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APPLICATION OF HPLC-PDA METHOD USING TWO DIFFERENT EXTRACTION PROCEDURES FOR THE DETERMINATION OF ALKYLRESORCINOLS IN CEREALS

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Cereals, especially barley, are an important source of vitamins, minerals, dietary fibre and various phytochemicals, such as alkylresorcinols (ARs). Cereal ARs are a group of phenolic lipids located in the outer parts of grain, particularly in rye and wheat, but not found in refined flour or in refined products from cereals. This study focuses on the comparison of different extraction procedures applied for the determination of the content of ARs (C15:0 – C23:0) in grain of Latvian barley genotypes. The content of ARs in 1 rye and 16 barley samples grown with different amounts of fertilier was determined by High Performance Liquid Chromatography method with Photodiode Array detection (HPLC-PDA) developed by us. Two different extraction methods were compared: accelerated Soxhlet extraction and 24-hour extraction. Aside from validation of the extraction procedures, validation parameters for the HPLC-PDA based quantitation method were provided. The coefficients of variation for repeatability and intermediate precision were < 9% and < 3%, respectively. The content of ARs determined with the HPLC-PDA method in conjunction with accelerated Soxhlet extraction was up to 1.5 times higher than using 24-hour extraction. AR content varied from 2.11 ± 0.04 to 3.80 ± 0.10 mg·100 g^{-1} for 24-hour extraction and from 2.66 ± 0.06 to 5.70 ± 0.20 mg·100 g^{-1} for accelerated Soxhlet extraction, indicating the increased efficiency of this procedure in analysis of ARs.

Key words: *rye, barley, HPLC-PDA, accelerated Soxhlet extraction methods.*

INTRODUCTION

The consumption of whole grain foods is strongly associated in many epidemiological studies with a reduced incidence of diet-related diseases, e.g. cardiovascular disease, type II diabetes and certain cancer types (Montonen *et al.*, 2003; Munter *et al.*, 2007; Gil *et al.*, 2011).

Alkylresorcinols (ARs) are phenolic lipid compounds from the bran fraction in cereals such as rye, wheat, triticale, and barley (Zarnowski and Suzuki, 2004a; Ross, 2012a). ARs are 3-dihydroxy-5-alkylbenzene derivatives with odd-numbered, mostly saturated hydrocarbon side chains typically ranging from C15 to C25. More than 99% of ARs are located in the intermediate layer of the grain (Landberg *et al.*, 2008). ARs have been suggested as potential biomarkers for whole grain wheat, rye, triticale and barley in food products (Ross *et al.*, 2012b) and have been used in multi-analyte methods for determining the presence of different cereal fractions in foods (Barron *et al.*, 2011).

The potential health benefits and biological activities of ARs have generated significant scientific interest. For example, ARs from wheat bran may contribute to colon cancer prevention, as indicated by *in vitro* and *in vivo* animal studies (Ross, 2012b). These compounds can incorporate into membranes and inhibit the activity of some enzymes (Stasiuk and Kozubek, 2010). ARs have antigenotoxic and antioxidant activity in protecting lipids against air-induced oxidation under *in vitro* conditions (Korycinska *et al.*, 2009). Dietary ARs also regulate γ-tocopherol and cholesterol levels in rat liver (Ross *et al.*, 2004). In addition, ARs can be used as a method for checking contamination of non-gluten containing cereals with gluten containing cereals (wheat, rye, and barley) (Ross, 2012b). ARs are not destroyed during food processing (Gunenc *et al.*, 2013a) and are well absorbed in humans (Ross *et al.*, 2003).

ARs in cereal grain and products have been quantified using high performance liquid chromatography HPLC–DAD

(Geerkens *et al.*, 2015), (HPLC)-Coularray-Based Electrochemical Detection (Ross and Kochhar, 2009), ultra-high-pressure liquid chromatography (UHPLC) (Ross, 2012a), gas chromatography (GC) (Ross *et al.*, 2001; Landberg *et al.*, 2009; Athukorala *et al.*, 2010; Gunenc *et al.*, 2013b) and colorimetric method by using Fast Blue B BF_4 (Korycinska *et al.*, 2009). Various extraction methods are used: 24-h extraction with acetone (Gunenc *et al.*, 2013b) or ethyl acetate (Ross *et al.*, 2001; Landberg *et al.*, 2008) and continuous stirring at room temperature, by soaking plant material three times with the same amount of acetone for 24 h each (Zarnowski *et al.*, 2002), 48 h extraction with acetone by continuous stirring at room temperature (Korycinska *et al.*, 2009), extraction using the Soxhlet apparatus (Zarnowski and Suzuki, 2004b); a two-step sequential supercritical carbon dioxide ($SC–CO_2$) extraction technique (Athukorala *et al.*, 2010) and a rapid ultrasound-assisted extraction procedure was used recently (Geerkens *et al.*, 2015)**.**

Barley grain was chosen for this study because of its growing popularity in human consumption, high content of fibre, phenolic compounds and a low amount of ARs, which is very useful for method validation. Rye grain was included because of its high amount of ARs and for comparison of recovery parameters with barley samples.

