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Fibre and short-chain carbohydrate composition in rye varieties, novel industrial milling fractions and breads

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ABSTRACT

Rye is an important raw material of bread due to tradition and its favourable nutritional and technological qualities. Despite the beneficial fibre composition, a special group of short-chain carbohydrates, the so called FODMAPs (fermentable oligo-, di-, monosaccharides and polyols) may cause problems for patients with irritable bowel syndrome. The aim of our work was to investigate the non-starch carbohydrate (dietary fibre compounds, short-chain carbohydrates) composition of rye varieties, and of their novel milling fractions obtained from industrial milling trials and test loaves made from them. Regarding fibre and short chain carbohydrate composition, rye varieties did not show significant differences. In new subfractions, fibre and FODMAP composition were described, among profiles most of them differ from commonly used flours, independently from variety. The yeast fermentation and baking caused a decrease in water-extractable arabinoxylan content, at the same time increased the substitution pattern of water-extractable arabinoxylans. Furthermore, breadmaking process decreased the fructan content, and therefore increased

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the fructose level, thus modifying the short-chain carbohydrate composition. Based on our knowledge, this research is among the first ones investigating the fibre and short-chain composition of rye from the seeds to the consumable final products.

KEYWORDS

rye varieties, novel milling fractions, rye bread, arabinoxylans, short-chain carbohydrates, FODMAPs

1. INTRODUCTION

Rye is used for bread baking traditionally mainly in Northern Europe. It is easy to grow due to its low agronomical needs, good resistance to diseases and abiotic stress factors. In addition, rye grain has high fibre content, offering many nutritional benefits into the human diet (Hansen et al., 2004; Andersson et al., 2014).

The macro component composition of rye grains is similar to that of wheat. The crude protein content of the whole rye is between 12–15%. The lipid content of both rye and wheat ranges from 2 to 3%, and the ash content is around 2%. From the viewpoint of food safety, secalin and secalinin proteins of rye are also responsible for triggering hypersensitivity reactions such as celiac disease, non-celiac gluten sensitivity, and wheat allergy (Békés and Wrigley, 2015; Schalk et al., 2017). The total carbohydrate content of rye (71–87%) is almost the same as wheat, but the differences in composition cause the divergence between varieties. The most abundant polysaccharide is starch with a range from 58 to 64% (Hansen et al., 2004; Buksa, 2018).

From nutritional point of view, the main benefit of whole grain rye is the relatively high dietary fibre (DF) content (total DF 14.7–20.9%, water-soluble DF 3.4–6.6%) (Hansen et al., 2004; Ward et al., 2008). As in almost all cereals, the arabinoxylan and β-glucan are the most important non-starch polysaccharides in rye. Arabinoxylans are composed of a chain of β-D-xylopyranose residues, connected by β-1,4-glycosidic linkages, which can be substituted at C3 and/or at C2 position by α-L-arabinofuranosyl residues. Based on extractability by water, water-extractable (WEAX) and water-unextractable arabinoxylans (WUAX) are distinguished, and the sum of them gives the total arabinoxylan content (TOTAX). In the parts of rye white flour and bran, the WEAX contents are nearly 1%. The TOTAX content in rye flour varies between 3.1 and 4.3%, in bran it can be four times higher, the range is 12–15% (Shewry et al., 2008). The composition of arabinoxylan – determined by the amounts and ratio of monomers, ferulic acid content, crosslinks *via* ferulic acid, size of macromolecules, etc. - is different in endosperm, aleurone and seed coat parts, moreover, they also depend on the variety and environmental effects. The arabinoxylan content and composition influences the technological properties and the end-product quality (Buksa et al., 2010; Németh and Tömösközi, 2021). However, the raw material is rarely tested for several varieties along with the end products. Andersson et al. (2009) investigated the variability of the arabinoxylan composition of some rye varieties' wholemeal flour, but only one product made from a single variety was tested.

Other important fibre components in rye are the β-glucans. These non-starch polysaccharides commonly occur in the endosperm and aleurone cell walls. The β-glucan content of rye kernel varies between 1 and 3%. It also affects the technological and nutritional value of the raw materials and products (Andersson et al., 2014).

