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The Potential of Common Cereals to form Retrograded Resistant Starch

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Abstract: Resistant starch (RS) has been recognised as a functional fibre with many health-promoting effects. RS exists in four forms – RS_1, RS_2, RS_3, and RS_4. The RS_3 type is generated by amylose retrogradation typically resulting from food processing procedures. The aim of this study was to demonstrate the potential ability of six agriculturally important cereals to generate type RS_3 resistant starch after retrogradation of their amylose. In comparison with all tested cereals, the statistically significant highest level of RS_3 (5.28% ± 0.68) was detected in triticale, mainly in the Pinokio, Presto, Tricolor, and Kendo cultivars. Significant highly content was also found in rye (4.93% ± 0.73), especially in Selgo, Esprit, Dankowskie Nowe, and Apart cultivars, in comparison with wheat, spring and winter barley, tritordeum and oat. There were unsignificant differences between triticale and rye in RS_3 levels. Wheat contained less RS_3 (3.87% ± 0.55) in comparison to triticale and rye. The best wheats with this trait were the Athlet, Boka, Trane, Versailles, and Torysa cultivars. The content of RS_3 in barley was not high (2.35% ± 0.45 in winter barley, 2.51% ± 0.25 in spring barley), similar to tritordeum (2.26% ± 0.36). The RS_3 content in high amylose barley mutant Glacier was two-fold higher than in other tested barley cultivars ($P < 0.01$). Only minimal level of RS_3 can be generated from oat seed starch (0.41% ± 0.09). These results indicate that plant species as well as crop cultivar screening are important for the identification of suitable natural sources of resistant starch. Concerning the production of functional foods, it is important to recognize that valuable bread cereals such as wheat and rye are superior sources of this type of dietary fibre and are highly beneficial to the human health. However, it is advisable to continue for other years in these annual results and localities too.

Keywords: resistant starch; wheat; barley; rye; triticale; tritordeum; oat

The reduction of food carbohydrate intake and low-carb diet has increased the interest in products containing resistant starch (RS) as a low-carb ingredient. RS has been recognised as a source of functional fibre with an important role in the physiology of digestion. Due to its physiological effects, RS has also been considered a prebioticum (Annison & Topping 1994) with multiple health-promoting effects. Metabolites which arise during its fermentation, particularly short-chain fatty acids, are necessary for normal physiological bowel function (Silvester *et al.* 1995). Butyrate resulting from this process is an important source of energy for colonocytes and also activates cell proliferation (Mortensen & Clausen 1996). RS represents a barrier with both protective and nutritive functions, protecting against the development of colorectal cancer and aiding in the deterrence of ulcerative colitis (Hill 1995). Foods abundant in RS have a low (GI) glycemic index (Liljeberg & Björck 1994) and are capable of preserving normal glucose (Willett *et al.* 2002),

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insulin (Pereira *et al.* 2002), triacylglycerols, and cholesterol (Liu *et al.* 2001) blood concentrations in humans. A prolonged low-GI diet also protects against the development of non-insulin dependent diabetes mellitus (Björck *et al.* 2000) as well as cardiovascular diseases (Behall & Hove 1995) in healthy subjects. RS will become a component of functional foods (Niba 2002), defined as foods similar in appearance to conventional foods, consumed as part of a normal diet but which demonstrate physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions (Huggett & Schliter 1996).

RS has been defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans (Englyst & Kingman 1992) and escaping enzymatic digestion in the small intestine. Based on the mechanism that prevents this digestion, RS is classified into four types, namely RS_{1-4} (Eerlingen & Delcour 1995). Type RS_3 resistant starch is of particular interest. This type is produced by gelatinization followed by retrogradation of starch molecules. The dispersed amylose and amylopectin molecules spontaneously re-associate and form crystallites resistant to enzymatic digestion upon cooling (Sievert & Pomeranz 1989). Retrograded RS_3 generally results during food processing that utilize heat and moisture (conservation, baking, cooking) and subsequent cooling (storage, refrigeration, freezing). The most important source of RS, and especially RS_3, includes the starchy seeds of cereal and legume species exploited for ingredient and food production. The path to functional foods containing RS_3 begins with the potential of plant species and cultivars to create starch with different ratios of amylose and amylopectin. No relevant information exists within the published scientific literature on the potential of the most important agricultural crops to generate RS_3 retrograded resistant starch, and consequently to contribute to the functionality of produced foods. The content of retrograded RS_3 in six underutilized crops was analysed in our earlier study (Mikulíková *et al.* 2005). In this study, it was demonstrated that the highest potential for RS_3 generation was found in chickpea and buckwheat seeds. Both crops are insufficiently utilized for food production, mainly in developed countries, and the aim of this study was to determine and compare the content of RS_3, soluble and total starch, respectively, in seeds of commonly grown cereals crutially exploited by the food industry.