The main purpose of this work was to validate our developed accelerated Soxhlet extraction method for extraction of ARs from cereals and to compare the results with the 24-h extraction procedure, which was conducted on a shaker table. Also we sought to simultaneously verify the applicability and efficiency of our HPLC-PDA method for the quantitation of ARs homologues in the grain extracts, including the most common validation parameters. To our knowledge there have been no reports about validation of the accelerated Soxhlet extraction method in conjunction with HPLC-PDA.

MATERIALS AND METHODS

Chemicals. Authentic AR standards C15:0 (5-*n*-pentadecylresorcinol), C19:0 (5-*n*-nonadecylresorcinol), C21:0 (5-*n*-heneicosylresorcinol) and C23:0 (5-*n*-tricosylresorcinol) were purchased from Fluka (St. Louis, USA) and were ?95% purity. All standards were prepared as 2.5 $mg \cdot ml^{-1}$ stock solutions in methanol/ethanol mixture and were stored in a freezer at –21 °C. Methyl behenate (internal standard) was purchased from Fluka (St. Louis, USA). All solvents were of HPLC grade (Sigma-Aldrich, St. Louis, USA). Deionised water was used throughout the analysis.

Raw material and sample preparation. Barley were cultivated both organically and conventionally, and rye only conventionally. Organic field. The soil type was sod-podzolic (PVv), sandy loam and loamy sand. Organic matter content in soil was 20.2–21.6 mg/kg, the soil pH_{KCl} was 5.27–5.89, the content of plant-available phosphorus P_2O_5 138–164 mg/kg, and potassium K_2O 130–175 mg/kg. The common agronomic practices for organic management were used during the vegetation period. Conventional field. The soil type in the conventional field was sod-podzolic (PVv), sandy loam, content of organic substance 21–24 mg/kg, soil pH KCl 5.4–5.8, available phosphorus P_2O_5 137.0–158.8 mg/kg, and potassium K_2O 211.0–175.7 mg/kg. The experimental treatment consisted of three N rates — N80, N120, N160 in conventional growing conditions. Complex mineral fertiliser was used as a basic fertiliser at the rate 725 kg/ha (pure matter N – 80 kg/ha, P – 28.6, K – 112.4 kg/ha. The N application was split, part of the N being applied at the time of sowing and the remaining half at the end of tillering stage (growing stage/GS 29) of the crop. Ammonium nitrate (N 34%) was used a top-fertiliser in the following amount: 40 kg of N per ha (N120) and 80 kg of N per ha (N160). The treatments were laid out in a randomised complete block design; the plot size was 10 m^2, four replicates.

Barley and rye genotypes were sown with a compact trial drill 'Hege 80' in a well prepared seedbed at a rate of 500 germinating seeds per m². The plot size was 10 m^2, four replicates.

Cereal grain samples (16 barley samples and one rye sample) harvested in 2013 were provided by the State Stende Cereal Breeding Institute (SSCBI) (Latvia) (Table 1). All grain samples were milled and sieved in order to obtain the required particle size of 0.7 mm.

The 24-h procedure for extraction of ARs. According to a slightly modified method of Ross *et al.* (2001), ARs were extracted from a 0.50 ± 0.01 g samples of cereal grains with 20 mL of ethyl acetate for 24 h with continuous shaking on a shaker table (B Braun Biotech Certomat SII 886252/4, Germany) at 270 rpm and room temperature. The extract was centrifuged at 4500 rpm for 10 min and the supernatant (4 mL) was then evaporated to dryness. Methanol (0.5 mL) was added and the samples were filtered through 0.2 μm PES (Vivaspin 500, Sartorius Stedim Biotech GmbH, Germany) filters and centrifuged for 5 min at 3000 rpm before injection into the HPLC.

