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ORIGINAL PAPER

Canola Quality Indian Mustard Oil (*Brassica juncea*) is More Stable to Oxidation than Conventional Canola Oil (*Brassica napus*)

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Abstract Canola-quality Indian mustard (*Brassica juncea*) is being developed as a complimentary oilseed crop to canola (*Brassica napus*) for cultivation in hot and low-rainfall areas, where canola does not perform well. In Australia, several *B. juncea* breeding lines have been developed for commercial cultivation and for eventual processing as canola oil. However, there still are significant species-based differences in the fatty acid composition with *B. juncea* containing lower levels of linoleic acid and higher levels of oleic and linolenic acids compared with *B. napus*. This has raised concern about possible oxidative stability differences between the oils. Oils (unrefined) extracted from different breeding lines of each species were subjected to accelerated autoxidation, and development of oxidative rancidity was assessed by four separate techniques: depletion of polyunsaturated fatty acids, depletion of tocopherol, development of primary oxidation products, and development of secondary oxidation products (propanal and hexanal). All the tests showed that the newly developed *B. juncea* oils are more stable to autoxidation than conventional canola (*B. napus*) oil, despite containing marginally higher linolenic levels. Oxidative stability does not appear to be a barrier to using oils from these emerging lines of *B. juncea* for partial or full replacement of conventional canola oil.

Keywords Autoxidation · *Brassica napus* · *Brassica juncea* · Canola oil · Linolenic acid · Mustard oil · Oxidative stability · Tocopherol · Volatile oxidation products

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Introduction

The potential benefits of developing canola-quality Indian mustard (*Brassica juncea*) are recognized by a number of northern hemisphere countries, particularly Canada, where there are major breeding programs focused on its development [1]. *B. juncea* has been grown traditionally as a mustard crop in Canada as it shows good adaptation to the hotter, drier regions of western Canada. *B. juncea* was originally chosen for conversion to canola (*B. napus*) quality because it exhibits better heat and drought tolerance, disease resistance, and pod shatter resistance compared to *B. napus* [2, 3].

Canola-quality *B. juncea* is currently being developed for cultivation in low rainfall areas of Australia through the National Brassica Improvement Program in association with the Saskatchewan Wheat Pool. Recent successes in plant breeding have led to the development of canola-quality *B. juncea* that would be interchangeable with conventional canola for oil processing. *B. juncea* cultivars adapted to Australian conditions are now available for commercial cultivation. These are expected to succeed as an oilseed crop in regions of the southern wheat-belt that are too dry for canola, and also may become the major *Brassica* crop in northern NSW and Queensland [4].

Concerns about the detrimental health effects of *trans* fatty acids [5–7] have led to a growing interest in using *trans*-free natural vegetable oils such as canola for deep-frying food. In Australia, although there are no government regulations restricting the use of *trans* fats in food, many fast food restaurants appear to have voluntarily moved towards using *trans*-free natural vegetable oils such as canola for deep-frying [8]. Until now, partially hydrogenated vegetable oils, which are rich in *trans* fatty acids, have been used for deep-frying because of their excellent oxidative stability compared to non-hydrogenated oils.

Most vegetable oils, including canola, contain significant amounts of polyunsaturated fatty acids (PUFA). Such polyunsaturated oils have been seen as desirable from the health point of view, but are highly susceptible to oxidative deterioration. New Australian breeding lines of *B. juncea* have achieved fatty acid compositions very similar to that of conventional canola. However, several of the these oils earmarked for commercial cultivation still contain higher levels of the oxidatively unstable linolenic acid compared to conventional *B. napus* canola. In this paper, we show that despite the higher level of linolenic acid, oils from the newly developed *B. juncea* breeding lines have greater oxidative stability than conventional *B. napus* canola oil.

Experimental Procedures

Seed Samples

Seeds of newly released and promising lines of *B. juncea* and *B. napus* were obtained from the Department of Primary Industries, Victorian Institute for Dryland Agriculture, Horsham, Victoria, Australia. The following breeding lines were represented: *B. juncea*: JR055, JC05002, JC06011, JC06016, and JC06019; *B. napus*: AV-Opal, AV-Jade, RT057, RT058, and RT076.