MATERIAL AND METHODS

Seed samples of 98 cultivars and lines of six analysed cereals were used for starch characterization. The seeds of 22 wheat (*Triticum aestivum* L.), 32 barley (*Hordeum vulgare* L.), 11 rye (*Secale cereale* L.), 13 triticale (*X Triticosecale* Wittmarck), 9 tritordeum (*Tritordeum Aschers & Graebn*) and 12 oat (*Avena sativa* L.) cultivars were analysed. All cultivars (excepting high amylose barley mutant Glacier) were grown in the locality of Piešťany and harvested in the summer of 2003.

Groats from all analysed plant species were rough-grinded to identical particle size (0.9 mm) and hydrothermally treated in accordance with Skrabanja and Kreft (1998). The measurement of RS_3 and soluble starch was performed by the Resistant Starch Assay Kit (Megazyme Int., Ireland) based on the McCleary *et al.* (2002) method. Internal control kit samples of resistant starch from kidney beans as well as our own control wheat, rye, barley, triticale, tritordeum, and oat samples were used. Resistant, soluble and total starch were calculated for each sample. Sample results were calculated on the groat dry weight basis (dwb). They were presented as the means of 3 replications ± standard deviations (SD) for each cultivar and plant crop too. Basic statistical tests were used for evaluation: Student's *t*-test (STATGRAPHICS 6.1.) between species and analysis of variance (ANOVA) within species. The analyses were carried out at the 0.05 and 0.01 significance levels.

RESULTS AND DISCUSSION

The process of treatment and its effect on RS_3 for groat samples optimized according to Skrabanja and Kreft (1998). The approach recommended by them was also jointly used for our samples. This approach is very similar to the usual sample processing for food. Also Parchure and Kulkarni (1997) state that pressure cooking and boiling results in the generation of the highest levels of retrograded RS. By growing our plants in one season and one locality, using identical sample preparation, treatment and analysis, we were able to compare the potential for RS_3 generation between and within six crops. The mean values of RS_3 (dwb) ± standard deviations (SD) of individual cereals were as follows: triticale 5.28% ± 0.68, rye 4.93% ± 0.73, wheat 3.87% ± 0.55, spring barley 2.51% ± 0.25, winter barley 2.35% ± 0.45, tritordeum 2.26% ± 0.36, and oat 0.41% ± 0.09. There were found statistically significant differences in RS_3 content between triticale or rye and other species. Differences between triticale and rye were unsignificant.

Triticale, rye, and wheat possess seed starch composition favourable for RS_3 generation after groats treatment. The efficiency of this trait is similar to or higher than chickpea which was the best in our earlier study (Mikulíková *et al.* 2005).

The mean ± SD of total starch (dwb) in all six crops were as follows: rye 70.38% ± 2.47, wheat 69.90% ± 1.71, triticale 69.61% ± 3.15, tritordeum 65.14% ± 3.26, spring barley 63.17% ± 2.81, winter barley 60.41% ± 3.71, and oat 47.45% ± 8.37. There were significant differences between individual plant crops.

The content of total starch within cultivars from all species was relatively uniform. However, several cultivars possess significant higher amount (Tables 1–7 and 9). The highest differences were found within oat cultivars where the variance between the Ábel cultivar and the lowest three was 22%. High variation within oat cultivars was due to the presence of four naked oats (Ábel, Detvan, Izak, and Jakub) which were different when compared to the means of others.

The proportion of RS_3 to total starch was as follows: triticale 7.6%, rye 7.0%, wheat 5.6%, spring barley 4.0%, winter barley 3.9%, tritordeum 3.5%, and oat 0.9%. Sources with a proportion greater than 4.5% are considered suitable sources of resistant starch (Liljeberg-Elmståhl 2002).

