Sensory and Oxidative Quality of Screw-Pressed Flaxseed Oil

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ABSTRACT: Cold-pressed flaxseed oil is an excellent source of dietary α-linolenic acid (ALA). However, breakdown of ALA in the oil, either in the seed before or during storage, or as a result of processing, may result in unacceptable flavors. Screw-pressed flaxseed oil from four seed lots and a commercial sample were analyzed for headspace volatiles (HV) by solid-phase microextraction; for nutty, painty, and bitter flavors; and for overall quality. HV and sensory analyses were performed after storage for 7 d at room temperature and again after 15 wk at 4°C. Marked, significant differences were observed between samples for painty and bitter flavors and overall quality, but only slight differences for nutty flavor ($P < 0.05$). Samples remained stable between the two storage periods. HV traces showed distinct differences between samples in number of peaks and in peak heights. Areas under peaks corresponding to select retention times were positively correlated ($P < 0.01$) with nutty, painty, and bitter flavors. Therefore, the HV analysis by solid-phase microextraction may be a useful tool for screening flaxseed lots to be used for the production of screw-pressed oil.

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KEY WORDS: Alpha-linolenic acid, flaxseed, headspace volatiles, screw press, sensory analysis, solid-phase microextraction.

Flaxseed oil is recognized and accepted as a healthful, edible oil with an outstanding content of alpha-linolenic acid. This FA, a type of n-3 FA, and its metabolites EPA and DHA may protect against heart disease and other illnesses (1,2). Oils high in n-3 FA should be processed at the lowest temperatures possible to maintain high quality and storage life (3). Consequently, flaxseed is screw-pressed with little or no pretreatment and little or no external heating to the press. Furthermore, the oil undergoes no refining beyond sedimentation or filtration. Fresh, unrefined flaxseed oil from good seed has an attractive golden color, a pleasant, nut-like flavor, and mild odor. However, flaxseed oil may develop a strong, unpleasant odor and flavor, because of either poor seed quality or unfavorable process or storage conditions.

Flaxseed oil quality was recently evaluated in a study of novel processes for fractionating flaxseed for food uses. Flaxseed lignans appear to protect against some cancers (4); therefore, a flaxseed fraction high in lignans was recovered before pressing the remaining seed embryo for oil (5). Some of the methods for preparing the seed embryo for pressing resulted in higher levels of products of lipid oxidation and hydrolysis (6); however, the oil quality tests used in that study—PV, FFA content, and conjugated diene value—were insensitive indicators of quality. Such tests reportedly are not useful predictors of sensory quality (Cardwell, D., personal communication, 2003).

If an accurate, fast test could be developed for flaxseed oil flavors, such a test could be used to improve practices for processing, handling, and storage, as well as to develop new varieties. The use of a trained panel to evaluate oil flavor provides the best measure of oil quality. However, much time and expense are required to obtain useful results. Routine oil analysis for process research requires a faster, less subjective method. Alpha-linolenic acid is susceptible to oxidation through a variety of pathways resulting in a large number of compounds, many of which are volatile and some of which strongly influence the sensory characteristics of the oil (7). Volatile compounds quantified in cold-pressed rapeseed oil by the purge-and-trap method were used for detecting sensory defects (8). A relatively new and simple approach to quantifying such compounds uses a solid-phase microextraction (SPME) fiber to adsorb volatile compounds from the headspace within a vial of sample and then provides for the GC analysis of those compounds. A high total content of volatile compounds in vegetable oils, as determined by SPME, generally corresponded to the least-acceptable sensory scores (9).

The purpose of this study was to quantify the sensory characteristics of different flaxseed oils, using a trained panel to compare the sensory characteristics with those of the same oil batches after extended refrigeration, and to correlate sensory data with headspace volatile data obtained by SPME.

