td>22-6</td>
<td>18-1</td>
</tr>
<tr>
<td>AC</td>
<td>13-6</td>
<td>12-3</td>
</tr>
<tr>
<td>AX</td>
<td>15-2</td>
<td>14-1</td>
</tr>
<tr>
<td>AE</td>
<td>16-3</td>
<td>15-4</td>
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<tr>
<td>AD</td>
<td>23-6</td>
<td>23-1</td>
</tr>
<tr>
<td>BX</td>
<td>24-3</td>
<td>23-0</td>
</tr>
</tbody>
</table>

\(\text{a Varieties in first samples group. b varieties in bold type are from second amplest group.}\)

RESULTS AND DISCUSSION

It is now accepted, that soluble non-starch polysaccharides, in particular the arabinoylan (wheat) and P-glucan (barley) fractions are the main determinants of intestinal viscosity in broilers. Soluble arabinoyxans are responsible for generating high viscosities when in solution, with molecular weight of the macromolecule being a prime contributory factor. The inclusion of xylanase and P-glucanase enzymes counteracts the anti-nutritive effects of soluble NSPs by partial depolymerisation of the
polysaccharide with consequential reduction in in vitro/in vivo viscosity. The use of exogenous enzymes in wheat based poultry diets has thus become widespread and is a proven means of counteracting high in vivo viscosity and improving bird performance. Recent reports detailing the presence of endogenous wheat arabinoxylanase, xylosidase and endoxylanase and the ability of these flour extracts to progressively depolymerise wheat arabinoxylan could be a relevant factor in reported improvements of AME of new season wheat grain on storage.

All wheat used in this study were milled as described in the sample preparation section and a thorough breakdown and mixing of the grain components was achieved. Plant cell wall degrading enzymes such as endoglycanases (endo-O-glycosyl-hydrolases) are known to target internal bonds of polysaccharides. This type of enzymatic attack brings about a rapid reduction in the molecular weight and, hence, the viscosity of polysaccharides. Bonnin et al. showed that the mature wheat grain contained enzymes with a relatively broad spectrum of arabinoxylan-hydrolysing activities such as endoxylanase, arabinoxylanase and xylopyranosidase. Various milling fractions, obtained in their laboratory, demonstrated gradients of enzyme activity increasing from the starchy endosperm to the pericarp. Their results confirm that hydrolytic enzyme synthesis is mostly within the aleuronic cells of cereals. Milled grain, as used in this study, would therefore contain any endogenous enzymes distributed throughout the representative sample. It is not possible to say if the endogenous enzymes in the wheat used were inactivated in the presence of the low pH pepsin treatment. In retrospect, it would have been advantageous to have the wheat assayed for endogenous enzyme activity. However, the Bedford and Classes method for IW is designed to mimic avian digestion and the wheat is subject to conditions similar to that occurring in the broiler gut. Additionally, as all wheat samples were milled immediately prior to IW determination (the exception being PM&S samples, which were milled at 0 week and the same sample tested at 4 weekly intervals) and the same method used for determining IW, it would seem reasonable to assume that the reduction in IW in stored wheat is due to storage. It is possible that hydrolysing enzymes could be synthesised during storage and act during digestion. If it can be argued that endogenous enzyme inactivation were likely in a low pH environment used for sample digestion, then all samples will be affected and the effect across all the samples is therefore cancelled. If on the other hand the endogenous enzymes are active during the IW assay then hydrolysis of NSP may take place during digestion and not during storage. An alternate means of plant cell wall polysaccharide breakdown or controlled loosening in vivo is oxidative scission due to hydroxyl radicals generated in the presence of L-ascorbate under appropriate conditions. The
mechanism described above could be a factor in physiological plant processes such as germination, fruit ripening and abscission. It is debatable whether it could be relevant to the present study.

Overall the results of this present study indicate that stored milled wheat IW increased with time for wheat grown at two of the three sites. This is believed to be the first report of such an effect.

The increase in viscosity of PM&S samples may be the result of endogenous enzymes losing activity as a result of post-grinding storage and/or due to the oxidation of arabinoxylans with resultant cross-linking among the polymers. In vitro viscosity was lowest in refrigerated grain throughout, followed by grain stored at room temperature. It is not clear why IW of refrigerated grain should have fallen more quickly than that of grain at RT. On a hypothetical basis one would be inclined to assume that room temperature would benefit enzyme activity and therefore promote lower IW, but it may also result in a loss of enzyme activity.

