Determination of arabinoxylans and beta-glucans in cereals and their fractions with near infrared techniques

S. Gergely,[a] K. Gebruers,[b] C.M. Courtin,[b] J.A. Delcour[b] and A. Salgó[a,*]

[a]*Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest 1111, Hungary.*
** E-mail: salgo@mail.bme.hu*
[b]*Laboratory of Food Chemistry and Biochemistry, and Leuven Food Science and Nutrition Research Centre, Catholic University of Leuven, Leuven 3001, Belgium*

Introduction

In the cereal grain and bakery products area the worldwide trend towards healthier eating and healthier products is leading to a growing market share of whole grain products and products high in dietary fibre. This includes products high in arabinoxylans (wheat, rye) and/or in glucans (oat, barley, rye), and products with high levels of other health promoting phytochemicals (the bioactive co-passengers of dietary fibre in whole grain). The nutritional benefits of arabinoxylans, glucans and other non-starch polysaccharide phytochemicals are quite diverse, being unsurpassed in maintaining regular intestinal function.

The arabinoxylans (AX), representing the largest part of the building blocks of cell walls[1] have received an increasing amount of attention as dietary fibre, because they may promote health by reducing the risk of different cancers, coronary heart disease and diabetes.[2,3] In addition, they are a major determinant of the functionality of cereals in biotechnological, food technological processes and applications. Specifically the water extractable fraction of arabinoxylans (WEAX) is favourable for bread making purposes.

β-glucans are predicted to become an important supplement in human nutrition for their proven cholesterol lowering effect[4] and augured positive effect on diabetes,[5] several immune diseases[6] and suppression of cancer development.[7]

The quantitative measurements of these bioactive compounds (total and soluble arabinoxylans, β-glucans) need complicated wet chemical methods including laborious (extraction, saponification, purification, derivatization separation, detection etc.) steps, which are time-consuming and expensive. The applications of near infrared methods to measure these non-starch polysaccharides have appeared in the scientific literature in the last five years.[8–12]

The aim of present study was to examine the potential of near infrared reflectance spectroscopy for the quantitative analysis of arabinoxylans and their fractions, as well as β-glucans in different cereals and milling fractions.

Experimental

200 varieties of different cereal species were grown on fields of the Agricultural Research Institute of the Hungarian Academy of Sciences at Martonvásár, (Hungary). Seeds were harvested in 2005 (200 samples). From the 200 varieties 26 wheat and 5 rye varieties were selected based on their dietary fibre and phytochemical contents, and were grown for two successive years on the same location (harvested in 2006 and 2007) and for one year on three other locations in Europe (harvested in 2007), i.e. Clermont-Ferrand (France), Dankow-Choryn (Poland) and Bury St. Edmunds (United Kingdom). In this way a representative (N = 355) sample set was created covering the effects of commodity, genetic and environmental factors on quality, to put as much variance as possible into the sample set.

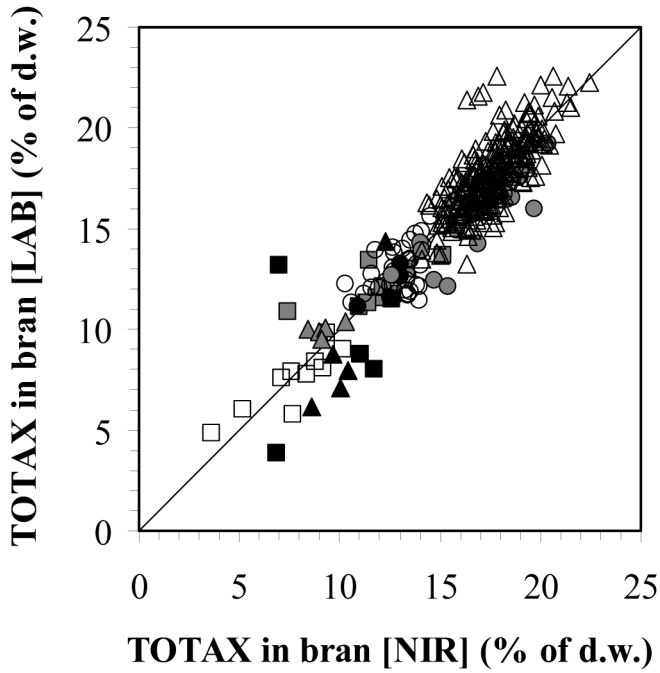

Figure 1. Scatter plots of reference versus predicted *values* regarding bran TOTAX of 2005, 2006 and 2007 samples using the 'all' (i.e. whole) sample set. Legend: △ winter wheat; ▲ monococcum; ▲ dicoccum; □ barley; ■ durum wheat; ■ oat; ○ rye; ● spring wheat; ● spelt. N = 353, R^2 = 0.8640, *SECV* = 1.3148, f = 10.