Table 1. Starch parameters in triticale cultivars

Cultivar	Soluble starch	RS_3	Total starch
Asper	63.4 ± 0.87	5.3 ± 0.07	68.7 ± 0.80
Benetto	56.5 ± 0.11	4.4 ± 0.05	60.9 ± 1.48
Colosal	66.4 ± 0.46	4.4 ± 0.05	70.8 ± 0.41
Kendo	63.0 ± 1.33	5.8 ± 0.04*	68.8 ± 1.29
Kolor	63.8 ± 0.53	5.1 ± 0.06	68.9 ± 0.59
Largus	68.4 ± 0.53	5.1 ± 0.09	73.6 ± 0.62*
Nargess	66.0 ± 0.39	5.2 ± 0.06	71.2 ± 0.45
Pinokio	63.0 ± 0.39	6.4 ± 0.05*	69.4 ± 0.34
Presto	62.7 ± 0.47	6.0 ± 0.05*	68.8 ± 0.52
Radko	67.3 ± 0.40	5.7 ± 0.09	73.0 ± 0.49*
Tewo	64.0 ± 0.26	4.2 ± 0.07	68.2 ± 0.33
Tricolor	64.8 ± 0.39	5.9 ± 0.10*	70.7 ± 0.50
Woltario	66.9 ± 0.27	5.2 ± 0.03	72.2 ± 0.29*

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

Table 2. Starch parameters in rye cultivars

Cultivar	Soluble starch	RS_3	Total starch
Albedo	65.4 ± 0.19	3.9 ± 0.05	69.3 ± 0.25
Apart	65.2 ± 0.35	5.3 ± 0.04*	70.6 ± 0.29
Aventino	68.7 ± 0.88	4.7 ± 0.03	73.4 ± 0.84*
Dankowskie Nowe	65.4 ± 0.27	5.5 ± 0.06*	70.9 ± 0.21
Esprit	63.7 ± 0.41	5.9 ± 0.09*	69.5 ± 0.50
Fernando	60.4 ± 0.41	4.4 ± 0.04	64.8 ± 0.37*
Matador	63.0 ± 0.82	4.9 ± 0.04	67.8 ± 0.86
Picasso	67.5 ± 0.54	4.0 ± 0.04	71.5 ± 0.58
Rapid	66.6 ± 0.47	4.9 ± 0.08	71.5 ± 0.56
Selgo	67.2 ± 0.41	6.2 ± 0.12*	73.4 ± 0.53*
Warko	66.9 ± 0.47	4.5 ± 0.11	71.4 ± 0.59

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

Our results, as well as these calculated proportions, show that triticale, rye, and wheat are potential and recommendable cereal sources for generation of retrograded RS_3.

The world-wide production of triticale as the first man-made cereal (wheat × rye) has steadily increased over the past two decades. Due to its environmental flexibility, superior tolerance to biotic and abiotic stress and improved nutritional qualities compared to wheat, triticale should be more notable. Its disadvantage in food production is a weaker baking quality in comparison to wheat but better then in rye. However, triticale flour used in mixtures of wheat flour (1:1) results in dough with acceptable rheological properties and tortillas of good quality (Serna-Saldivar *et al.* 2004). The prospects of triticale as a functional component of food have increased in response. We identified the highest level of RS_3 only in this crop (Table 1). The highest content of RS_3 was found in the Pinokio, Presto, Tricolor, and Kendo cultivars. The range for total starch was similar to that in wheat and rye (Tables 2 and 3).

Rye is a crop of special importance with high dietary fibre content in starchy endosperm (Åman *et al.* 1997). This contributes to the intake of dietary fibre due to the fact that rye is consumed frequently in whole grain products. In all rye cultivars analysed in our study, relatively high levels of RS_3 were found (Table 2), the highest in the Selgo, Esprit, Dankowskie Nowe, and Apart cultivars, respectively. A good alternative source for the generation of RS_3 in products undergoing starch retrogradation is the Selgo cultivar seed due to its starchy composition. The phenomenon of resistant starch generation in rye breads has been reported by Liljeberg and Björck (1994). This effect has beneficial influence on the postprandial glucose and insulin response (Juntunen *et al.* 2003) and explains the lower postprandial insulin response to rye bread in comparison to wheat bread.