Accelerated Soxhlet extraction method (ASE). The extraction was performed with a Soxtec™ 2055 Fat Extraction System (FOSS Analytical, Denmark). Grain samples (2.0 ± 0.1 g) were weighed into a cellulose thimble (33 × 80 mm) together with glass wool to obtain a clear extract, which was inserted into the extraction unit. Dichloromethane and hexane as 1 : 1 mixture (75 mL) was added to the system. The cups were heated on an electrical hot plate to 130 °C. The four-step extraction procedure consisted of boiling (30 min), rinsing (30 min), recovery (10 min) and pre-drying (3 min). The extracts were evaporated under slow nitrogen stream and methanol (1.0 mL) was added. The samples were shaken and ultrasonicated for 5 minutes, then centrifuged at 4500 rpm for 10 min. Portions of extract (200 μL) were filtered through 0.2 μm PES filters and centrifuged for 5 min at 3000 rpm before injection into the HPLC instrument. Dichloromethane and hexane were chosen as more

Table 1

DESCRIPTION OF BARLEY AND RYE SAMPLES

Coding system*		Lines	Description	Additional information
Cultivar / genotype +	Growing system			
Barley				
1185	Grown organically:	Simba/Wanubet	hulless barley breeding line	SSCBI** breeding material
Kornelija	'+BIO'	IC360	hulless barley breeding line	bred at SSCBI, 2014
1165		Gainer/Freedom	hulless barley breeding line	SSCBI breeding material
Ansis	Grown conventionally:	-	widely grown malting barley variety	bred at SSCBI, 1999
	'+80'–N supply 80 kg/ha			
Rye	'+120'–N supply 120 kg/ha			
Kaupo	'+160'–N supply 160 kg/ha	Tulvi/ Vambo/CHD-181/Muro		bred at PCBI***, 1998

* An example of coding system: 1185+BIO – barley genotype grown organically; 1185+120 – barley genotype grown conventionally with N supply 120 kg/ha; ** SSCBI, State Stende Cereal Breeding Institute; *** PCBI, Priekuļi Crop Breeding Institute

appropriate solvents due to better compatibility with the ASE apparatus.

All samples were extracted in duplicate, and the results are reported based of fresh weight (FW). AR homologues C15:0, C19:0, C21:0 and C23:0 were quantified using an external standard method and were identified also according to their spectra (Kulawinek and Kozubek, 2008). All quantifications were performed using linear calibration curves generated with authentic standards (from 0.5 to 600 $\mu g \cdot mL^{-1}$) and were based on peak area.

HPLC-PDA analysis. The analyses were carried out on a Alliance 2695 HPLC (Waters, USA) with a Waters Photodiode Array Detector 2996 scanning between 260 and 295 nm. Separation of AR homologues was achieved on a Luna C18 column (4.6 × 150 mm, 5 µm) from Phenomenex (USA). The gradient programme used was: flow rate of 1.00 $mL \cdot min^{-1}$ starting with a mobile phase of methanol/water (80/20) for 5 min, followed by methanol/water (99.2/0.8) for 25 min. The temperature of the column oven was set at 30 ^{o}C and 40 µL portions of each sample were injected into the chromatographic column.

Validation parameters for HPLC-PDA method. Signal linearity. Linearity of calibration curves was evaluated by the coefficient of correlation (R^2), testing pure standards of AR homologues (C15:0, C19:0, C21:0 and C23:0) in the concentration range from 0.5 $\mu g \cdot mL^{-1}$ to 600 $\mu g \cdot ml^{-1}$.

Limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values for C15:0, C19:0, C21:0 and C23:0 homologues were obtained from the standard deviation of ten replicates using 0.5 $\mu g \cdot mL^{-1}$ of authentic standard (the instrument LOQ/LOD) and ten replicates of barley sample containing 0.5 $\mu g \cdot mL^{-1}$ for 24-h extraction and 5.0 $\mu g \cdot ml^{-1}$ for ASE according to Magnusson and Ornemark (2014), where $LOD = 3 \cdot S_o/n^{0.5}$; $LOQ = 10 \cdot S_o/n^{0.5}$ (S_o – estimated standard deviation of single results at or near zero concentration; n – the number of replicates averaged in routine use).

Repeatability and intermediate precision. Repeatability was ascertained by two different methods. In the first method, repeatability was validated at three different concentrations (0.5, 5.0 and 100 $\mu g \cdot mL^{-1}$) of the reference substances C15:0; C19:0; C21:0 and C23:0 with three replicates according to the International guideline (International Conference on Harmonisation, 2005). The parameter was expressed by the coefficient of variation (CV) of means.

In the second method, repeatability was validated by seven independent determinations using two different concentrations (0.5 and 5.0 $\mu g \cdot mL^{-1}$) of the reference substances C15:0; C19:0; C21:0 and C23:0 with a two-day gap according to The Fitness for Purpose of Analytical Methods (2014). In addition, repeatability experiments with barley and rye samples were conducted also with the standard addition method:

a) A 1 ml aliquot of 5.0 $\mu g \cdot ml^{-1}$ methanolic standard (C15:0; C19:0; C21:0 and C23:0) was evaporated to dryness in the extraction flask and 24 h extraction with HPLC analysis were carried out as described above (in six replicates).

b) Aliquots of 40 µl (100 $\mu g \cdot mL^{-1}$) and 160 µl (400 $\mu g \cdot mL^{-1}$) from 2.5 $mg \cdot ml^{-1}$ methanolic standards of C15:0 and C23:0 were added to barley and rye samples before applying the ASE method, respectively (in three replicates).