Chemicals and Solvents

HPLC grade (Merck Lichrosolv) hexane, isooctane, isopropanol and methanol were obtained from Crown Scientific (Melbourne, Australia). HPLC grade *tert*-butyl methyl ether was obtained from Riedel-deHaen (Sigma Aldrich) (Sydney, Australia). α- and γ-Tocopherol standards were obtained from Sigma Aldrich (Sydney, Australia). The tocopherol calibration standards were prepared in the same solvent used for HPLC (eluent A, see below).

Oil Extraction

Approximately 20 g samples of seed were crushed using a laboratory blender and extracted with hexane for 3 h using a Soxhlet extractor. The oil was recovered by removal of most of the solvent using a rotary evaporator, followed by purging with dry nitrogen to remove the final traces of solvent.

Lipid Oxidation

Oils extracted from each breeding line (ten samples in all) were subjected to accelerated autoxidation without further processing. A sample of oil (3.5 g) was placed in an uncapped, amber-colored 10 ml headspace vial and heated at 60 °C inside a fan-forced oven in the dark for 21 days. During this oxidation period, samples were withdrawn periodically and analyzed for the extent of oxidation by techniques described below.

Headspace Analysis

Volatile products of oxidation were analyzed by headspace solid phase micro extraction (SPME) using a divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/Carboxen/PDMS, 50/30 μm coating, Supelco, Sydney, Australia). Samples of oil (150 mg) withdrawn from the oxidation oven (see above) were sealed in amber-colored glass vials with Teflon-coated silicone septa, and heated at 60 °C for 30 min with the SPME fiber inserted into the sample headspace. The fiber was then withdrawn and transferred to the GC injector and held for 2 min to desorb the extracted volatile compounds on to the GC column. The fiber was allowed to remain in the injector for a further 5 min for conditioning the fiber for the next analysis. The entire series of events was performed using a Shimadzu AOC-5000 auto-injector (CTC Analytics).

Gas chromatography (GC) was performed on a VOC fused silica capillary column (60 m, 0.32 mm i.d., 1.8 μm film thickness, Agilent, Melbourne, Australia) using an Agilent Model 6890 N instrument fitted with a flame ionization detector (FID). Helium was used as the carrier gas at a constant flow rate of 2.0 ml/min. The injector was initially operated in the splitless mode and switched to the split mode (50:1) 2 min after sample injection. The temperature of the injector and the FID detector were both held at 230 °C. The oven temperature was programmed from 40 to 230 °C at the rate of 22 °C/min and held at the final temperature for a further 12 min. The volatile compounds were identified by their retention times. When in doubt, identities were confirmed by GC-MS analysis using the same analytical column and GC operating conditions. Data analysis was performed using Chemstation software (Agilent, Melbourne, Australia).

Fatty Acid Composition

Fatty acid composition was determined by GC-FID using a BPX-70 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness, SGE, Melbourne, Australia). The GC column temperature was programmed as follows: 60–170 °C at the rate of 20 °C/min, then to 190 °C at the rate of 1 °C/min, and finally to 220 °C at the rate of 10 °C/min. The injector and the FID detector were maintained at 220 and 250 °C, respectively. Fatty acid methyl esters (FAME) were prepared by transesterification with potassium hydroxide/methanol according the procedure of [9]. Each oil sample was analyzed in duplicate. The variation between the duplicate analyses was less that 1%. The FA composition was calculated using GC peak area responses. The percentage loss of PUFA (linoleic acid and linolenic acid) during oxidation was also calculated from peak areas and expressed as below:

$$\begin{aligned}&\%\,\text{Loss of PUFA}_{dx}\\&\quad=\{(\text{Peak area of PUFA}_{d0}/\text{Peak area of } 16:0_{d0})\\&\qquad-(\text{Peak area of PUFA}_{dx}/\text{Peak area of } 16:0_{dx})\}\\&\qquad\times 100/(\text{Peak area of PUFA}_{d0}/\text{Peak area of } 16:0_{d0}),\end{aligned}$$

where d_0 and d_x refer to oxidation time of zero and x days, respectively.