Table 3. Determination of starch parameters in wheat cultivars

Cultivar	Soluble starch	RS_3	Total starch
Alana	65.3 ± 0.47	3.7 ± 0.08	69.1 ± 0.39
Alka	66.9 ± 0.61	3.3 ± 0.05	70.2 ± 0.66
Armelis	66.2 ± 0.54	4.1 ± 0.06	70.3 ± 0.59
Asta	65.6 ± 1.75	3.9 ± 0.11	69.4 ± 0.45
Astella	66.0 ± 0.61	4.1 ± 0.07	70.1 ± 0.68
Athlet	61.6 ± 0.27	5.8 ± 0.08*	67.4 ± 0.18
Axis	65.6 ± 0.47	3.6 ± 0.03	69.2 ± 0.44
Blava	64.7 ± 1.31	3.7 ± 0.06	68.3 ± 0.54
Boka	67.4 ± 0.54	4.8 ± 0.06*	72.3 ± 0.61*
Bonita	65.2 ± 0.40	3.8 ± 0.03	68.9 ± 0.37
Brea	66.1 ± 0.41	4.0 ± 0.06	70.2 ± 0.35
Contra	69.3 ± 0.61	3.5 ± 0.10	72.8 ± 0.71*
Ebi	63.4 ± 0.81	3.8 ± 0.05	67.2 ± 0.87
Estica	67.0 ± 0.41	3.7 ± 0.06	70.7 ± 0.47*
Ilona	64.4 ± 0.21	3.3 ± 0.06	67.6 ± 0.26
Malyska	66.8 ± 0.40	3.4 ± 0.04	70.2 ± 0.45
Solara	66.2 ± 0.41	3.7 ± 0.05	69.9 ± 0.35
Torysa	67.1 ± 1.29	4.3 ± 0.05*	71.4 ± 1.34
Trane	67.9 ± 0.74	4.5 ± 0.03*	72.4 ± 0.77*
Vanda	65.4 ± 0.34	3.8 ± 0.04	69.2 ± 0.30
Versailles	69.2 ± 0.47	4.5 ± 0.06*	73.7 ± 0.53*
Vlada	65.6 ± 0.40	3.7 ± 0.04	69.4 ± 0.44

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

Based on RS_3 content, it is evident that wheat is a good source of retrograded resistant starch. The highest levels of RS_3 among the analysed wheat cultivars were detected in the Athlet, Boka, Trane, Versailles, and Torysa (Table 3). Four of these are known to be cultivars of low bread-baking quality, with the exception of the Boka cultivar. Wheat starch contains 20–25% of amylose (Shi *et al.* 1991). Amylose content significantly influences processing and post-processing attributes, namely texture, dough properties, baking quality (Park & Baik 2003), etc. Starches with high amylose content formulate resistant starch; however, this is accompanied by deterioration in baking quality (Morita *et al.* 2002). Bread products made from wheat flour do not undergo the same conditions during the production process as was simulated by hydrothermal treatment in this study. Therefore RS and consequently also RS_3 content should be lower. Liljeberg-Elmståhl (2002) detected very low levels of RS (0.6% ± 0.1) in wheat flat bread. The addition of rye flour increased the content to 6.0% ± 1.2. Smaller baking products (rolls, pretzels, etc.) are subjected to lower baking temperatures and shorter times, therefore, the generation of RS_3 is lower. In addition to the analyses of RS_3 content in seed samples, we also measured RS_3 content in bread loaves. Wheat loaves sold on the market, weighing two kilograms and produced by classical bread-making practices in commercial bakeries, contained 2.2% of RS_3 (unpublished). This demonstrates that wheat seed is a suitable source of retrograded starch; that significant dif-

Table 4. Determination of starch parameters in winter barley cultivars)

Cultivar	Soluble starch	RS_3	Total starch
Babylone	56.7 ± 0.74	2.6 ± 0.03	59.2 ± 0.76
Bogesa	56.1 ± 0.40	2.4 ± 0.04	58.5 ± 0.36
Haller	58.5 ± 0.53	2.1 ± 0.03	60.6 ± 0.57
Hanna	59.2 ± 0.54	2.9 ± 0.07*	62.1 ± 0.61
Luran	53.9 ± 0.74	2.2 ± 0.08	56.2 ± 0.67
Luxor	59.7 ± 0.60	3.0 ± 0.04*	62.7 ± 0.56
Monako	58.1 ± 0.19	1.6 ± 0.04	59.7 ± 0.16
Montana	54.5 ± 0.61	1.9 ± 0.03	56.4 ± 0.57
Polana	65.9 ± 0.47	2.4 ± 0.04	68.3 ± 0.50*

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species ferences exist between registered cultivars; and that, wheat flour should be utilized in functional baking products. Furtheremore, daily intake of RS can be attained by consuming relatively small quantities (120–150 g) of bread.