Repeatability was expressed by the coefficient of variation (CV) of mean values.

Intermediate precision was ascertained by six independent determinations with a two-week gap. This experiment was conducted with authentic standards only by using 0.5

$\mu g \cdot mL^{-1}$ concentration of the reference substances C15:0; C19:0; C21:0 and C23:0, respectively. The parameter was expressed by the coefficient of variation of mean values.

Recovery. (a) The 24-h extraction recovery for barley and rye flour was determined by adding authentic standards (C15:0, C19:0; C21:0 and C23:0), using concentrations in the range of 5.0–100 $\mu g \cdot ml^{-1}$. An aliquot of 1 mL methanolic standard solution was evaporated to dryness in the extraction flask followed by 24-h extraction and HPLC analysis as described above; (b) accelerated Soxhlet extraction recovery was determined by addition of authentic C15:0 and C23:0 standards at concentrations of 20 and 100 $\mu g \cdot ml^{-1}$ for barley; 100 and 400 $\mu g \cdot ml^{-1}$ for rye.

Recovery experiments were conducted in triplicate and evaluated according to Causon (1997). The parameter was expressed by the CV of mean values.

RESULTS

Method validation. All calibration curves of AR standards C15:0, C19:0, C21:0 and C23:0 showed linearity ($R^2 > 0.99$) of the PDA signal at 275 nm over a wide range of concentrations: 0.5–600 $\mu g \cdot mL^{-1}$. Instrument LOD and LOQ were the lowest for C15:0 with 0.67 and 2.2 ng on column. Method LOD_{24h} and LOQ_{24h} were the lowest for C15:0 with 1.7 and 5.8 ng on column and method LOD_{ASE} and LOQ_{ASE} were also the lowest for C15:0 with 13 and 45 ng on column (see Table 2).

Averaged recoveries varied from 91% to 95% for all ARs tested, and for both extraction methods (Table 3) with CV < 3.7%. An exception was observed for C15:0 (88.5%) in a barley sample and for C19:0 (89.3%) in a rye sample when using the 24-h extraction method.

Coefficients of variation of repeatability for pure standard experiments (according to two different international guidelines) were < 3.0%, with the best value for C15:0 (CV < 1.6%). The CV values for repeatability parameters when using the 24-h extraction method varied from 2.7% to 9.1% for barley and rye samples, and from 0.8% to 5.0% for both samples when using the ASE method. As shown in Table 2, the obtained coefficient of variation for intermediate precision for all homologues of ARs was < 3.4%.

Application of the developed method for the quantification of ARs in barley and rye grains. Sixteen barley samples and one rye sample were analysed by our developed HPLC-PDA method in conjunction with accelerated Soxhlet extraction system for quantification of AR concentration in grains. The 24-h extraction method was used to compare the results with ASE and also for validation. All data were converted to a fresh weight (FW), considering that the moisture content of cereals is expected to be ≥ 14% according to Commission Regulation (EC) 687/2008. The results

Table 2

LIMIT OF DETECTION AND QUANTIFICATION, COEFFICIENT OF CORRELATION (R^2) AND REPEATABILITY OF THE HPLC-PDA METHOD

ARs homologue	LOD Instrument, ng	LOQ Instrument, ng	LOD 24h method, ng	LOD ASE method, ng	LOQ 24h method, ng	LOQ ASE method, ng	R2	Concentration range of pure standard[b], $\mu g\ mL^{-1}$	Intermediate precision, CV (%)	Repeatability, CV (%)					
									Pure standard	Pure standard[d]	Pure standard[e]	Barley flour[f]	Barley flour[g]	Rye flour[f]	Rye flour[g]
C15:0	0.67	2.23	1.73[a]	13.4[a1]	5.77[a]	44.8[a1]	0.9994	0.5-100; 400	< 1.7[c] < 1.5[c1]	< 1.6	< 3.2	2.7	0.8	7.3	2.1
C19:0	1.14	3.80	3.04	16.0	10.2	53.2	0.9982	0.5-100	< 2.1 < 3.4	< 2.9	< 3.0	6.1	-	9.1	-
C21:0	1.76	5.85	2.31	18.0	7.70	60.0	0.9990	0.5-100	< 2.0 < 3.4	< 2.3	< 3.1	5.5	-	6.3	-
C23:0	0.86	2.87	2.79	13.6	9.30	45.3	0.9998	0.5-100; 400	< 2.5 < 1.9	< 1.7	< 1.7	3.2	2.7	7.1	5.0

[a] Averaged recovery from ten replicates (barley samples) of C15:0, C19:0, C21:0 and C23:0 was 95.5%, when using the 24 h extraction method (0.5 $\mu g \cdot mL^{-1}$).