Determination of Tocopherols and Oxidation Products by HPLC

Tocopherols and lipid oxidation products were analyzed by normal phase HPLC using an Agilent Series 1050 HPLC system consisting of a quaternary HPLC pump, degasser, autosampler and diode array detector (DAD). A Shimadzu Model RF-10AXL fluorescence detector (FD) was connected after the DAD. The signal from the FD was acquired via a HP-35900 ADC interface. ChemStation software (LC-3D Rev A.09.03) was used for data acquisition and instrument control. The DAD collected data at 234, and 292 nm, as well as spectra between 200 and 400 nm. The FD detector was operated at the lowest sensitivity setting (×1), and the fluorescence excitation and emission wavelengths were 298 and 325 nm, respectively.

The oil samples (60–200 mg) were dissolved in isooctane/isopropanol (99.5:0.5, v/v, 1.2 ml) and analyzed on an Alltech normal phase Allsphere Cyano column [150 mm × 4.6 mm ID (5 μm)], using the following quaternary solvent gradient: A: Isooctane/Isopropanol (99.5:0.5, % v/v); B: Isooctane/Isopropanol (96:4, % v/v); C: *tert*-butyl methyl ether; D: Methanol. The following gradient was used. 0–10 min: 100% A; 15 min: 100% B; 20 min: 100% C, 22 min: 100% C; 25 min: 100% D; 29 min: 100% D; 32 min: 100% C; 34 min: 100% C; 37 min: 100% A; 53 min: 100% A.

The flow rate was constant at 1.0 ml/min, with a total run time of 53 min.

Tocopherols were identified based on retention time and DAD spectra. They were quantified by FD using external calibration standards. FD was used in preference to UV, due to possible interference from lipid oxidation products in UV measurements [10]. Semi-quantitative changes in a cluster of oxidation product peaks were followed by UV (234 nm) detection. UV spectra of the oxidation products were also collected with the DAD.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the data for 18:2 and 18:3 (day 14), propanal and hexanal (day 14), and tocopherols (day 6) using the statistical package R version 2.4.1 [11].

Results and Discussion

The FA composition of the *B. juncea* and *B. napus* oils used in the study are shown in Table 1. The *B. juncea* samples contained higher levels of linolenic (18:3n-3, mean 12.7%, range 11.8–14.1%) and lower levels of linoleic (18:2n-6, mean 14.4%, range 13.8–15.8%) than the *B. napus* samples, the corresponding values for which were: 18:3n-3 (mean 11.6%, range 11.2–11.9%) and 18:2n-6 (mean 21.3%, range 20.3–21.8%). Overall, the content of the PUFA, i.e. 18:2n-6 and 18:3n-3, was lower in *B. juncea* with an average value of 27.1% compared with 32.9% *B. napus*. However, oleic acid (18:1n-9) content was higher in *B. juncea* (65.1%) than in *B. napus* (59.2%).

When subjected to accelerated autoxidation (60 °C, in the dark) the concentrations of 18:2 and 18:3 rapidly decreased as oxidation progressed. Figure 1 shows the percentage fall in the levels of 18:3 and 18:2 (relative to 16:0), at two time points (7 and 14 days) during the course of oxidation. After 14 d, the fall in 18:2 was greater than 50% for *B. napus* and less than 20% *B. juncea* ($p < 0.001$). A similar trend was observed for 18:3 with falls of greater than 70% for *B. napus* and less than 30% for *B. juncea*. A similar species effect on the rate of oxidation was observed after oxidation for 7 days. The results suggested that the oils from the *B. juncea* samples had greater oxidative stability than those from the *B. napus* samples.

During the course of oxidation, development of a range of volatile secondary oxidation products including hexanal and propanal was also monitored (Fig. 2). Propanal and hexanal are secondary lipid oxidation products, which are derived from initially formed lipid hydroperoxides. The concentrations of both propanal and hexanal increased to a

Table 1 Fatty acid composition (g/100 g FAME) of oils from *B. juncea* and *B. napus* breeding lines