Within the analysed barley cultivars, the highest levels of RS_3 were found in three spring cultivars – Kompakt, Jubilant, Karát, and two winter cultivars – Luxor and Hanna. However, barley is not as considerable a source of resistant starch and retrograded RS_3 as triticale, rye, or wheat. No statistically significant differences using Student's *t*-test were detected between spring (2.51% ± 0.25) and winter barley (2.35% ± 0.45) cultivars or between malting (2.47% ± 0.32) and feed barley (2.55% ± 0.14) cultivars (Tables 4, 5 and 8). Two genes are known to be responsible for starch composition in barley (Schondelmaier *et al.* 1992). The gene for high amylose content, *amo1*, is located on chromosome 1H. The *waxy* gene for high amylopectin content is located on chromosome 7H. In order to estimate the direct relationship between amylose content and ability to generate retrograded RS_3 starch, the barley genotype Glacier was included in the set of analysed barley cultivars. This barley possesses a recessive *amo1* gene which encodes for high amylose content. Repeated autoclaving-cooling cycles can increase RS content to 26% in Glacier starch (Szczodrak & Pomeranz 1991). The RS_3 content in Glacier after simple hydro-

Table 5. Determination of starch parameters in spring barley cultivars

Cultivar	Soluble starch	RS_3	Total starch
Amos (C)	61.5 ± 0.80	2.7 ± 0.06	64.2 ± 0.74
Cyril (C)	63.2 ± 0.74	2.4 ± 0.07	65.7 ± 0.67
Denar (B)	62.9 ± 0.61	2.5 ± 0.08	65.4 ± 0.69
Donum (C)	64.8 ± 0.40	2.6 ± 0.05	67.4 ± 0.46*
Dregerův (B)	57.6 ± 0.54	2.2 ± 0.03	59.9 ± 0.52
Export Ratborský (B)	56.0 ± 1.08	2.2 ± 0.02	58.3 ± 1.06
Galan (B)	63.4 ± 0.74	2.7 ± 0.08	66.2 ± 0.83
Horal (C)	59.1 ± 0.61	2.5 ± 0.08	61.6 ± 0.69
Jubilant (A)	63.8 ± 0.34	3.0 ± 0.04*	66.8 ± 0.30*
Karát (A)	62.8 ± 0.68	2.9 ± 0.03*	65.7 ± 0.65
Kompakt (A)	64.3 ± 0.54	3.0 ± 0.06*	67.3 ± 0.48*
Kosan (C)	62.0 ± 0.28	2.6 ± 0.02	64.8 ± 0.04
Krystal (A)	58.6 ± 0.54	2.3 ± 0.05	61.0 ± 0.59
Michalovický (B)	59.0 ± 0.41	2.2 ± 0.07	61.2 ± 0.35
Proskovcův (A)	59.7 ± 0.81	2.3 ± 0.04	62.0 ± 0.86
Slovenský kvalitný (A)	62.8 ± 0.34	2.2 ± 0.06	65.1 ± 0.39
Svit (C)	61.8 ± 0.94	2.7 ± 0.05	64.5 ± 0.89
Tatry 1995 (B)	56.9 ± 0.27	2.2 ± 0.05	59.1 ± 0.33
Tolar (C)	58.0 ± 0.87	2.5 ± 0.01	60.5 ± 0.86
Viktor (C)	58.5 ± 0.54	2.6 ± 0.09	61.1 ± 0.44
Zefír (A)	58.6 ± 1.49	2.5 ± 0.03	61.1 ± 1.47
Židlochovický (A)	58.9 ± 0.55	2.4 ± 0.04	61.3 ± 0.50
Glacier (mutant)	49.7 ± 0.55	5.2 ± 0.04*	54.9 ± 0.58 *