[a1] Averaged recovery from ten replicates (barley samples) of C15:0, C19:0, C21:0 and C23:0 was 94.1%, when using the ASE extraction method (5.0 $\mu g \cdot mL^{-1}$).

[b] for repeatability experiments different concentrations were tested (see Section 2.4.3.).

[c] CV of intermediate precision parameter

[c1] CV of intermediate precision parameter after two weeks

[d] according to international guideline (International Conference on Harmonisation, 2005.)

[e] according to The Fitness for Purpose of Analytical Methods (2014).

[f] 24 h extraction method.

[g] accelerated Soxhlet extraction method (ASE).

Table 3

RECOVERY OF ARS FROM BARLEY AND RYE FLOUR

Food matrix	Extraction method	AR homologue	Concentration spiked, $\mu g \cdot mL^{-1}$	Recovery, %	Averaged recovery, %
Barley flour	24-h method	C15:0	5.0	75.2 ± 2.7	88.5 ± 3.6
			25	93.9 ± 4.2	
			100	96.4 ± 4.0	
	ASE method	C15:0	20	97.5 ± 2.9	91.4 ± 1.9
			100	85.2 ± 0.8	
	24-h method	C19:0	5.0	97.4 ± 6.1	95.3 ± 3.7
			25	95.4 ± 2.1	
			100	93.1 ± 2.8	
	24-h method	C21:0	5.0	91.4 ± 5.5	95.0 ± 3.1
			25	95.2 ± 2.7	
			100	98.4 ± 1.0	
	24-h method	C23:0	5.0	94.3 ± 3.2	92.8 ± 3.9
			25	92.3 ± 3.6	
			100	91.9 ± 5.0	
	ASE method	C23:0	20	98.1±0.4	91.3 ± 1.6
			100	84.5±2.7	
Rye flour	24-h method	C15:0	5.0	88.0 ± 7.3	93.7±4.7
			25	94.8 ± 1.6	
			100	98.3 ± 5.1	
	ASE method	C15:0	100	94.7 ± 5.8	94.1 ± 4.0
			400	93.4 ± 2.1	
	24-h method	C19:0	5.0	98.2 ± 9.1	89.3 ± 6.7
			25	95.6 ± 2.7	
			100	74.1 ± 8.4	
	24-h method	C21:0	5.0	89.0 ± 6.3	92.3 ± 4.0
			25	97.7 ± 2.0	
			100	90.2 ± 3.8	
	24-h method	C23:0	5.0	97.7 ± 7.1	93.1 ± 4.0
			25	95.4 ± 3.0	
			100	86.1 ± 1.9	
	ASE method	C23:0	100	92.6 ± 6.1	94.1 ± 5.6
			400	95.6 ± 5.0	

of AR analysis (Table 4) varied from 2.11 ± 0.04 to 3.8 ± 0.1 $mg \cdot 100\ g^{-1}$ FW in the case of 24-hour extraction and from 2.66 ± 0.06 to 5.7 ± 0.2 $mg \cdot 100\ g^{-1}$ FW in the case of accelerated Soxhlet extraction.

DISCUSSION

Method validation for analyses of ARs in cereals. Validation of the elaborated method demonstrated that all calibration curves of AR standards showed good linearity ($R^2 > 0.99$) over a wide range of concentrations. Instrument LOD and LOQ for C15:0 were 0.67 and 2.23 ng on column, whereas Geerkens *et al.* (2015) reported a lower sensitivity for C21:0 with LOD 1.2 ng and LOQ 3.6 ng on column. Method LOD_{24h} and LOQ_{24h} for C15:0 were 1.73 and 5.77 ng on column and method LOD_{ASE} and LOQ_{ASE} for C15:0 were 13.4 and 44.8 ng on column, while Ross (2012a) reported the best sensitivity of LOD = 0.5 ng on column and LOQ = 1.25 ng on column by applying ultra-high-performance chromatography with UV detection at 276 nm.

The elaborated method demonstrated an appropriate recovery rate for all ARs tested, and for both extraction methods with CV < 3.7%. Repeatability was determined according to international guidelines (Anonymous 2005) (Table 2) and the obtained coefficients of variation of repeatability for pure standard experiments were even below 3.0% and this criteria indicated full appropriateness for determination of ARs in grain samples.

Different CV values were obtained for repeatability parameters when between the 24-h extraction method (from 2.7% to 9.1%) and ASE method (from 0.8% to 5.0%). Therefore, the procedure proposed within the current study demonstrated clearly improved repeatability. In a previous study, Ross and Kochhar (2009) showed improved repeatability for decreased alkyl chain length from C25:0 to C21:0. In our research, this tendency was observed when using the ASE method (Table 2) — from C23:0 to C15:0 for both cereal samples.