MATERIALS AND METHODS

Sample preparation. Seven lots of brown-hulled flaxseed were obtained from David Cardwell (Barlean's, Ferndale, WA). Lots were judged by Cardwell to represent qualities ranging from high quality to marginal quality. Oil temperatures were measured with Type T thermocouples at the time of pressing, and the data logged continuously to a CR10X datalogger (Campbell

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Scientific Instruments, Logan, UT). Flaxseed was pressed at North Dakota State University (NDSU) with a Komet screw press (6), clarified by gravity settling and decantation, and then collected in dark high-density polyethylene (HDPE) bottles in two portions.

Four of the seven lots—those arbitrarily numbered before as 3, 4, 5, and 7—were selected for sensory analysis on the basis of headspace volatile (HV) analysis results. The HV profiles of samples 3, 4, 5, and 7 were distinctly different from one another; whereas the profile for sample 1 resembled that of sample 3, and profiles from samples 2 and 6 resembled that of sample 7. Selection of samples 3 and 7 over their respective, similar samples was random. Commercial flaxseed oil (Barlean's) purchased through a local retail outlet served as a fifth sample for each type of analysis. This purchased sample was not subjected to the room-temperature holding step, was pressed 4 wk before purchase, and was refrigerated during the intervening storage before purchase.

The first portion of oil underwent HV and sensory analysis after completion of the 7-d room-temperature (23°C) storage and was also analyzed for PV and FFA content. Apart from this room-temperature holding step, the oil was stored at −18°C and then warmed to room temperature just before analysis. The second portion of the oil from each of the four flaxseed lots was stored 15 wk at 4°C in dark HDPE bottles. A portion of the commercial flaxseed oil was stored frozen (−18°C) during this same period. After storage, these five samples underwent HV, PV, FFA, and sensory analysis.

Sample analysis. Analyses for PV and FFA followed AOCS Official Methods (10) Cd 8-53 and Ca 5a-40, respectively.

Sensory evaluation of flaxseed oil samples for nutty, painty, and bitter flavors, and overall quality was performed by a semitrained panel using a continuous, linear 15-cm intensity-scale marking procedure (11). Sensory analysis of oil in part 1 (oil stored 7 d at room temperature) was performed midafternoon on two separate, consecutive days. Analysis of oil in part 2 (oil stored 15 wk at 4°C) was performed midmorning on three separate days within a 9-d period. Eight panelists (6 Caucasian and 2 Asian; 4 men and 4 women) participated in both parts. Panelists were faculty, staff, and students who volunteered from the Cereal & Food Sciences Department and Agricultural and Biosystems Engineering Department at NDSU. Three additional panelists (two Caucasian women, one Caucasian man) participated in part 2. Panelists were trained to mark each sensory characteristic relative to references. Training was conducted on the day preceding analysis of oil from the 7-d storage and reviewed immediately before analysis of oil from the 15-wk storage. Refined, bleached, deodorized corn oil was used as a low reference for all flavors. High references for nutty and bitter flavors were chopped walnut meats and bitter melon extract, respectively. Bitter melon extract was prepared by boiling 442 g fresh, chopped bitter melon in 1180 g water for 8 min, then decanting and freezing the liquid until use. Cold-pressed walnut oil (Loriva, San Leandro, CA) was used as a midlevel reference for nutty flavor (marked at the midpoint of the nutty-flavor scale). Heat-stressed ("oxidized") flaxseed oil was used as a midlevel reference for painty flavor (marked 6 cm from the low end of the painty-flavor scale). Heat-stressed oil was prepared by microwave heating (1500 W, 2450 MHz) of commercial flaxseed oil (80 mL) for 7 min. The oil was then refrigerated (4°C) in dark glass bottles until used. Panelists were instructed to gently swirl the bottle contents, open the bottle, gently sniff the headspace two or three times but not taste the sample, close the bottle, and then pass to the next panelist. Samples (10 mL) and references were presented in 30-mL covered cups randomly placed on a paper plate at room temperature. Panelists were also provided spoons, saltine crackers with unsalted tops, and a cup of water. Evaluation stations were set before the arrival of panelists, and evaluation was completed within 30 min. Three-digit random numbers were marked on sample cups, and panelists were free to choose the order of sample evaluation. During sample evaluation, panelists first smelled the headspace within the sample cup and then consumed a portion of the sample. Intensity was marked on the scale and identified by a three-digit number. Discussions were not allowed. Results were tabulated by measuring the distance in cm from the left end of the line (low reference), and dividing by 1.5. Thus, sensory scores were reported on a continuous 10-point scale (0 = low, 10 = high).

