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NMR metabolomics of ripened and developing oilseed rape (*Brassica napus*) and turnip rape (*Brassica rapa*)

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ABSTRACT

The oilseeds of the commercially important oilseed rape (*Brassica napus*) and turnip rape (*Brassica rapa*) were investigated with ^{1}H NMR metabolomics. The compositions of ripened (cultivated in field trials) and developing seeds (cultivated in controlled conditions) were compared in multivariate models using principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA). Differences in the major lipids and the minor metabolites between the two species were found. A higher content of polyunsaturated fatty acids and sucrose were observed in turnip rape, while the overall oil content and sinapine levels were higher in oilseed rape. The genotype traits were negligible compared to the effect of the growing site and concomitant conditions on the oilseed metabolome. This study demonstrates the applicability of NMR-based analysis in determining the species, geographical origin, developmental stage, and quality of oilseed *Brassicas*.

1. Introduction

As a raw material for food oils, animal feed, and biofuels, the oleiferous plants of genus *Brassica* are versatile crops. Oilseed rape (*Brassica napus* L. subsp. *oleifera*) is one of the most cultivated oil crops in the world, while turnip rape (*Brassica rapa* L. subsp. *oleifera*) is grown only in limited areas in Northern Europe, and more precisely in Finland, where the summer is too short for oilseed rape. The oils extracted from their seeds are naturally rich in α-linolenic acid, resulting in an ideally low ratio of $n-6/n-3$ fatty acids for human diet (Simopoulos, 2002). However, the abundance of unsaturated bonds can lead to the production of oxidised species during storage, cooking, and digestion, which may cause adverse health effects through oxidative stress and inflammation (Awada et al., 2012; Tarvainen, Phuphusit, Suomela, Kuksis, & Kallio, 2012). The oil content and quality of the raw material used for oil is determined by the genome of the oil plant (mainly through maternal factors) and interactions with the environment (weather, soil, cultivation techniques, biotic and abiotic stresses) (Weselake et al., 2009). The oil content of *Brassicas* is also associated with protein and fibre content and seed colour (Abbadi & Leckband, 2011; Snowdon, Lühs, & Friedt, 2007). Crushed seeds and seed extrudates are important sources of plant protein, but antinutrients, such as glucosinolates, have shadowed their use as feedstuffs. The breeding of *Brassica* has managed to produce seeds with high yield and oil content, free of erucic acid, glucosinolates, sinapic acid esters, and chlorophyll (Abbadi & Leckband, 2011). Glucosinolates are the main group of secondary metabolites, along with phenolic choline esters. The differences in the composition and concentrations of triacylglycerols between *B. napus* and *B. rapa* are not usually significant (Vuorinen et al., 2014). Therefore, the examination of minor components of the oilseeds may be of great importance, since they are more susceptible to exogenous and endogenous variation (Spyros & Dais, 2013).

Many of the nuclear magnetic resonance (NMR) spectroscopy studies on vegetable oils have focused on classification, authentication, and determining of the geographical origin of olive oils with proton NMR (Alonso-Salces et al., 2010; Longobardi et al., 2012; Sacchi et al., 1998; Sacco et al., 2000). ^{13}C and ^{31}P NMR techniques have also been successful in oil analyses (Hatzakis, Koidis, Boskou, & Dais, 2008; Sacchi, Addeo, & Paolillo, 1997; Vigli, Philippidis, Spyros, & Dais, 2003). Apart from studies by Chen, Li, Lei, Zhu, and Zhang (2010), little attention has been paid to the NMR metabolomics of rapeseed oils *per se*, although ^{1}H NMR fingerprinting has been used to distinguish rapeseed (or canola) oil from other vegetable oils and detect its adulteration by animal fats (Fang, Goh, Tay, Lau, & Li, 2013). However, other analytical methods, such as pulse-NMR and near-infrared spectroscopy (NIR), have also been

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used in oilseed analyses (Barthet, 2013). The *B. rapa* leaf composition has been studied using 1D and 2D NMR techniques by Abdel-Farid, Kim, Choi, and Verpoorte (2007) with respect to the effect of cultivar and developmental stage.

The aim of the present work was to study the composition of oilseed rape and turnip rape seeds after a facile extraction procedure, by using NMR spectroscopy and chemometrics. The effects of species, developmental stage, geographical origin, and related growing conditions on the seed composition were specifically viewed.