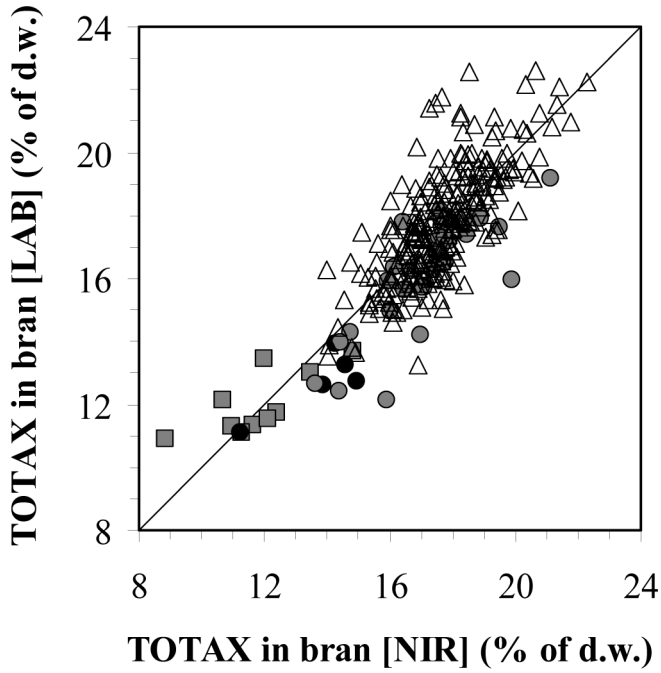

Figure 2. Scatter plots of reference *versus* predicted values regarding bran TOTAX of 2005, 2006 and 2007 samples using the 'wheat only' (i.e. selected) sample set. Legend: △ winter wheat; ■ durum wheat; ● spring wheat; ● spelt. $N = 293$, $R^2 = 0.7133$, $SECV = 1.2474$, $f = 9$.

Flour and bran fractions were prepared by grinding cereals on a Chopin CD2 Mill (CHOPIN Technologies, Villeneuve-la-Garenne, France) equipped with 0.9 mm and 0.25 mm sieves. Whole meal samples were prepared by grinding cereals in a Perten Laboratory Mill 3100 (Perten Instruments, Huddinge, Sweden), equipped with a 0.5 mm sieve.

Bran, and flour, as well as whole meal samples were analysed in order to follow the compositional and physical changes of test materials during milling and separation procedures. Spectra were recorded from each sample (two independent replicates) in reflectance mode at room temperature using two NIRSystems Model 6500 monochromator systems (Foss NIRSystems, Silver Spring, MD, USA). One of them was equipped with a sample transport module (STM) and the other one was equipped with a rapid-content analyzer (RCA). Standard sample cups equipped with threaded backs were used. Samples were scanned (32 scans co-added) from 400 to 1098 nm (silicon detector) and from 1100 to 2498 nm (lead sulphide detector). Data were collected every 2 nm (1050 data points per spectrum). The aim of parallel scanning of samples on the different instruments was to put the instrumental variations into the spectroscopic data set, and to evaluate the physical differences between similar instruments.

Spectral data were processed using WinISI II 1.50 (Infrasoft International, Port Matilda, PA, USA) and Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA) softwares.

Table 1. Results of MPLS calibrations calculated without and *with* repeatability file using the "all" (i.e. whole) sample set.

Fraction	Bran				Flour				Whole meal	
Constituent	TOTAX (% of DW)		WEAX (% of DW)		TOTAX (% of DW)		WEAX (% of DW)		Glucan (% of DW)	
Instrument	STM	RCA	STM	RCA	STM	RCA	STM	RCA	STM	RCA
N	353		353		352		352		352	
Range	3.83 – 22.60		0.15 – 1.53		1.05 – 4.31		0.15 – 1.94		0.24 – 6.54	
Mean	16.259		0.489		2.061		0.579		0.966	
Std. dev.	3.280		0.244		0.481		0.294		0.964	
R^2	0.849 *0.864*	0.843 *0.868*	0.833 *0.871*	0.836 *0.861*	0.771 *0.929*	0.791 *0.905*	0.712 *0.837*	0.710 *0.687*	0.917 *0.952*	0.900 *0.909*
SECV	1.325 *1.315*	1.416 *1.395*	0.108 *0.098*	0.111 *0.103*	0.236 *0.153*	0.238 *0.196*	0.160 *0.134*	0.179 *0.178*	0.317 *0.258*	0.337 *0.335*
f	10 *10*	13 *13*	13 *13*	14 *14*	13 *15*	11 *16*	11 *13*	11 *7*	13 *15*	13 *10*

Results and discussion

Figure 1 shows the scatter plot of equation developed for determination of total arabinoxylans (TOTAX) content in the combined sample set. The robust model shows strong commodity effects and resulted in a fast and reliable routine screening method providing semi-quantitative results.