The determination of the composition of short-chain carbohydrates (SCC, Dp60>) is analytically complex and their nutritional assessment is divisive. Among cereals, rye contains the highest level of fructans (3.6–6.4%), which belong to dietary fibres. However, individuals suffering from e.g., irritable bowel syndrome (IBS) should avoid their consumption and have to follow a strict low-FODMAP diet (Gibson and Halmos, 2019; Atzler et al., 2021). Fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are also components of FODMAPs (Benkeblia, 2013). Studies have mainly investigated the fructans, FOS, and GOS content of rye products (soft and crispy bread, crackers, etc.), some of them also examined the wholemeal rye flours (Biesiekierski et al., 2011; Ispiryan et al., 2020). In several studies the genetic variability of mono- and disaccharide composition were also investigated (Kaur et al., 2021; Németh and Tömösközi, 2021).

Short-chain carbohydrate compositions can be found in the literature, but there is very little information about FODMAP variability. Generally, data are given only for commercial rye flours and different products made from them (Ispiryan et al., 2020; Pitsch et al., 2021; Schmidt and Sciurba, 2021). However, there is no study available examining the SCC and FODMAP variability together with fibre components in rye varieties, milling fractions and in the main rye product, bread.

In this study we aimed to characterise the fibre and short-chain carbohydrate composition (including FODMAPs) of rye varieties and novel milling fractions, as well as to investigate the effect of harvest year and processing (breadmaking) on these parameters.

2. MATERIALS AND METHODS

2.1. Sample collection

Dankowskie Diament (referred as Diament later), Ryefood, and Wibro rye varieties are widely used for agricultural production in Hungary. The properties of these three varieties and a commercial rye of average quality parameters were examined. The three varieties were grown at the same location (Galgahévíz, Hungary) by GalgaAgrár Ltd. in 2019. Diament and Ryefood from harvest year of 2020 were also investigated.

Conventional wholemeal flour (Fraction 1), white flour (Fraction 2), bran (Fraction 3), and new experimental rye milling fractions (Subfractions 1–6) obtained from industrial milling trials described by Jaksics et al. (2022) were investigated. The commercial rye was a mixture that year from rye grown in the country, so this sample well represents the average quality found in industrial rye milling practice.

2.2. Baking test procedure

The small-scale baking test was adopted from Németh et al. (2019). The ingredients were 50 g (Fraction1, -2) or 40 g (Subfraction1, -2, and -5) flour (14% moisture), 1.4% dry yeast, 1.5% salt, 1.5% sugar. The added water was determined according to the water absorption defined for the different dough consistencies during the mixing measurements. All ingredients were mixed by Farinograph-E (Brabender, Duisburg, Germany) for 5 min, and then the mass of dough equivalent to 45 g of flour/doughs were removed and slightly rounded by hand. Dough pieces were put in small-scale baking pans and proofed for 40 min at 30 °C and 85% relative humidity.

Finally, loaves were baked for 15 min at 210 °C. Baking trials were performed in triplicate measurements. The samples were freeze dried with a Christ Alpha 1-4 LDC-1M freeze dryer and were ground for further analysis with a Retsch Grindomix GM200 Knife Mill for 10 s at a speed of 800 r.p.m.

2.3. Determination of dietary fibre, β-glucan, and fructan content

The soluble (SDF), insoluble (IDF), and total (TDF) dietary fibre contents were measured according to the AOAC (1995) 991.43 method using Fibertec1023 (Foss-Tecator, Sweden). β-glucan content of the samples was determined by enzymatic colorimetric method according to AACC (2010), Method 32-23.01. The fructan content was measured by Megazyme Fructan HK Assay. The sample preparation was carried out with a minor modification, reducing the required sample amount to 200 mg (Megazyme, 2020).

2.4. Arabinoxylan quantification

The total and the water-extractable AX (TOTAX and WEAX) contents of the rye fractions were measured separately by gas chromatography (GC) using pre-column derivatisation. Sample preparation was described in detail by Török et al. (2019).