2. Materials and methods

2.1. Sample material

Ripened seeds of low erucic acid spring rape (oilseed rape; *Brassica napus* L. subsp. *oleifera*) and spring turnip rape (*Brassica rapa* L. subsp. *oleifera*) of the 2011 crops were received *via* MTT Agrifood Research Finland from the official variety test trial sites in Hauho, Inkoo, Jokioinen, Maaninka, and Pernaja (Kangas et al., 2011). The trial sites situate in different cultivation zones according to their geographical location, Inkoo (60.08°N, 24.89°E) and Pernaja (60.45°N, 26.15°E) belonging to zone 1, Jokioinen (60.81°N, 23.50°E) and Hauho (61.15°N, 24.59°E) to zone 2, and Maaninka (63.14°N, 27.32°E) to zone 3, respectively (Supplementary Fig. S1). Classification of zones is based on the temperature sum accumulation of a region during growing season. The turnip rape genotypes chosen for this study were Aurea CL, Bor 05075, Bor 05100, Bor 07010, Cordelia, Juliet, SW Petita, and Viikki 11, while the oilseed rape genotypes were Belinda (hybrid), DLE 1006, DLE 1107, Highlight, Marie, Mirco CL (hybrid), Proximo, Majong (hybrid), SW Q2865, Trapper (hybrid), Tamarin, Brando (hybrid), Early Bird, and Lunedie. Weeds and pests were controlled according to the protocol of the test sites. Plots were harvested when fully matured and the yield obtained was dried after harvest to a moisture content of approximately 9%. Seeds from one or two block samples were randomly selected for extraction. The weather data for each experimental site was calculated based on the data collected by the Finnish Meteorological Institute (Helsinki, Finland) (Table 1).

The oilseed rape genotypes Marie and Bor 01000, and the turnip rape genotypes SW Petita and Bor 05075 were cultivated in controlled conditions as described by Vuorinen et al. (2014). Optimal growing conditions were created in a growth room and in a greenhouse at 22 °C with 16 h day length and at 15–20 °C with 16–19 h day length. Stress conditions were created with the reduced temperature (15 °C, 16 h) in a growth chamber. The siliques were harvested at different time points (2, 3, and 4 weeks after flowering, WAF, with ±3 days marginal per weekly time point) after the start of flowering and stored at −20 °C until further treatment.

2.2. Chemicals

Analytical grade cyclohexane was purchased from Lab-Scan (Dublin, Ireland). Chloroform-*d* ($CDCl_3$, 99.8 atom % D) was from Sigma–Aldrich (St. Louis, MO), and methanol-d_4 (CD_3OD, 99.8 atom % D) from Sigma–Aldrich and VWR International Oy (Espoo, Finland).

2.3. Sample preparation

Ripened seeds (100 mg) were homogenised and extracted with 1.5 ml cyclohexane using an ULTRA-TURRAX T 25 homogeniser (IKA Works, Wilmington, NC), equipped with an 8 G dispenser element. The cyclohexane extract, separated after centrifugation of

Table 1
Accompanying information on the growing sites. Average values of the weather parameters were calculated separately for *B. napus* (*BN*) and *B. rapa* (*BR*). The parameters were calculated (from sowing to ripening) based on the data provided by the Finnish Meteorological Institute (Helsinki, Finland).

Growing site				Weather station[a]		Sowing date	Growing time (days)		Number of hot days[b]		Average daily temperature (°C)		Temperature sum (°C)		Growing degree days (°Cd)[c]		Global radiation sum (10^4 kJ/m^2)		Average relative humidity (%)		Precipitation sum (mm)	
Site	Coordinates	Zone	Soil type	Coordinates	Altitude		*BN*	*BR*	*BN*	*BR*	*BN*	*BR*	*BN*	*BR*	*BN*	*BR*	*BN*	*BR*	*BN*	*BR*	*BN*	*BR*
Inkoo	60.08°N, 24.89°E	1	Sandy clay	60.21°N, 24.74°E	31	May 6th	110	101	23	23	17	17	1850	1690	1300	1190	220	207	72	71	166	143
Pernaja	60.45°N, 26.15°E	1	Sandy clay, silty clay	60.39°N, 25.61°E	22	May 9th	127	114	25	25	17	17	2130	1950	1490	1370	234	221	74	72	285	234
Jokioinen	60.81°N, 23.50°E	2	Sandy clay, silty clay	60.81°N, 23.50°E	104	May 22nd	116	94	26	25	16	17	1870	1570	1290	1100	185	166	81	79	342	281
Hauho[d]	61.15°N, 24.59°E	2	Coarse silt	61.05°N, 25.04°E	125	May 9th	116	94	26	24	16	16	1860	1520	1240	1020	185	166	75	72	201	175
Maaninka	63.14°N, 27.32°E	3	Fine sandy till, till	63.14°N, 27.31°E	90	May 24th	–	77	–	21	–	17	–	1310	–	923	–	147	–	71	–	228

[a] Weather stations with closest match to the growing sites were chosen. The radiation data for Inkoo and Pernaja, and the precipitation data for Inkoo were from a substituting weather station at coordinates 60.33°N, 24.96°E (altitude 51 m). The radiation data for Hauho was calculated using the data recorded at the station closest to Jokioinen.
[b] Daily maximum temperature above +25 °C.
[c] The effective temperature sum (the sum of the positive differences between average daily temperatures and +5 °C).
[d] Growing time for Hauho was estimated using the data from the nearest site in Jokioinen, because the ripening dates were not recorded in Hauho.