	16:0	18:0	18:1n-9	18:2n-6	18:3n-3	20:0	20:1	22:0
B. juncea								
JR055	3.3	2.1	62.2	15.8	14.1	0.7	1.5	0.3
JC05002	3.8	2.5	65.7	14.1	11.8	0.7	1.2	0.3
JC06011	3.2	2.4	65.9	15.6	10.8	0.7	1.3	0.3
JC06016	3.1	2.1	66.7	13.8	12.2	0.6	1.3	0.2
JC06019	3.0	2.4	65.7	14.0	12.5	0.7	1.5	0.3
B. juncea average	3.3 ± 0.3	2.3 ± 0.2	65.2 ± 1.8	14.7 ± 1.0	12.3 ± 1.2	0.7 ± 0.0	1.4 ± 0.1	0.3 ± 0.0
B. napus								
AV OPAL	4.2	2.1	58.6	21.8	11.7	0.6	0.9	0.2
AV JADE	4.3	2.1	58.6	21.7	11.9	0.4	0.8	0.2
RT057	4.3	2.0	59.6	21.2	11.3	0.5	1.0	0.2
RT058	3.9	1.8	60.7	20.3	11.2	0.7	1.1	0.3
RT076	4.3	2.1	58.6	21.7	11.7	0.5	0.9	0.2
B. napus average	4.2 ± 0.2	2.0 ± 0.1	59.2 ± 0.9	21.3 ± 0.6	11.6 ± 0.3	0.5 ± 0.1	0.9 ± 0.1	0.2 ± 0.0

The standard deviation for the *B. juncea* and *B. napus* averages reflects the variation between the breeding lines. Concentration of erucic acid (22:1) did not exceed 0.2% in any of the samples

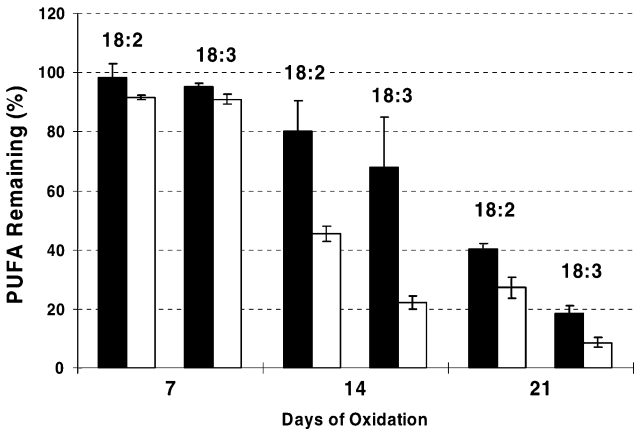

Fig. 1 Depletion of linoleic (18:2) and linolenic (18:3) acids in oils from *B. juncea* (*black*) and *B. napus* (*white*) breeding lines subjected to accelerated autoxidation (60 °C) for 7, 14, and 21 days. The percentage loss in linoleic/linolenic acid to palmitic acid ratios is plotted

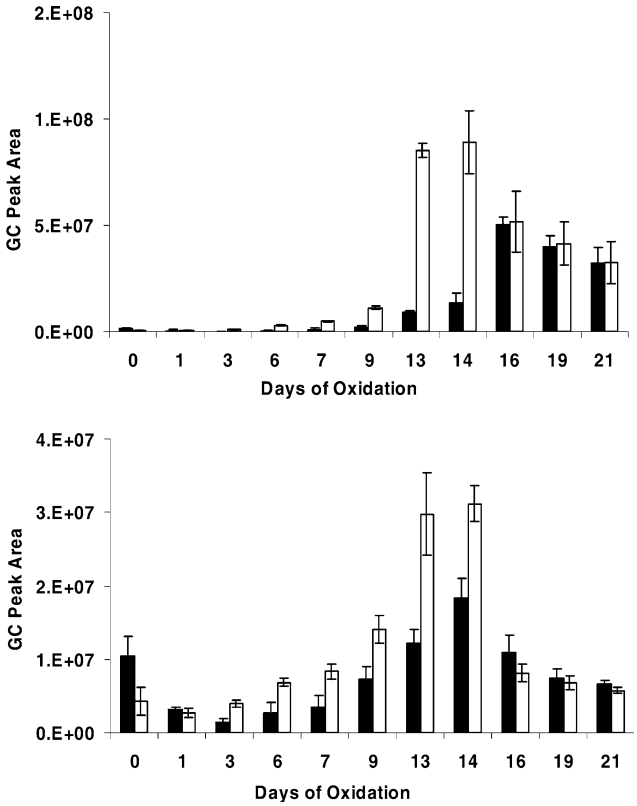

Fig. 2 Development of propanal (*bottom*) and hexanal (*top*) during accelerated autoxidation (60 °C) of *B. juncea* (*black*) and *B. napus* (*white*) oils. For each species, average values for five breeding lines are shown. The *error bars* represent standard deviation ($n = 5$) between the breeding lines within a given species

maximum before falling off. The fall in their concentration after attaining maximum values may be explained as follows. Propanal and hexanal oxidize to propanoic acid and hexanoic acid, respectively, and the net concentration of each aldehyde begins to fall when the rate of conversion to acids exceeds the rate of formation from hydroperoxides. This concurred with the presence of increasing amounts of both propanoic acid and hexanoic acid in the headspace of the oxidized oils (results not shown).