However, comparison of the moisture contents between three sets of refrigerated samples and their PM&S (comparison with grain at RT not available) counterparts revealed between 4 and 10-1 g/kg increases in moisture for the refrigerated samples. Barley acid extract viscosities were lowered in the presence of excess grain moisture (40%).

However, these changes were attributed to a reduction in the soluble P-glucan content and not related to the activity of enzymes in the grain or the extent of fermentation during storage. Moisture levels in the present study seldom exceeded 13% and therefore it is difficult to attribute the lowered IW to increased moisture content.

Growth location is known to have a significant effect ($P < 0.001$) on various chemical parameters and IW. However, this is, to our knowledge the first report of a significant ($P < 0.001$) site effect on IW relating to storage. Earlier studies on the effect of storage on wheat in this laboratory have also revealed a site effect of stored grain and milled grain IW. In vitro viscosity of PM&S samples (stored at RT) harvested and milled in September 2010 increased on average by 36-4, 43-7 and 14-9% for C, D and L samples respectively by September 2010.

The values for stored grains (at RT) milled in 2010 were lower than those for the September 2010 milled grain period of 4 weeks from the initial IW (0 week) measurement (17-5mPa.s) a significant ($P < 0.001$) decrease was observed in the samples stored refrigerated (15-5 mPa.s) and after 8 weeks both PM&S and FR values were significantly different ($P< 0.001$) from the initial IW. Thereafter FR-IW remained relatively constant and RT values fell to similar levels. PM&S samples, on the other hand, maintained significant increases in IW during the storage period.
A significant \((P < 0.001)\) interaction was seen between growth site and type of storage (Table 1).

The analysis of meaned IW values for the initial sample (initial IW), PM&S, RT, FR samples and frozen grain over the 6 month storage period showed that the initial IW (17.5 mPa.s) was significantly higher \((P < 0.001)\) than the FR-IW (144 mPa.s).

The PM&S samples gave the highest IW value (18.7 mPa.s) with significant differences \((P < 0.001)\) being noted between these and grain stored at RT (15.8 mPa.s), FR (14.4 mPa.s) or frozen (15.8 mPa.s). The effect of type of storage over a period of 24 weeks on wheat IW is shown in Figure 1. After a period of 4 weeks from the initial IVV (0 week) measurement (17.5 mPa.s) a significant \((P < 0.001)\) decrease was observed in the samples stored refrigerated (15.5 mPa.s) and after 8 weeks both PM&S and FR values were significant different \((P < 0.001)\) from the initial IVV. Thereafter FR-IVV remained relatively constant and RT values fell to similar levels. PM&S samples, on the other hand, maintained significant increases in IVV during the storage period.

**Table 4**

<table>
<thead>
<tr>
<th>Site</th>
<th>Initial</th>
<th>PM&amp;S</th>
<th>RTC</th>
<th>FRd</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>19.2</td>
<td>18.4</td>
<td>15.3</td>
<td>14.3</td>
<td>15.2</td>
</tr>
<tr>
<td>D</td>
<td>19.8</td>
<td>22.8</td>
<td>17.9</td>
<td>16.6</td>
<td>18.4</td>
</tr>
<tr>
<td>L</td>
<td>13.5</td>
<td>15.0</td>
<td>14.0</td>
<td>12.3</td>
<td>13.9</td>
</tr>
<tr>
<td>First sample</td>
<td>16.4</td>
<td>17.1</td>
<td>14.0</td>
<td>9.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Second samples</td>
<td>18.6</td>
<td>20.4</td>
<td>17.6</td>
<td>15.9</td>
<td>17.4</td>
</tr>
</tbody>
</table>

\(a\) PM&S = Pre-milled and stored. \(c\) RT = Room temperature. \(b\) FR = Refrigerator.

**CONCLUSIONS**

Cultivar, harvest year and substrate solubility are known to determine the level of activity of endogenous arabinoxyylan degrading enzymes.

The evidence presented above clearly suggests that, in general, stored whole wheat IW tends to fall, whereas milled wheat at RT shows the opposite trend. However, growth site is a factor associated with the specific effect on IW in both cases. Storage temperature is also a factor related to the changes associated with whole grain storage, with low temperature \((0 - 5 \, ^\circ C)\) proving more effective in reducing IW than room temperature. The interactions of variety and storage temperatures (Table 3) are an indicator of the impact variety may have on the outcome of storage temperature on IW. However, none of the changes were large and it
would appear from this study (a) that storage effects are difficult to predict and (b) are not large enough to be likely to have much effect on the nutritive value of the wheat.

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