2.5. Determination of short-chain carbohydrate composition

The applied methodology for measuring small molecular weight carbohydrates (including FODMAPs) based on Muir et al. (2009) with minor modifications. Monomers and sugar alcohols were separated by a ligand exchange column (a). The DP2-DP4 oligomers (sucrose, maltose, trehalose, kestose, raffinose, nystose, and stachyose), were measured by HILIC column (b). The sample preparation was executed according to Muir et al. (2009)., with the only modification of sample amount from 1 g to 100 mg.

The HPLC apparatus was an Agilent 1260 Infinity II Prime LC. The data analysis system was OpenLab (Agilent). An Agilent Hi-Plex Ca (Duo) 8 μm, 6.5 × 300 mm with HPLC Guard Column Hi-Plex Ca (Duo), 8 μm, 7.7 × 50 mm used for method (a). The mobile phase consisted of HPLC-grade filtered water (Millipore). The pump flow rate was 0.6 mL min^{-1}, the column temperature was 80 °C, N_2 gas flow was 1.6 NSL, the evaporator temperature was 50 °C, the nebuliser temperature was 80 °C, and the injection volume was 20 μL. For the method (b) an Agilent Carbohydrate 5 μm, 4.6 × 150 mm column with Agilent Cartridge Guard-Column, Zorbax NH2 5 μm, 4.6 × 12.5 mm was used. The mobile phase consisted of 82% ACN. The pump flow rate was 1 mL min^{-1}, the column temperature was 55 °C, N_2 gas flow was 2 NSL, the evaporator temperature was 55 °C, the nebuliser temperature was 55 °C, and the injection volume was 20 μL.

2.6. Statistical evaluation

The analytical results were statistically evaluated by Pearson's correlation analysis, LS Means, ANOVA using TIBCO Statistica 14.0.0 (TIBCO Software Inc., Palo Alto, USA). Experimental factors were considered as random factors (Variety, Harvest year, Fractionation) in the analysis.

3. RESULT AND DISCUSSION

3.1. Characterisation of short-chain carbohydrate and fibre composition in wholemeal flours of rye varieties

The wholemeal flours of three varieties and commercial rye samples from 2019 harvest year were investigated. The dietary fibre results have already been published by Jaksics et al. (2022). These results are used here for comparison with the detailed fibre composition. Among the varieties, Ryefood had the highest total dietary fibre value (17.18%), while Diament had the lowest one (15.73%). The soluble dietary fibre content varied in a very narrow range, from 2.60 to 2.83%. The water-extractable arabinoxylan content of the wholemeal flours showed no significant differences, ranging from 1.92 to 1.99%, which is lower than reported in the literature (3.4–6.6%) (Kaur et al., 2021; Németh and Tömösközi, 2021). In contrast, the water-unextractable and thus the total arabinoxylan content moved in a wide range. The lowest TOTAX value was recorded by commercial rye (7.31%) and the highest one by Wibro (8.23%). Arabinoxylan substitution ratios of the samples were similar (TOT-A/X: 0.58–0.64; WE-A/X: 0.64–0.68). The β-glucan content of varieties showed low variability (2.01–2.31%). Wibro and Ryefood varieties had similar fructan values (4.40, 4.43%), while that of Diament was significantly lower (3.53%). These were lower than the values found in the literature (4.5–6.4%) (Kaur et al., 2021; Németh and Tömösközi, 2021). Among the oligosaccharides, the amount of DP4 nystose varied in a wider range (0.43–1.06%). The raffinose and kestose content of the varieties were similar and varied in narrow ranges (1.24–1.29% and 1.00–1.17%, respectively). The trehalose disaccharide (0.68–0.75%) took much lower values than maltose (2.00–2.60%) and sucrose (1.93–2.43%). None of the varieties had an excess of fructose (0.09–0.21%) compared to glucose (0.11–0.23%). The amount of galactose was below the limit of detection in all cases.