Malting quality: A – high malting quality, B – medium malting quality, C – non-malting (feed) barley

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

Table 6. Determination of starch parameters in tritordeum cultivars

Cultivar	Soluble starch	RS_3	Total starch
HT 31-1	57.4 ± 0.79	1.9 ± 0.04	59.3 ± 0.76
Linda	61.2 ± 0.33	2.2 ± 0.03	63.4 ± 0.29
Planet	64.0 ± 0.47	2.2 ± 0.06	66.3 ± 0.52
HT 119	62.3 ± 0.67	1.9 ± 0.05	64.2 ± 0.07
HT 129	58.6 ± 0.74	1.8 ± 0.04	60.4 ± 0.69
Maris Dove	64.3 ± 0.46	2.6 ± 0.04*	66.9 ± 0.51*
Rena	65.6 ± 0.33	2.6 ± 0.05*	68.2 ± 0.38*
AC Vilmot	66.5 ± 0.67	2.5 ± 0.06*	68.9 ± 0.73*
HTC 1380	66.1 ± 0.79	2.7 ± 0.06*	68.8 ± 0.74*

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

Table 7. Determination of starch parameters in oat cultivars

Cultivar	Soluble starch	RS_3	Total starch
Ábel	62.4 ± 0.48	0.4 ± 0.06	62.8 ± 0.42*
Ardo	40.3 ± 0.33	0.5 ± 0.02	40.8 ± 0.35
Atego	40.2 ± 0.59	0.6 ± 0.02	40.8 ± 0.61
Auron	41.4 ± 0.58	0.4 ± 0.07	41.8 ± 0.52
Detvan	59.2 ± 0.88	0.4 ± 0.04	59.6 ± 0.84*
Euro	41.3 ± 0.59	0.3 ± 0.02	41.6 ± 0.57
Expander	42.6 ± 0.39	0.3 ± 0.02	42.8 ± 0.37
Izak	47.9 ± 0.79	0.5 ± 0.09	48.4 ± 0.88*
Jakub	59.4 ± 0.73	0.4 ± 0.06	59.8 ± 0.79*
Kanton	44.4 ± 0.91	0.5 ± 0.03	44.8 ± 0.95
Master	40.5 ± 0.46	0.3 ± 0.06	40.8 ± 0.40
Zvolen	45.0 ± 0.59	0.3 ± 0.02	45.4 ± 0.57

% dwb – dry weight basis of groat, *statistically significant differences at $P < 0.01$ against the mean of species

thermal treatment, as employed in this study, was about two-fold higher than in other tested barley cultivars (Table 5), $P < 0.01$.

Tritordeum hexaploid is the fertile amphiploid ($2n = 6x = 42$, $AABBH^{ch}H^{ch}$, between *Hordeum chilense* and durum wheat. Chromosomes A and B descend from wheat and H^{ch} from barley. Tritordeum is similar to wheat both morphologically and agronomically (Martin *et al.* 1999). Total starch in tritordeum accessions was higher (Table 6) when compared to both spring as well as winter barley (Tables 4 and 5), but similarly low as in wheat (Table 3). RS_3 level in tritordeum is similar to barley. Increases in total and resistant starch levels were identified in the tritordeum Maris Dove, Rena, AC Vilmot and HTC 1380 accessions.

Oat is poor in RS as well as in total starch in comparison with other crops studied (Table 7). The proportion of amylose to amylopectin in oat starch is approximately 30:70. Amylose bonds significant amount of phospholipids (Zhou *et al.* 1998). Due to this phenomenon, the solubilisation of oat starch granules is different from starches from other crops. Both lipid content and lipid composition have been documented as affecting pasting properties of oat (Zhou *et al.* 1999). This is likely the main reason why the process of oat amylose retrogradation to resistant starch is blocked.