Figure 2 represents the scatter plot of model calculated for wheat samples only. The dedicated model has lower R^2 values (compared to general models) due the reduced total variance of spectroscopic data, and narrower ranges of reference values. The standard error of cross validation (*SECV*) values were smaller (the accuracy increased) compared to the overall models because the commodity effects were avoided. The optimal number of latent variables (i.e. factors, *f*) was also slightly decreased.

Statistical values (Table 1 and Table 2) confirmed that TOTAX can be detected in wheat fractions with acceptable accuracy, the precision of the WEAX model was significantly poorer compared to TOTAX. The apparent good calibration model for the prediction of β-glucan in

Table 2. Results of MPLS calibrations calculated without and *with* repeatability file using the 'wheat only' (i.e. selected) sample set.

Fraction	Bran				Flour				Whole meal	
Constituent	TOTAX (% of DW)		WEAX (% of DW)		TOTAX (% of DW)		WEAX (% of DW)		Glucan (% of DW)	
Instrument	STM	RCA	STM	RCA	STM	RCA	STM	RCA	STM	RCA
N	293		293		293		293		292	
Range	10.89–22.60		0.27–0.92		1.31–2.74		0.24–1.07		0.24–0.96	
Mean	17.317		0.427		1.952		0.514		0.673	
Std. dev.	2.197		0.092		0.270		0.141		0.117	
R^2	0.685 *0.713*	0.679 *0.731*	0.433 *0.515*	0.430 *0.462*	0.558 *0.821*	0.573 *0.721*	0.173 *0.452*	0.206 *0.459*	0.267 *0.552*	0.349 *0.507*
SECV	1.270 *1.247*	1.318 *1.257*	0.073 *0.071*	0.073 *0.072*	0.184 *0.132*	0.188 *0.163*	0.128 *0.115*	0.128 *0.120*	0.103 *0.091*	0.099 *0.093*
F	9 *9*	11 *11*	11 *10*	10 *9*	11 *13*	9 *10*	7 *8*	6 *9*	7 *12*	8 *10*

whole-meal samples reflected a strong commodity effect, and in spite of acceptable statistical results this model did not measure the concentration of glucan effectively, so the model is really ranking the different commodities based only on their glucan level.

Conclusions

The calibration models for TOTAX and WEAX provide fast routine screening methods, providing either quantitative or semi-quantitative results. These calibration models would be suitable for use by plant-breeders, for assessment of the AX level and type in seeds; by millers to evaluate the efficiency of separation and mixing procedures; and by food technologists to select milling products or fractions for manufacturing products (with dedicated functional properties) with designed composition of bioactives. The models are transferable between different instruments but a validation process would be needed following the calibration transfer.

Acknowledgements

The authors gratefully acknowledge Pierre Dardenne (Walloon Agricultural Research Centre, CRA-W) for his invaluable help during the personal consultations. This work was supported by HEALTHGRAIN Integrated Project (FOOD-CT-2005-514008).

References

1. C.J.A. Vinkx and J.A. Delcour, *J. Cereal Sci.* **24,** 1 (1996).
2. G. Dongowski, *Food Chem.* **104,** 390 (2007).
3. A.L. Garcia, B. Otto, S.C. Reich, M.O. Weickert, J. Steininger, A. Machowetz, N.N. Rudovich, M. Möhlig, N. Katz, M. Speth, F. Meuser, J. Doerfer, H.J.F. Zunft, A.H.F. Pfeiffer and C. Koebnick, *Eur. J. Clin. Nutr.* **61,** 334 (2007).
4. K.M. Behall, D.J. Scholfield and J. Hallfrisch, *Am. J. Clin. Nutr.* **80,** 1185 (2004).
5. K.M. Behall, D.J. Scholfield and J. Hallfrisch, *Nutr. Res.* **26,** 644 (2006).
6. A. Estrada, C.H. Yun, A. van Kessel, B. Li, S. Hauta and B. Laarveld, *Microbiol. Immunol.* **41,** 991 (1997).
7. S. Modak, G. Koehne, A. Vickers, R.J. O'Reilly and N.K.V. Cheung, *Leuk. Res.* **29,** 679 (2005).
8. A.B. Blakeney and P.C. Flinn, *Mol. Nutr. Food Res.* **49,** 546 (2005).
9. P. Robert, M. Marquis, C. Barron, F. Guillon and L. Saulnier, *J. Agric. Food Chem.* **53**, 7014 (2005).
10. R.M. de Sá and G.H. Palmer, *J. Inst. Brew.* **112,** 9 (2006).
11. M. Sohn, D.S. Himmelsbach, F.E. Barton, C.A. Griffey, W. Brooks and K.B. Hicks, *Appl. Spectrosc.* **62,** 427 (2008).
12. J. Schmidt, S. Gergely, R. Schönlechner, H. Grausgruber, S. Tömösközi, A. Salgó and E. Berghofer, *Cereal Chem.* **86,** 398 (2009).