3.2. Characterisation of short-chain carbohydrate and fibre composition in white flour and new milling fractions

Milling experiments with three varieties and a commercial rye sample were fulfilled in 2019 and published by Jaksics et al. (2022). Similar trends were found for all varieties, therefore the compositional characterisation of white flour and experimental subfractions is presented here through the results of the commercial rye samples (Fig. 1A). Approximately half value of total dietary fibre was measured in white flour (8.34%) compared to wholemeal. SDF ranged from 2.17 to 6.07%. Subfraction6 had similarly low dietary fibre content as white flour. TDF content of subfractions 1-4 were similar to wholemeal. The result of Subfraction5 was close to bran value (34.43%), which was the highest. In the case of soluble dietary fibre results, white flour was the lowest (2.17%) again, but Subfraction5 (6.07%) was significantly higher than bran and Subfraction2. The total arabinoxylan content ranged from 4.07 to 15.09%, which means that the Subfractions were positioned between the white flour and bran values. The water-extractable arabinoxylan results for wholemeal, Subfraction4, and white flour were very close to each other (1.97–2.07%). Subfractions 1, -2, -3, and -6 also formed a group (2.41–2.75%), and Subfraction5 was the significantly highest with 3.91%. One of the possible explanations for this is that this subfraction may contain the highest amount of aleurone layers. The β-glucan contents of the

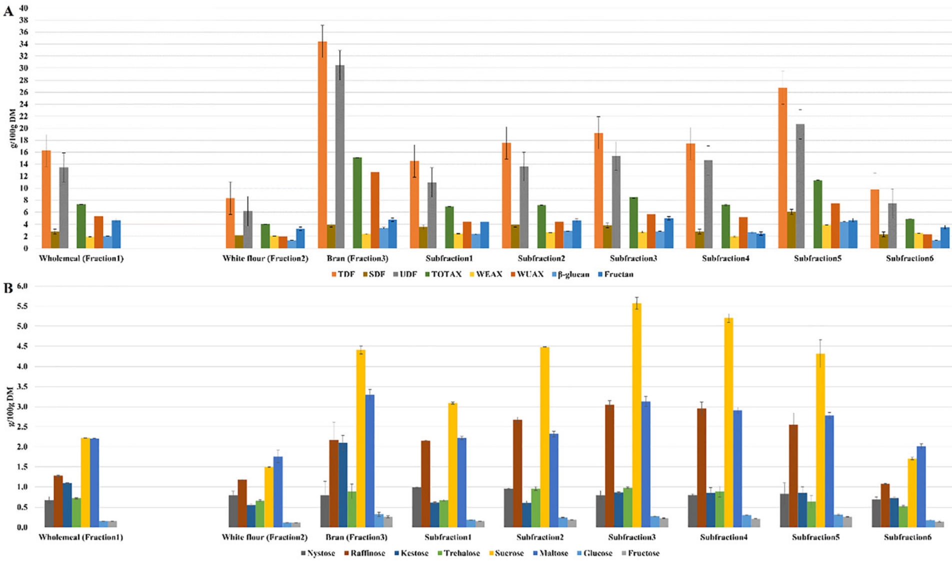

Fig. 1. Dietary fibre (A) and short-chain carbohydrate (B) composition of experimental fractions obtained from industrial milling of commercial rye samples in harvest year 2019

Fraction2 (1.35%) and Subfraction6 were lower than the wholemeal. Subfraction1-4 ranged between 2.40 and 2.91%. Bran had high β-glucan content (3.40%), but Subfraction5 was significantly higher at 4.41%. In the case of fructans, Subfraction4 (2.46%) had significantly lower contents than white flour and other fractions. Subfraction3 (5.04%) had the highest fructan content.

The composition of short-chain carbohydrates below DP4 in the fractions is shown in Fig. 1B. The composition of these components largely depends on the condition and properties of the grain at the time of harvest (enzyme activity, grain moisture content) and storage. Thus, the results are only indicative. The variability of nystose in the milling fractions was quite narrow (0.68–0.98%). In contrast, the raffinose (Subfraction6 and -3 0.87–1.06%) and kestose (Fraction2 and -3 0.56–2.10%) contents varied in a wider range. Regarding disaccharides, the variability of trehalose between fractions was narrow (0.53–0.98%). In contrast, the content of maltose (1.76–3.30%) and sucrose (1.49–5.57%) varied in a wider range. White flour had the lowest fructose and glucose contents, both around 0.12%. The highest values were obtained by bran and Subfraction5, uniformly 0.32% glucose and 0.26% fructose.