Content of ARs in barley and rye grains. Low instrument detection limit (≤1.76 ng on column) and good repeatability of authentic standards (CV ≤ 3.0%) make an elaborated HPLC-PDA method an appropriate choice for AR detection in cereals. The results obtained with the HPLC-PDA method in conjunction with accelerated Soxhlet extraction

Table 4

ARS CONCENTRATIONS IN BARLEY AND RYE GRAINS OBTAINED BY THE APPLICATION OF TWO DIFFERENT EXTRACTION METHODS, mg·100 g^{-1} FW

Genotype		ARs homologue									
		C15:0		C19:0		C21:0		C23:0		Total	
		24 ha	ASE[b]	24 h	ASE	24 h	ASE	24 h	ASE	24 h	ASE
Barley grains	1185 + 80	n.d.[c]	n.d.	0.46 ± 0.02[d]	0.49 ± 0.03	1.08 ± 0.03	1.30 ± 0.04	0.83 ± 0.07	0.92 ± 0.03	2.37 ± 0.04	2.71 ± 0.03
	1185 + 120	n.d.	n.d.	0.38 ± 0.02	0.52 ± 0.01	1.04 ± 0.01	1.30 ± 0.04	0.82 ± 0.02	0.96 ± 0.03	2.24 ± 0.02	2.78 ± 0.02
	1185 + 160	n.d.	n.d.	0.44 ± 0.01	0.50 ± 0.10	1.05 ± 0.08	1.30 ± 0.01	0.79 ± 0.01	0.86 ± 0.04	2.27 ± 0.04	2.66 ± 0.06
	1185 + Bio	n.d.	n.d.	0.52 ± 0.01	0.50 ± 0.10	1.08 ± 0.08	1.29 ± 0.04	0.87 ± 0.01	0.90 ± 0.07	2.47 ± 0.03	2.69 ± 0.08
	Kornelija + 80	n.d.	n.d.	0.37 ± 0.01	0.36 ± 0.03	1.08 ± 0.01	1.41 ± 0.02	0.96 ± 0.03	1.01 ± 0.07	2.41 ± 0.02	2.77 ± 0.04
	Kornelija + 120	n.d.	n.d.	0.26 ± 0.01	0.25 ± 0.01	0.98 ± 0.01	1.48 ± 0.02	0.90 ± 0.10	0.97 ± 0.04	2.11 ± 0.04	2.70 ± 0.02
	Kornelija + 160	n.d.	n.d.	0.30 ± 0.01	0.22 ± 0.02	1.00 ± 0.10	1.24 ± 0.01	1.17 ± 0.05	0.93 ± 0.01	2.49 ± 0.05	2.38 ± 0.01
	Kornelija + Bio	n.d.	n.d.	0.37 ± 0.06	0.40 ± 0.04	1.25 ± 0.02	1.60 ± 0.02	1.01 ± 0.02	1.30 ± 0.10	2.63 ± 0.03	3.35 ± 0.06
	1165 + 80	n.d.	n.d.	0.72 ± 0.10	0.76 ± 0.05	1.55 ± 0.10	1.94 ± 0.07	0.80 ± 0.10	1.15 ± 0.05	3.10 ± 0.10	3.85 ± 0.06
	1165 + 120	n.d.	n.d.	0.73 ± 0.02	0.74 ± 0.04	1.44 ± 0.01	2.39 ± 0.03	0.88 ± 0.01	1.60 ± 0.07	3.05 ± 0.01	4.73 ± 0.05
	1165 + 160	n.d.	n.d.	0.76 ± 0.01	0.75 ± 0.02	1.52 ± 0.08	1.90 ± 0.10	1.00 ± 0.20	1.20 ± 0.02	3.31 ± 0.09	3.88 ± 0.05
	1165 + Bio	n.d.	n.d.	0.82 ± 0.07	0.78 ± 0.03	1.72 ± 0.01	1.90 ± 0.40	1.17 ± 0.02	1.20 ± 0.20	3.72 ± 0.03	3.90 ± 0.20
	Ansis + 80	n.d.	n.d.	0.79 ± 0.04	0.94 ± 0.07	1.61 ± 0.09	2.50 ± 0.40	1.11 ± 0.07	1.50 ± 0.10	3.51 ± 0.07	4.90 ± 0.20
	Ansis + 120	n.d.	n.d.	0.90 ± 0.10	0.90 ± 0.10	1.60 ± 0.10	2.18 ± 0.07	1.15 ± 0.01	1.39 ± 0.02	3.64 ± 0.09	4.45 ± 0.07
	Ansis + 160	n.d.	n.d.	0.70 ± 0.01	0.75 ± 0.05	1.24 ± 0.01	1.76 ± 0.07	0.93 ± 0.01	1.10 ± 0.10	2.87 ± 0.01	3.61 ± 0.09
	Ansis + Bio	n.d.	n.d.	0.96 ± 0.03	1.30 ± 0.10	1.90 ± 0.20	2.88 ± 0.05	0.91 ± 0.08	1.50 ± 0.30	3.80 ± 0.10	5.70 ± 0.20
Rye grain	Kaupo + 80	0.33 ± 0.01	2.38 ± 0.05	23.00 ± 0.50	26.2 ± 0.3	16.0 ± 2.0	16.0 ± 0.5	9.10 ± 0.70	9.40 ± 0.50	48.0 ± 1.0	54.0 ± 0.3