Hexanal levels grew much more rapidly in *B. napus* oils than *B. juncea* oils ($p < 0.001$ for 16 days oxidation) reaching maximum levels after 14 and 16 days for *B. napus* and *B. juncea*, respectively. This result was not surprising, because hexanal is produced from the degradation of 18:2 hydroperoxides, and the content of 18:2n-6 was higher in *B. napus* (21.4%) than *B. juncea* (14.6%). Interestingly,

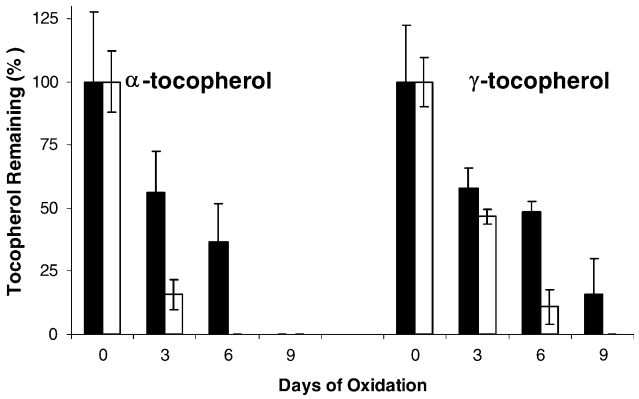

Fig. 3 Depletion of tocopherols in *B. juncea* (*black*) and *B. napus* (*white*) oils during accelerated autoxidation (60 °C). For each species, average values ($n = 5$) for five breeding lines are shown. α-Tocopherol was not detected beyond 6 days

however, the development of propanal which is derived from 18:3n-3 hydroperoxide, was also significantly higher ($p < 0.001$ for 16 days oxidation) in *B. napus*, despite the higher abundance of 18:3n-3 in *B. juncea* (12.3%) than in *B. napus* (11.6%). The faster production of propanal in *B. napus* was in agreement with the faster depletion of 18:3n-3 observed for this species (Fig. 1).

Also observed was a significant ($p < 0.001$ for 6 days oxidation) species-dependant difference in the depletion of the natural tocopherols during the course of oxidation. γ-Tocopherol was present at approximately the same level (600 μg/g) in both *B. juncea* and *B. napus* oils. During oxidation, γ-tocopherol was used up more rapidly by the *B. napus* oils, where it was totally consumed after 9 days, whereas 16% still remained in the *B. juncea* oils at this time (Fig. 3). The half-lives of γ-tocopherol in the *B. juncea* and *B. napus* oils were approximately 6 and 3 days, respectively. The same trend was observed for α-tocopherol, a significant part (36%) of which still remained after 6 days in the *B. juncea* oils but not in the *B. napus* oils, even though the initial concentration of α-tocopherol was higher in the *B. napus* oils. α-Tocopherol, together with γ-tocopherol protects oil from oxidation, but γ-tocopherol is more effective than α-tocopherol at reducing oxidative breakdown of vegetable oils [12, 13]. Since these tocopherols act as antioxidants, oils in which they degrade more rapidly would be expected to exhibit lower stability. It has been shown that oils with faster rates of tocopherol degradation during deep frying also tend to have faster rates of total polar compound production [14]. Thus, the slower rate of degradation of tocopherols in the *B. juncea* oils can be taken as further evidence for their greater oxidative stability compared with *B. napus* oils.