In the following, the characteristic differences of varieties will be discussed. Dietary fibre differences between varieties resulted from the variability of individual fibre components. The WUAX and TOTAX values of Ryefood were lower in bran and new fractions compared to the others. The Diament variety was characterised by a higher WEAX content. In terms of β-glucan content, Diament behaved uniquely. Varietal characteristics can be observed in the fructan results, so Diament generally had the lowest and Wibro had the highest content.

3.3. The effects of harvest year on short-chain carbohydrate and fibre composition in fractions – stability studies

The varieties Wibro and Diament were grown in 2019 and 2020, and the milling experiments were carried out on the samples grown in both years (Supplementary Material 1–2, to be found at the server of Publisher). Therefore, we had the opportunity to investigate both the effect of a complex environmental factor (harvest year) and the varietal effect on the carbohydrate composition of rye milling fractions. The harvest year affected the non-starch carbohydrate composition, but the effect of harvest year on the fractions was different. The most significant changes appeared in the bran fraction, i.e., the WUAX content was lower by 15% in 2019 compared to 2020, in contrast the fructan content that was higher by 30%. Different harvest year effects were identified in the carbohydrate pattern of new subfractions. The quantitative ratio of Diament and Wibro reversed regarding the SDF, β-glucan, and fructans of white and wholemeal flours. In most cases, there was a drop in dietary fibre and its components in the 2020 harvest year compared to 2019. On the other hand, the fructan content increased in 2020.

The ANOVA provides an opportunity for joint assessment of variety, harvest year, and fractionation factors and their interactions (Fig. 2) (Supplementary Material 3). The total deviation of the estimated variance components is illustrated in the pie charts in case of random factors. The effect of fractionation appeared with all components. In the case of soluble dietary fibre (Fig. 2A), this represented 57%. In addition, the interaction of the three factors (variety × harvest year × fractionation) was significant, followed by the variety effect (13%) and the harvest year (4%). The harvest year factor has not affected the degree of arabinoxylan substitution, nystose, kestose, and sucrose contents (Fig. 2D). The raffinose content was the most influenced by the harvest year effect (29%). The effect of genetics was not considerable for many components, but due to the low number of varieties, this is not authoritative. From the point of view of the target-specific selection, the combined effect of variety × fractionation would be interesting (Fig. 2B and C). This effect appears just in a few components (i.e., trehalose, fructan, glucan, and WEAX). The variety × harvest year effect was generally decisive for short-chain carbohydrates (15–32%), i.e., fructose, less for fibre components (0–11%). The harvest year × fractionation affects fructan content (26%) (Fig. 2C). The interaction of the three factors influenced the development of all components except WE-A/X.

3.4. Investigation of short-chain carbohydrate and fibre composition of rye breads made from experimental milling fractions

Baking trials were carried out from five flour fractions of the three varieties from 2019 harvest year: wholemeal (Fraction1), white flour (Fraction2), and three new flours (Subfraction1, -2, -5) (Supplementary Material 4). We experienced almost similar results and trends for all varieties as in case of the flours earlier, therefore as an example, Fig. 3 shows the results of Diament's bread. The total dietary fibre contents of wholemeals' breads were (15.85–17.70%) similar to the literature (18%) (Andersson et al., 2009). The TDF in white flour's bread was around 10%. Subfraction2 of Diament and Wibro almost reached 20%, and Subfraction5 surpassed both of them (22.63–23.63%). The SDF values of the wholemeal breads (2.88–3.46%) were lower than those found in the literature (3–7%) (Andersson et al., 2009; Buksa et al., 2010). Subfractions1 and -2 breads had higher SDF values than the wholemeal breads, moreover Subfraction5 only

