Determination of alkylresorcinols and sterols in cereal wholemeals with near infrared techniques

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Introduction

The bioactive compounds of cereals and their milling fractions have different nutritional benefits covering a broad spectrum of physiological, immunoprotective, immunostimulatory or disease prevention effects. The different morphological layers of cereal seeds or fortified fractions prepared by mixing could have dedicated compositions from a bioactive component point of view and could be used as potential functional food ingredients. The different commodities, plant tissues and fractions show a broad spectrum of lipophilic (tocols, phenolic compounds, alkylresorcinols, sterols, lignins) and hydrophilic (folates, tannins, lignins, arabinoxylans, glucans, other non-starch polysaccharides) bioactive components varying in a concentration range between some mg kg^{-1} and 25% (w/w). Alkylresorcinols (ARs) are phenolic lipids present in high amounts in the bran layer. The AR family comprises compounds with lipid side-chains of various lengths. They are not present in the endosperm, which means that ARs can be used as biomarkers for people who eat foods containing wholegrain wheat and rye rather than cereal products based on white flour.[1] *In vitro* studies have shown that ARs may prevent cells turning cancerous but that they do not have any effect on cells that are already cancerous.[2] The quantitative analysis of AR content can be carried out by gas chromatography (GC) after time-consuming extraction of the samples.[3]

Phytosterols include both plant sterols and stanols, which differ in their chemical structures. The three most common forms of phytosterols in foods are β-sitosterol, campesterol and stigmasterol. As plant components, phytosterols may offer protection against cancer by several different means. These include inhibiting cell division, stimulating tumour cell death and modifying some of the hormones that are essential for tumour growth.[4] Phytosterols also lower cholesterol in two ways: firstly, they block the absorption of dietary cholesterol that is circulating in the blood and secondly, they reduce the re-absorption of cholesterol which the body naturally produces from the liver.[5] The composition of sterols in the wholemeal of cereal lines has been determined by GC analysis. Sterols are extracted from wholemeal samples after direct acid and alkaline hydrolyses.[6] This study focuses on the development of NIR analytical methods for lipophilic-type bioactive components such as total alkylresorcinols and total sterols in cereal wholemeals.

Materials and Methods

Samples

Varieties (n=200) 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. 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 further, successive years at the same location (harvested in 2006 and 2007) and for one year at 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 so as to include as much variance as possible into the sample set. Wholemeal samples were prepared by grinding cereals (Perten Laboratory Mill 3100 equipped with a 0.5 mm sieve; Perten Instruments, Huddinge, Sweden).

Determination of total AR content by extraction, silylation and GC analysis with 2 replicates was performed at the Swedish University of Agricultural Sciences and expressed as μg g^{-1} of dry weight.[7] Determination of total sterol content by acid hydrolysis, saponification, purification of unsaponifiable fraction by solid phase extraction, silylation and GC analysis with 2 replicates was performed at the University of Helsinki and expressed as μg g^{-1} of fresh weight.[8]

Reference paper as:
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Near infrared spectroscopy

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 9.1 (Statsoft, Inc., Tulsa, OK, USA) software. Calibration models were developed using the modified partial least squares (mPLS) method with 4-segment cross-validation (CV) using standard normal variate (SNV) and detrend pre-processing of first derivative spectra.

Results and Discussion

Figures 1 and 2 show the scatter plots of equations developed for determination of total AR and total sterol content respectively in the combined sample set. The robust models show strong commodity effects and provide a fast and reliable routine screening method producing semi-quantitative results.

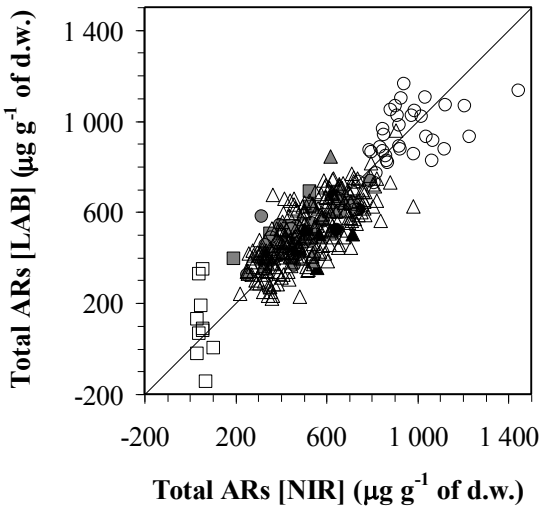

Figure 1. Scatter plots of reference versus predicted values for total ARs of 2005, 2006 and 2007 samples using the whole sample set. Legend: △ winter wheat; ▲ monococcum; ▲ dicoccum; □ barley; ■ durum wheat; ■ oat; ○ rye; ● spring wheat; ● spelt. N = 347, R^2 = 0.81, SECV = 108.8, f = 15.