[a] 24-h extraction method.

[b] ASE, accelerated Soxhlet extraction method.

[c] n.d., not detected.

[d] Concentrations are expressed by the mean value of two replicates ± standard deviation (CV 10%).

were up to 1.5 times higher than the results obtained using 24-hour extraction of barley samples (Fig. 1). Data obtained for barley samples in the current study are in line with previously reported results: 3.9 mg·100 g^{-1} FW for C19:0, C21:0 and C23:0 by Landberg *et al.* (2009), 4.6 mg·100 g^{-1} by Zarnowski and Suzuki (2004b) for C15:0, C19:0, C21:0 and C23:0 homologues, although higher than reported by Bobere *et al.* (2013) (≤ 1.67 mg·100 g^{-1} FW for C21:0 and C23:0). However, such variations are probably due in part to differences in analytical methods and also the AR content of cereals appears to be highly variable, depending on cultivar and environmental conditions.

The AR content in the rye sample was higher in the case of using the accelerated Soxhlet extraction method: 54.0 ± 0.3 mg·100 g^{-1} FW for ASE and 48.0 ± 1.0 mg·100 g^{-1} FW for 24 h, respectively. These values are comparable to those reported previously: 50.9 mg·100 g^{-1} FW by Geerkens *et al.* (2015), 57.3 mg·100 g^{-1} FW by Landberg *et al.* (2007), although lower than reported by Bobere *et al.* (2013), where the averaged content of C15:0, C19:0, C21:0 and C23:0 was 61.5 mg·100 g^{-1} FW.

Assessment of the AR content depending on barley genotype showed significantly higher concentration found in the 'Ansis' genotype (4.67 ± 0.87 mg·100 g^{-1} FW, followed by '1165' (4.09 ± 0.43 mg·100 g^{-1} FW), 'Kornelija' (2.80 ± 0.40 mg·100 g^{-1} FW) and '1185' (2.71 ± 0.05 mg 100·g^{-1} FW) genotypes. No statistically significant effect of growing conditions on AR content was observed in the current study.

Not all laboratories have modern chromatographic and extraction equipment available. The HPLC-PDA apparatus is suitable and easy to use by an expert. The accelerated Soxhlet extraction method developed in our study has only one drawback — it requires 75 mL of solvent, although dichloromethane and hexane have relatively low toxicity and low cost. The extraction time is only a little longer than one hour, and the extracted amounts are higher compared to 24-h extraction, repeatability is ≤ 5% for barley and rye grains, and the average recovery is ≥ 88% and ≥ 93% for barley and rye, respectively. Application of the ASE method and dichloromethane/hexane extraction is useful, as it is both a rapid and reliable preparative procedure. These advantages make ASE an excellent alternative to other extraction methods and will be also valuable as an extraction method for food and feed analyses.