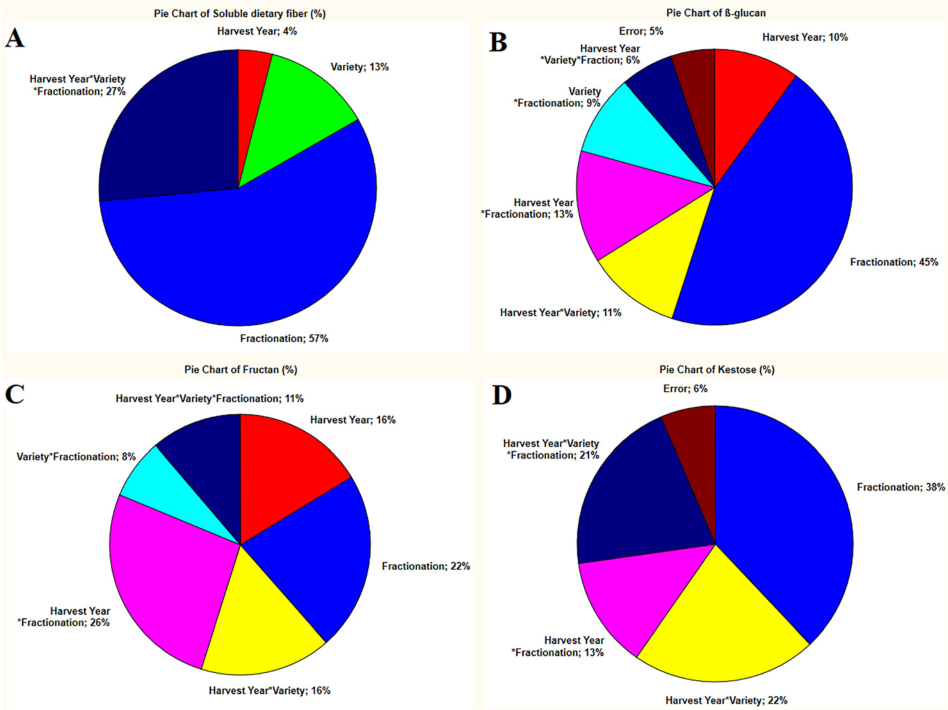

Fig. 2. Investigation of the effect of random factors (Variety [3], as genetic factor, Harvest year [2] as environmental factor, Fractionation [9] as milling process and their interactions) on the content of some individual non-starch carbohydrate components in rye milling fraction: soluble dietary fibre (A), β-glucan (B), fructan (C), kestose (D) (ANOVA, Pie charts show the variance of components. There were no biological or technical replications, there were two parallel measurements except for fructan)

ranged between 6.89 and 7.29%. The TOTAX results showed a similar trend as the TDF values. The wholemeal breads (8.94–9.43%) took higher values than the literary 8.5%. TOTAX values of the breads made from white flours were around 5%. TOTAX values of the breads from Subfraction5 were more variable (11.92–13.76%). The WEAX values of the breads made from Fraction1 and -2 were almost the same (1.17–1.27%), which is much lower than the value found in the literature (2.8%) (Andersson et al., 2009). On the other hand, the new subfractions had wide variability (0.81–2.81%), highest WEAX content was found in Ryefood's Subfraction5. The results of white bread were around 1.4%, Fraction1 2.25%, Subfraction1 2.6%, and Sufraction5 around 4.0%. In the β-glucan results only Subfraction2 showed wider variability (2.82–3.41%) among the breads of the three varieties. The results of Fractions1 and -2 were between 2.72 and 3.29%, Subfractions were between 3.66 and 4.20%. In any case, they were higher than any literature data found (Andersson et al., 2009; Rakha et al., 2010).