Products formed during oxidation of the *B. juncea* and *B. napus* oils were monitored by normal phase HPLC using UV detection at 234 nm. A cluster of peaks at retention time of approximately 17 min eluting after the triacylglycerol (TAG) peak, and absent from the un-oxidized oil, increased in intensity as the oxidation progressed. Figure 4 shows the development of this cluster of peaks for the *B. napus* breeding line AV-Jade. This cluster of peaks had an absorption maximum at 234 nm. Although we did not identify the components of this cluster, this UV absorption at 234 nm suggested that they were conjugated dienes, presumably lipid hydroperoxides. Figure 5 shows this cluster of peaks for different breeding lines of *B. juncea* and *B. napus* oils after oxidation for 6 days. The intensity of this cluster of oxidation peaks for *B. napus* breeding lines was at least twice as much as that for *B. juncea* breeding lines, further demonstrating the greater oxidative stability of *B. juncea* oils.

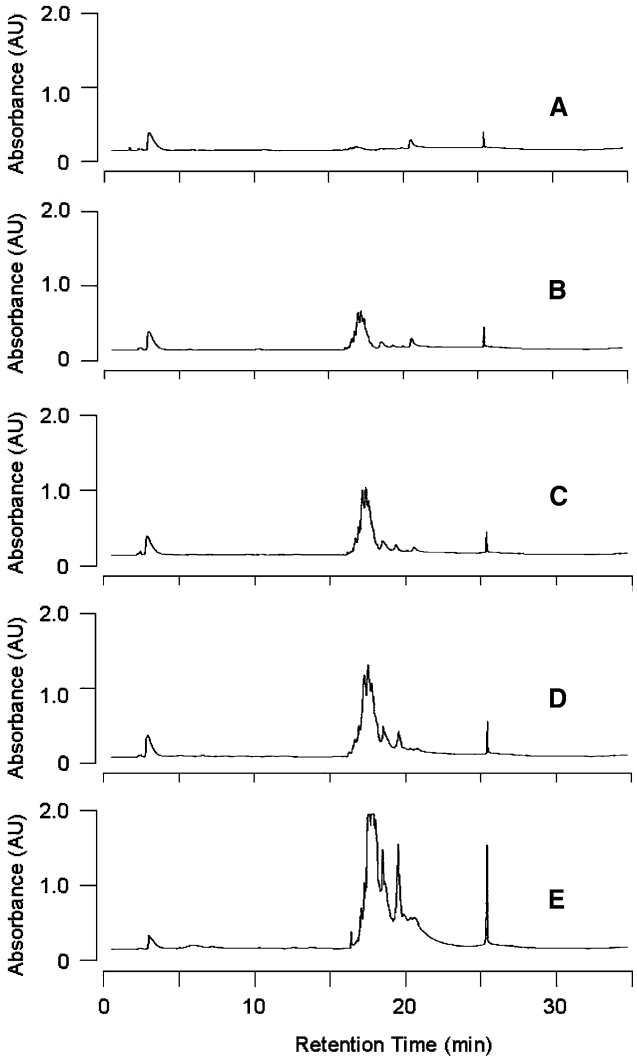

Fig. 4 Increase in cluster of oxidation products, as observed by normal phase-HPLC with UV (234 nm) detection. The results shown are for the *B. napus* breeding line AV-Jade after 0 (**a**), 3 (**b**), 6 (**c**), 9 (**d**) and 13 (**e**) days of oxidation