Oat cultivars are widely known for their high β-glucan content (Colleoni-Sirghie *et al.* 2004), similar to barley (Lee *et al.* 1997). The results from Ehrenbergerová *et al.* (2003) indicate that an inverse relationship exists between amylose

Table 8. Differences in RS_3 level between means of species, computed *t*-statistic

	Wheat	Spring barley	Winter barley	Rye	Triticale	Tritordeum	Oat
Wheat (3.87%)		1.36**	1.52**	1.06**	1.41**	1.61**	3.46**
Spring barley (2.51%)			0.16^{NS}	2.43**	2.77**	0.25*	2.10**
Winter barley (2.35%)				2.58**	2.93**	0.09^{NS}	1.94**
Rye (4.93%)					0.35^{NS}	2.67**	4.52**
Triticale (5.28%)						3.02**	4.87**
Tritordeum (2.26%)							1.85**
Oat (0.41%)							

Significant at $^{**}P < 0.01$, $^{*}P < 0.05$, ^{NS}not significant

Table 9. Differences in total starch level between means of species, computed *t*-statistic

	Wheat	Spring barley	Winter barley	Rye	Triticale	Tritordeum	Oat
Wheat (69.90%)		6.73**	9.49**	0.48^{NS}	0.29^{NS}	4.76**	22.45**
Spring barley (63.17%)			2.76*	7.21**	6.44**	1.97*	15.72**
Winter barley (60.41%)				9.97**	9.20**	4.73*	12.96**
Rye (70.38%)					0.77^{NS}	5.24**	22.93**
Triticale (69.61%)						4.47**	22.16**
Tritordeum (65.14%)							17.69**
Oat (47.45%)							

Significant at $^{**}P < 0.01$, $^{*}P < 0.05$, ^{NS}not significant

content and β-glucan content. In agreement with these results, low RS_3 levels were identified and limited to all oat and barley cultivars in our study (Tables 5 and 7).

In conclusion, this study demonstrates that for the most part, triticale, rye, and some wheat cultivars are highly suitable for the production of functional food components (Table 8), namely, retrograded resistant starch of the type RS_3 which contributes, as a component of total resistant starch, to a highly beneficial human health effect (Nugent 2005). The potential of triticale, rye, and wheat in the production of functional foods has been adequately demonstrated.

From our preliminary study it is advisable to continue in starch results for other years, eventually for different localities.

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References

Annison G., Topping D.L. (1994): Nutritional role of resistant starch: chemical structure vs physiological function. Annual Review of Nutrition, **14**: 297–320.

Áman P., Nilsson M., Andersson R. (1997): Positive health effects of rye. Cereal Foods World, **42**: 684–688.

Behall K.M., Howe J.C. (1995): Effect of long-term consumption of amylose vs amylopectin starch on metabolic variables in human subjects. The American Journal of Clinical Nutrition, **61**: 334–340.

Björck I., Liljeberg H., Östman E. (2000): Low glycaemic index foods. British Journal of Nutrition, **83**: 149–155.

Colleoni-Sirgie M., Jannink J.L., White P.J. (2004): Pasting and thermal properties of flours from oat lines with high and typical amount of β-glucan. Cereal Chemistry, **81**: 686–692.

Eerlingen R.C., Delcour J.A. (1995): Formation, analysis, structure and properties of type III enzyme resistant starch. Journal of Cereal Chemistry, **22**: 129–138.

Ehrenbegerová J., Vaculová K., Psota V., Havlová P., Šerhantová V. (2003): Effect of cropping system and genotype on variability in important phytonutrients content of the barley grain for direct food use. Plant, Soil and Environment, **49**: 443–450.

Englyst H.N., Kingman J.H. (1992): Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition, **46**: 33–50.

Hill M.J. (1995): Bacterial fermentation of complex carbohydrate in the human colon. European Journal of Cancer Prevention, **4**: 353–358.

Huggett A.C., Schliter B. (1996): Research needs for establishing the safety of functional foods. Nutrition Reviews, **54**: 143–148.

Juntunen K.S., Laaksonen D.E., Autio K., Niskanen L.K., Holst J.J., Savolainen K.E., Liukkonen K.H., Poutanen K.S., Mykkänen H.M. (2003): Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. The American Journal of Clinical Nutrition, **78**: 957–964.

Lee C.J., Horsley R.D., Manthey F.A., Schwarz P.B.(1997): Comparison of β-glucan content of barley and oat. Cereal Chemistry, **74**: 571–575.

Liljeberg H., Björck I. (1994): Biovailability of starch in bread products. Postprandial glucose and insulin response in healthy subjects *in vitro* resistant starch content. European Journal of Clinical Nutrition, **48**: 151–163.