The short-chain carbohydrate composition of wholemeal and white flour bread was similar. Considering the short chain carbohydrate composition of white and wholemeal breads, no stachyose was detected, the raffinose contents were similar to the literature (0.20–0.39%)

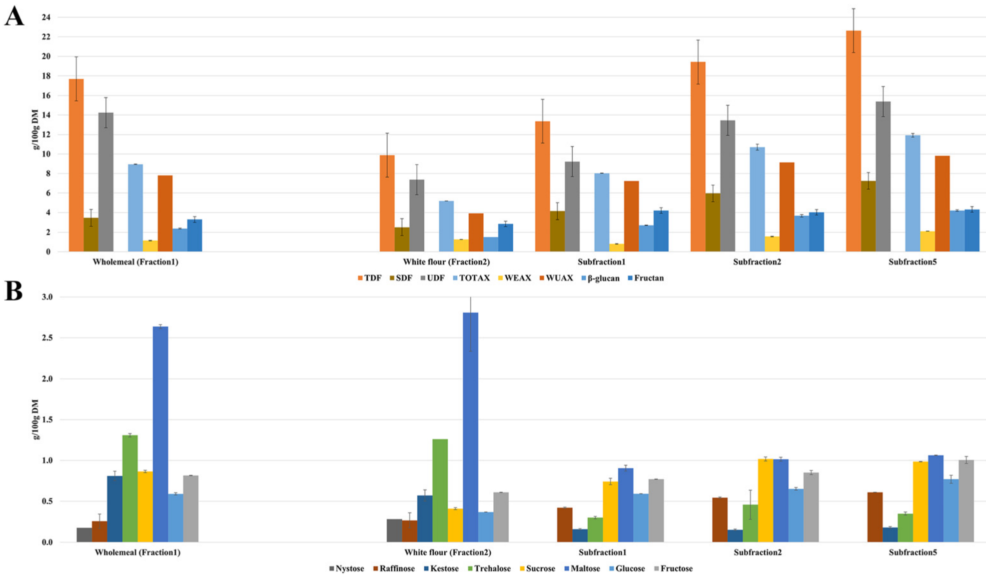

Fig. 3. Fibre (A) and short-chain carbohydrate (B) composition of breads made from experimental rye milling fractions (rye variety: Dankowskie Diament)

(Biesiekierski et al., 2011), but the new fractions showed higher values and were in a relatively wider range (0.42–0.66%). Kestose results ranged widely, it was generally true for all subfractions' bread that they had much lower kestose content (around 0.2%) than the breads made from traditional factions [Fraction1 (0.9%), Fraction2 (0.5%)]. A similar trend could be observed for trehalose and maltose. The glucose data of whole wheat breads corresponded to the literature (0.6%), while the fructose values were multiple times higher than those reported in the literature, ranging between 0.6 and 1.1% (Schmidt and Sciurba, 2021).

3.5. Baking effects on the short chain carbohydrate and fibre composition of the rye varieties and their products

The effects of processing (yeast fermentation and baking) are illustrated on LS Means diagrams (Fig. 4) (Supplementary Material 5). No change occurred in cases of TDF and β-glucan contents. The products had higher TOTAX values than the raw material (Fig. 4A). The reason for this is not clarified and it has to be elucidated in further studies. On the other hand, the water-extractable arabinoxylan content significantly decreased in all cases (Fig. 4B). It seems that during the fermentation and mainly during the baking, the structure of WEAX changed resulting decreased solubility. The degree of substitution of the total arabinoxylan content increased in breads made from wholemeal and white flour but decreased in Subfractions compared to traditional flours. The degree of substitution of WEAX in the products increased in all cases. It can be explained with the higher degree of substitution characteristic of unchanged WEAX polymers. The content of fructans was lower in the products than in the raw material (Fig. 4C), which means that the