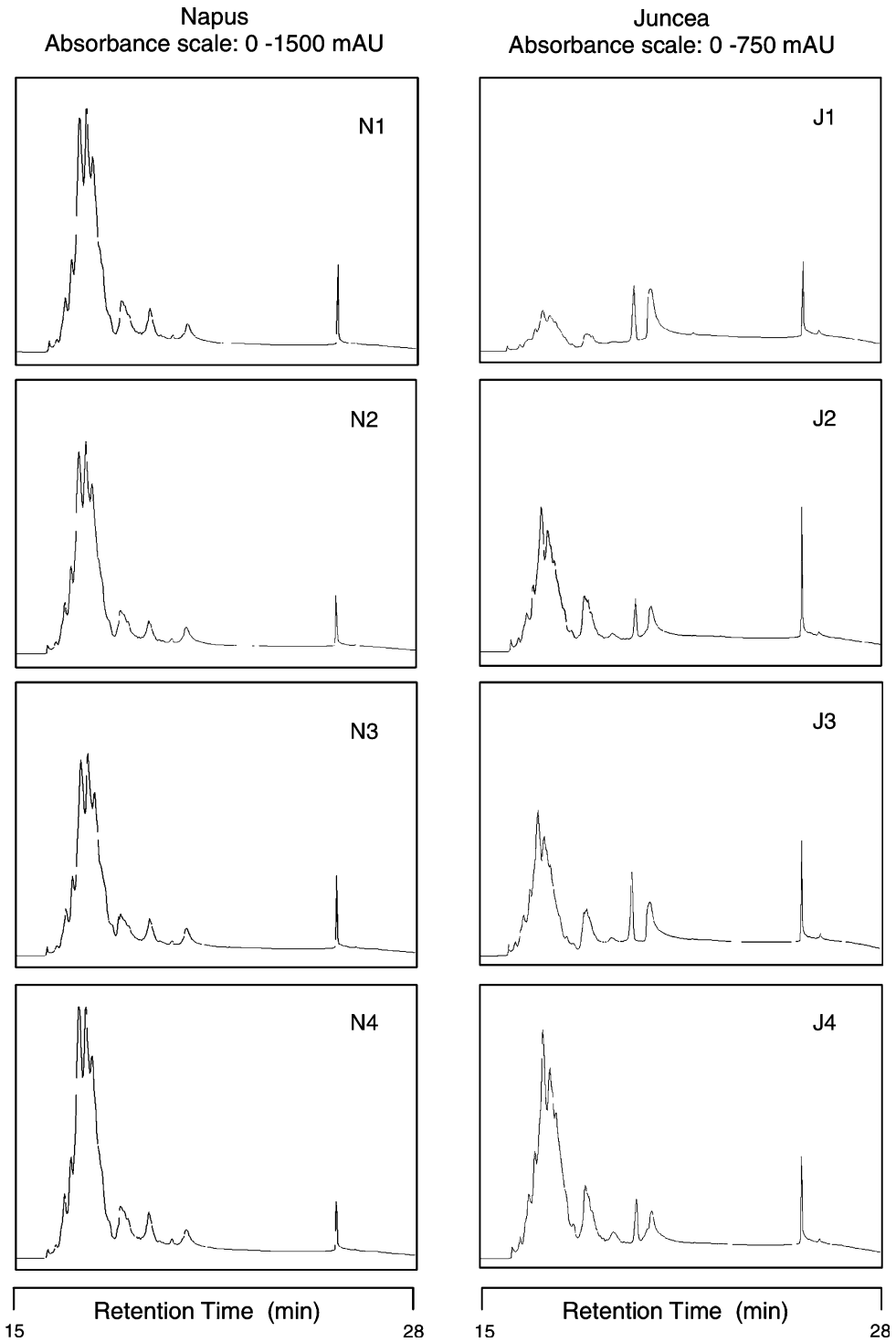

Fig. 5 Hydroperoxide levels in each *B. juncea* (J1–J4) and *B. napus* (N1–N4) breeding line after accelerated autoxidation (60 °C) for 6 days, as determined by HPLC with UV (234 nm) detection. The peak size for *B. juncea* is scaled up twofold compared to that for *B. napus*. J1 = JR055; J2 = JC06016; J3 = JC05002; J4 = JC06019; N1 = AV-Opal; N2 = AV-Jade; N3 = RT076; N4 = RT057

Not considering the effects of the native antioxidants, the susceptibility of oils to autoxidation is, by and large, determined by the type and amount of their constituent PUFA, i.e. 18:2n-6 and 18:3n-3 in the case of *B. juncea* and *B. napus* oils. This is because, in neat systems without added initiator, 18:2n-6 is approximately 40 times more reactive than 18:1n-9, and oxidizability of each PUFA increases approximately twofold for each bis-allylic

methylene group [12]. The average oxidizability values [12] for *B. junncea* and *B. napus*, which were calculated using the equation: oxidizability = [(%18:2 × 1) + (%18:3 × 2)]/100, were 0.39 and 0.45, respectively. The results concur with the oxidative stability results of the present study.

The results of this study highlight the inadequacy of using the content of linolenic acid as the sole criterion for assessing the oxidative stability of canola quality mustard (*B. juncea*) oils. Some of the newer breeding lines of *B. juncea* contain relatively high levels of oleic and correspondingly low levels of linoleic acid, which make them more resistant to oxidation than conventional canola oils. Therefore, the linolenic acid content should not be a barrier to using *B. juncea* oils as alternatives to conventional canola oil, either on their own or as blends with canola oil.

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