Liljeberg-Elmståhl H. (2002): Resistant starch content in a selection of starchy foods on the Swedish market. European Journal of Clinical Nutrition, **56**: 500–505.

Liu S., Manson J.E., Stampfer M.J., Holmes M.D., Hu F.B., Hankinson S.E., Willett W.C. (2001): Dietary glycemic load assesed by foodfrequency questionnaire in relation to plasma high-density-lipoprotein cholesterol and fasting plasma triacylglycerols. The American Journal of Clinical Nutrition, **73**: 560–566.

Martin A., Alvarez J.B., Martin L.M., Barro F. (1999): The development of tritordeum: a novel cereal for food processing. Journal of Cereal Science, **30**: 89–95.

McCleary B.V., McNally M., Rossiter P. (2002): Measurement of resistant starch enzymatic digestion in starch and selected plant materials: collaborative study. Journal of AOAC International, **85**: 1103–1111.

Mikulíková D., Čičová I., Antalíková G., Kraic J. (2005): Grains of nontraditional crops as source of retrograded resistant starch. Czech Journal of Genetics and Plant Breeding, **41**: 96–104.

Morita N., Maeda T., Miyazaki M., Yamamori M., Miura H., Ohtsuka I. (2002): Dough and baking properties of high-amylose and waxy wheat flours. Cereal Chemistry, **79**: 491–495.

Mortensen P.B., Clausen M.R. (1996): Short-chain fatty acid in the human colon. Relation to gastrointestinal health and disease. Scandinavian Journal of Gastroenterology, **31** (suppl. 261): 132–148.

Niba L.L. (2002): Resistant starch: a potential functional food ingredient. Nutrition and Food Science, **32**: 62–67.

Nugent A.P. (2005): Health properties of resistant starch. Nutrition Bulletin, **30**: 27–54.

Parchure A.A., Kulkarni P.R. (1997): Effect on food processing treatments on generation of resistant starch. International Journal of Food Sciences and Nutrition, **48**: 257–260.

Park C.S., Baik B.K. (2003): Significance of amylose content of wheat starch on processing and textural properties of instant noodles. Cereal Chemistry, **81**: 521–526.

Pereira M.A., Jacobs D.R., Pins J.J., Raatz S.K., Gross M.D., Slavin J.L., Seaquist E.R. (2002): Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. The American Journal of Clinical Nutrition, **75**: 848–855.

Schondelmaier J.A., Jacobi G., Fischbeck G., Jahoor A. (1992): Genetic studies on the mode of inheritance and localization of the *amo1* (high amylose) gene in barley. Plant Breeding, **109**: 274–290.

Serna-Saldivar S., Guajardo-Flores S., Viesca-Rios R. (2004): Potential of triticale as a substitute for wheat in flour tortilla production. Cereal Chemistry, **81**: 220–225.

Shi Y. C., Seib P.A., Lu S.P. (1991): Leaching of amylose from wheat and corn starch. Advances in Experimental Medicine & Biology, **302**: 667–686.

Sievert D., Pomeranz Y. (1989): Enzyme resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical and microscopical methods. Cereal Chemistry, **66**: 342–347.

Silvester K.R., Englyst H.N., Cummings J.H. (1995): Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. The American Journal of Clinical Nutrition, **62**: 403–411.

Skrabanja V., Kreft I. (1998): Resistant starch formation following autoclaving of buckwheat (*Fagopyrum esculentum* Moench) groats. An *in vitro* study. Journal of Agricultural and Food Chemistry, **46**: 2020–2023.

Szczodrak J., Pomeranz Y. (1991): Starch and enzyme-resistant starch from high amylose barley. Cereal Chemistry, **68**: 589–596.

Willett W., Manson J., Liu S. (2002): Glycemic index, glycemic load and risk of type 2 diabetes. The American Journal of Clinical Nutrition, **76**: 274–280.

Zhou M., Robards K., Glennie-Holmes M., Helliwell S. (1998): Structure and pasting properties of oat starch. Cereal Chemistry, **75**: 273–281.

Zhou M., Robards K., Glenie-Holmes M., Helliwell S. (1999): Effect of oat lipid on groat meal pasting properties. Journal of the Science of Food and Agriculture, **79**: 585–592.

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