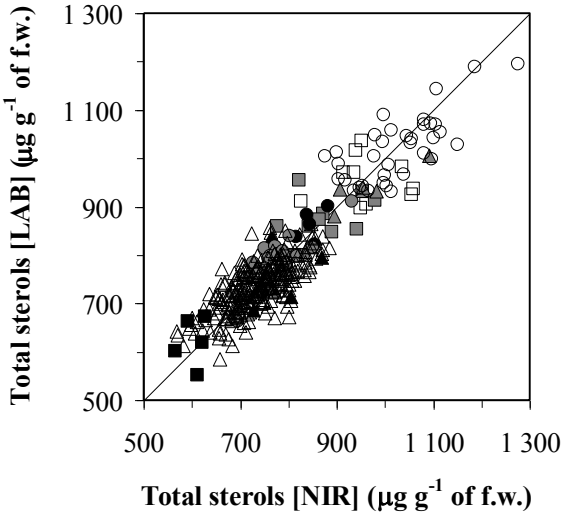

Figure 2. Scatter plots of reference versus predicted values for total sterols of 2005, 2006 and 2007 samples using the whole sample set. Legend: △ winter wheat; ▲ monococcum; ▲ dicoccum; □ barley; ■ durum wheat; ■ oat; ○ rye; ● spring wheat; ● spelt. N = 352, R^2 = 0.87, SECV = 46.98, f = 15.

Figures 3 and Figure 4 contain the scatter plots of models developed for wheat samples only. These models have lower R^2 values compared to the general models because of the 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 reduced.

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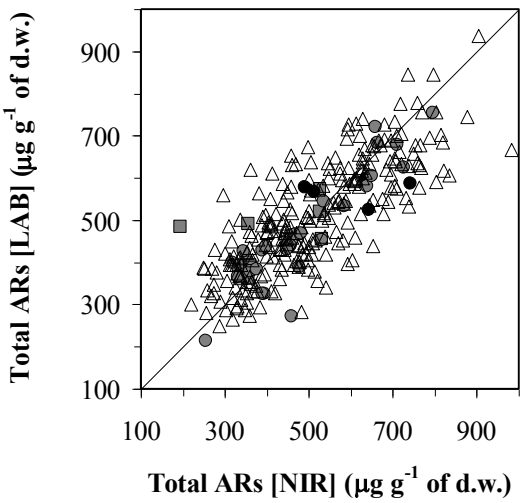

Figure 3. Scatter plot of reference versus predicted values for total ARs of 2005, 2006 and 2007 samples using the wheat sample set. Legend: △ winter wheat; ■ durum wheat; ● spring wheat; ● spelt. N = 292, R^2 = 0.70, SECV = 91.6, f = 11.

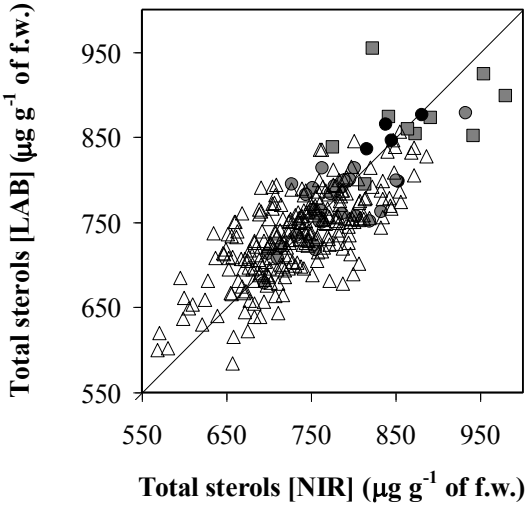

Figure 4. Scatter plot of reference versus predicted values for total sterols of 2005, 2006 and 2007 samples using the wheat sample set. Legend: △ winter wheat; ■ durum wheat; ● spring wheat; ● spelt. N = 292, R^2 = 0.66, SECV = 43.2, f = 11.

Statistical values (Table 1) confirmed that total ARs and total sterols can be detected in whole meals with acceptable accuracy. The R^2 of the total sterols model was slightly better than that for total ARs in the case of the complete sample set but the opposite was found for models calculated on wheat samples only.

Table 1. Results of mPLS calibrations calculated with and without repeatability file.

Sample set	whole				wheat			
Constituent	Total ARs (µg^{-1} g of d.w.)		Total sterols (µg^{-1} g of f.w.)		Total ARs (µg^{-1} g of d.w.)		Total sterols (µg^{-1} g of f.w.)	
Instrument	STM	RCA	STM	RCA	STM	RCA	STM	RCA
N	347		352		292		292	
Range	32.2 – 1444.0		567 – 1276		194.3 – 981.0		568 – 980	
Mean	538.9		780.5		505.3		744.4	
Std. dev.	214.7		117.4		151.5		67.2	
R^2	0.703 *0.813*	0.729 *0.815*	0.803 *0.872*	0.789 *0.810*	0.580 *0.699*	0.541 *0.665*	0.553 *0.663*	0.528 *0.565*
SECV	123.0 *108.8*	119.4 *110.0*	53.8 *46.9*	56.8 *56.1*	101.4 *91.7*	106.7 *99.8*	45.8 *43.2*	47.6 *45.6*
f	12 *15*	11 *14*	12 *15*	11 *10*	10 *11*	9 *10*	10 *11*	8 *6*

Conclusion

Although it is known that total sterol content can be measured non-destructively by NIR in winter rapeseed,[9] in this study it was also demonstrated for cereals. There is only one publication connected with ARs in cereals using GC-FTIR-MS after a laborious extraction and base-acid clean-up procedures. These calibration models would be suitable for use by plant-breeders for assessment of the total AR and total sterol level 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.

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