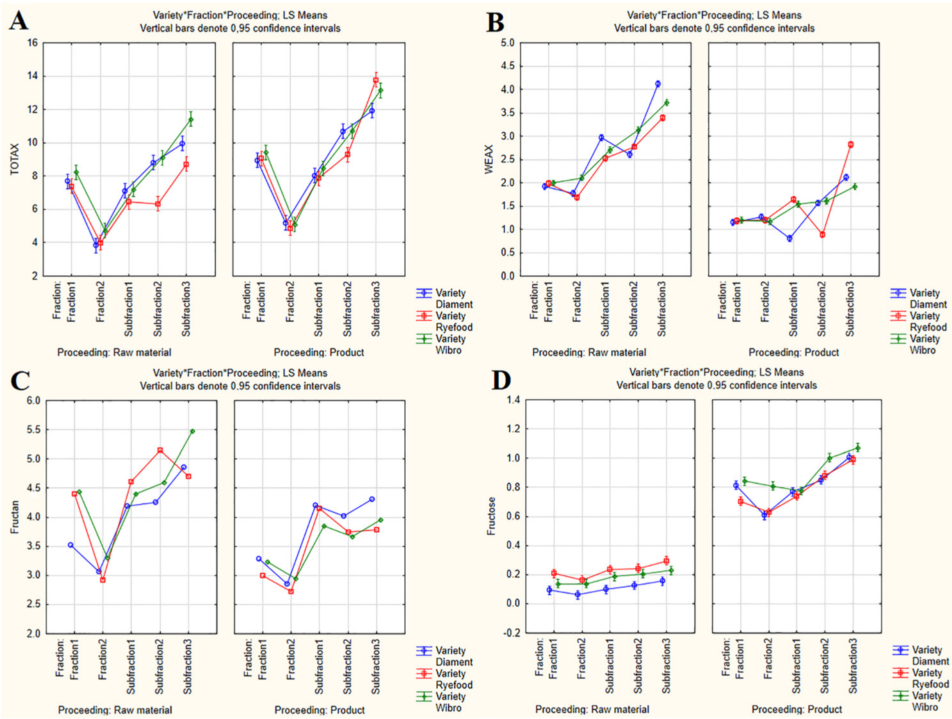

Fig. 4. Comparing least squares means results of raw materials and products in some components. TOTAX (A), WEAX (B), fructan (C), fructose (D)

polymers were hydrolysed during fermentation. Accordingly, the fructose level in the products increased (Fig. 4D). The amount of glucose in the products also increased, but to a lesser extent than fructose. Significant reductions were observed in the short-chain carbohydrates (di- and trisaccharides) levels of the products compared to the flours.

4. CONCLUSIONS

In this study, fibre and short-chain carbohydrate composition were examined in rye varieties, in special milling fractions, and experimental bread products. Our results show that fractions with significantly different fibre and short-chain carbohydrate composition can be identified from rye milling process. Regarding the rye flours, the composition of the fractions is primarily influenced by the applied milling operations, and only a minor effect of variety and harvest year was identified. The composition of non-starch carbohydrates in breads made from fractions changed as the result of short fermentation and baking. We found that the dietary fibre content did not change significantly, but their compositional changes, especially the decrease in quantity and the change in the degree of substitution were seen in WEAX, needs further explanations. The fructan content also decreased during bread making process, accordingly the increase of fructose was measured. The reasons can be related to the fermentation processes, enzymatic hydrolysis, changes in the proportions of monomers, and complex formation, which might take place under the influence of heat treatment (i.e., baking).

From nutritional point of view, Subfraction5 looks promising due to its high water-extractable and total fibre content. The Ryefood kept its high soluble fibre content particularly well during baking and had the lowest FODMAP composition of the three varieties. Diament seems to be a more stable variety in terms of several components, but we cannot say this for sure depending on two years. In addition to higher fibre content, the FODMAP composition was also higher in Diamant than that in Wibro. However, due to the total FODMAP content, the investigated fractions of the rye varieties cannot be included in the low FODMAP diet. Despite this, it can be used for adding to food to increase the fibre content due to its favourable high glucan and arabinoxylan contents.

Overall, we were among the first ones who describe the variability of the NSP composition of rye varieties, their conventional and new experimental rye fractions, and their products. Furthermore, we investigated the effects of milling and bread making processes. Our applied methods and achievements can be used for the development of health-supporting rye-based raw materials and products, and partly for understanding the molecular processes behind the phenomena.

Conflict of interest: The last author, S. Tömösközi, is a member of the Editorial Board of the journal. Therefore, the submission was handled by a different member of the editorial board, and he did not take part in the review process in any capacity.

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SUPPLEMENTARY MATERIALS

Supplementary data to this article can be found online at https://doi.org/10.1556/066.2022.00221.

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