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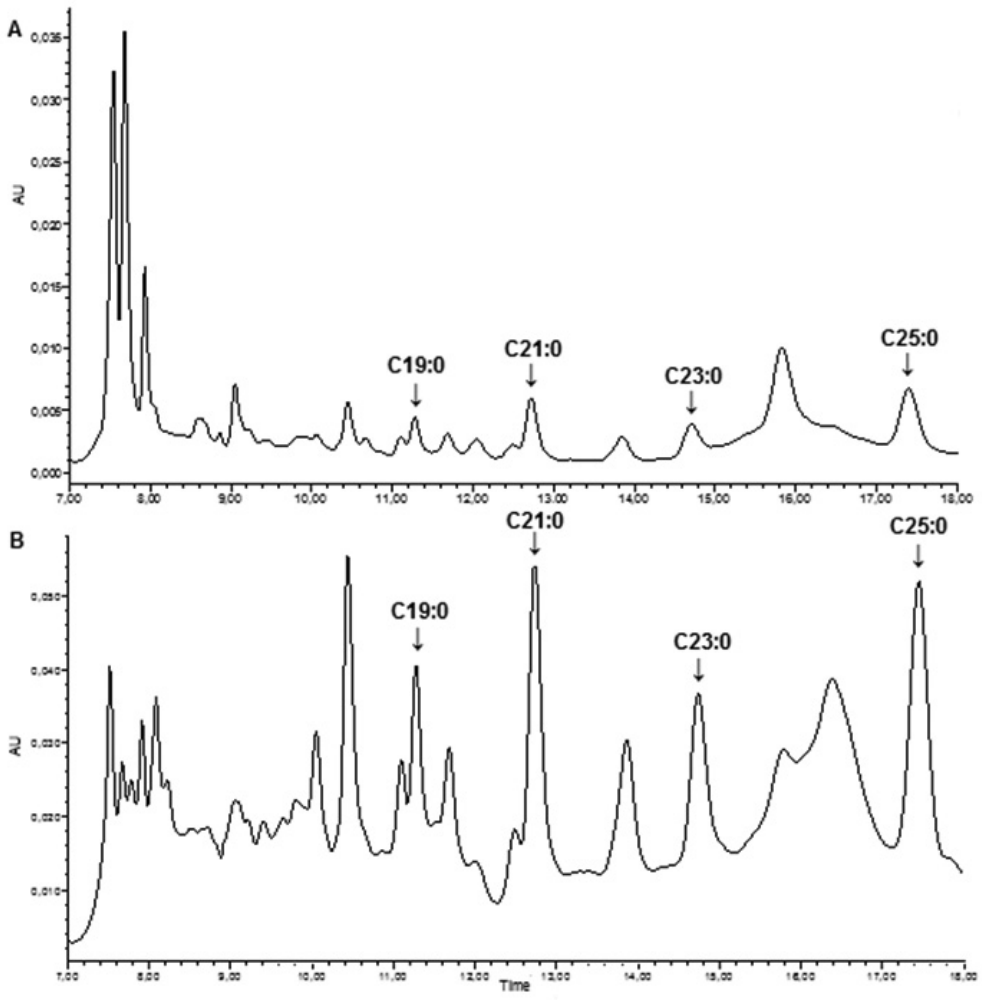

Fig. 1. HPLC-PDA chromatograms of AR homologues in barley Ansis after A) 24-h extraction with ethyl acetate and B) after accelerated Soxhlet extraction with dichloromethane/hexane mixture. See Section 2.3. for the HPLC conditions.

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HPLC-PDA PIEMĒROŠANA, IZMANTOJOT DIVAS EKSTRAKCIJAS METODES ALKILREZORCĪNU NOTEIKŠANAI GRAUDAUGOS

Graudaugi, īpaši mieži ir nozīmīgs vitamīnu, minerālvielu, šķiedrvielu, kā arī dažādu fitoestrogēnu, tai skaitā alkilrezorcīnu (AR), avots. Graudaugu AR pieder pie fenolu savienojumu grupas, kur viena molekulas daļa ir fenola atlikums, bet otra ir lipīdu atlikums. AR atrodami grauda ārējos slāņos galvenokārt rudzu un kviešu graudos, bet nav sastopami rafinētos miltos vai citos rafinētos graudaugu produktos. Šī pētījuma pamatā ir alkilrezorcīnu (C15:0 – C23:0) daudzuma noteikšana Latvijā selekcionētos graudaugu genotipos. Tika noteikts AR daudzums 1 rudzu un 16 miežu paraugos, kas audzēti ar dažādiem mēslojuma daudzumiem, izmantojot izstrādāto augsti efektīvās šķidruma hromatogrāfijas metodi ar fotodiožu matricas detektoru (HPLC-PDA). Tika salīdzinātas divas ekstrakcijas metodes — paātrinātā Soksleta ekstrakcija un 24 stundu ekstrakcija. Papildus ekstrakcijas metožu validācijai tika iegūti validācijas parametri kvantitatīvai AR noteikšanai graudaugos ar HPLC-PDA metodi. Metodes atkārtojamības variācijas koeficients un precizitāte attiecīgi ir < 9% un < 3%. Līdz pat 1,5 reizes lielāks AR daudzums tika noteikts ar HPLC-PDA metodi savienojumā ar paātrināto Soksleta ekstracijas metodi nekā ar 24 stundu ekstrakciju. Rezultāti variēja no 2,11 ± 0,04 līdz 3,80 ± 0,10 mg·100 g^{-1} 24 stundu ekstrakcijā un no 2,66 ± 0,06 līdz 5,70 ± 0,20 mg·100g^{-1}, izmantojot paātrināto Soksleta metodi. Šie dati norāda uz paātrinātās Soksleta ekstrakcijas metodes lielāku efektivitāti AR noteikšanas analīzē.