HV were determined by headspace SPME. HV were adsorbed onto a divinylbenzene/carboxene/poly(dimethylsiloxane) fiber (Supelco, Bellefonte, PA) recommended by Jeleń *et al*. (9) for vegetable oil HV analysis. The fiber was positioned in the headspace above 1 mL of oil contained within a 4-mL vial in an ultrasonic bath. The fiber resided in the headspace for 5 min at 60°C. Volatile compounds were desorbed into a gas chromatograph (Hewlett-Packard model 5890 with FID) at 180°C. The volatiles were resolved on a column packed with (14% cyanopropyl-phenyl)-methylpolysiloxane (J&W Scientific, Inc., Folsom, CA) using the following column oven program: 40°C for 5 min, 5°C/min ramp for 28 min, 180°C for 12 min. HV analysis of the first portion of oil samples was performed in duplicate with samples 1, 2, and 3, and with single determinations in the remaining samples. HV analysis of the second portion of oil samples was performed in duplicate with samples 3, 4, 5, and 7, and a single determination was performed with the commercial sample.

Statistical analysis. Two-way ANOVA was performed with a least significant difference *post-hoc* test using the General Linear Model Procedure (SAS System for Windows, release 8.02; SAS Institute, Cary, NC). Two-way ANOVA was used to analyze differences concurrently in sensory, PV, and FFA results between two storage conditions and five samples; each sensory input value represented the mean score from the eight panelists who had evaluated samples in both parts. Two-way ANOVA also was used to analyze differences concurrently in bitter scores after the 15-wk storage between 11 panelists and 5 samples; each sensory input value represented the mean score from 3 replicates. Linear regression was performed using the method of least squares on Microsoft Excel 2002 for the 15-wk

storage to determine (i) the correlation matrix between sensory values, PV, and FFA; and (ii) the relationships of selected headspace volatiles—identified by retention time—with nutty, painty, and bitter flavors. Each input represented the mean value of respective characteristics for one of the five oil samples, except where noted otherwise.

RESULTS AND DISCUSSION

Effect of sample type and storage time on sensory characteristics, PV, and FFA. Mean sensory scores from the combined storage periods showed marked differences between samples in painty and bitter flavors, but only slight differences in nutty flavor (Table 1). Sample 7 had the lowest nutty, painty, and bitter flavors and highest overall quality; in contrast, sample 5 had the highest painty and bitter flavors and lowest overall quality. The commercial flaxseed oil sample was similar to samples 3 and 4. Several panelists reported a musty flavor in sample 4. Buttery, banana, and burnt are additional flavor characteristics that have been attributed to pressed flaxseed oil (Fraley, P., personal communication, 2004).

All PV and FFA were ≤0.8 mequiv/kg oil and 0.12%, respectively, with the exception of PV for sample 5 (Table 1). PV and FFA were well below the recommended upper limits of 15 mequiv/kg oil and 2%, respectively (12). HV traces of all seven samples, obtained after storage for 7 d at room temperature, ranged from a few, small peaks (sample 7) to numerous peaks, including some large peaks (sample 5), as shown in Figure 1. The samples used for sensory analysis (3, 4, 5, and 7) appeared to represent the spectrum of HV traces adequately and provided a manageable number of samples for sensory analysis.

Oil temperature was monitored during pressing to determine whether this might have influenced oil quality. Oil temperature during pressing depends strongly upon seed moisture content. However, seed moisture contents for samples 3, 4, 5, and 7 were all within the narrow range of 7.3 to 7.5% at the time of pressing, and pressed oil temperatures were found to range from 50 to 55°C for these same four lots. This range was deemed sufficiently narrow to rule out press conditions as a cause of oil quality variation. Therefore, differences between oil samples likely resulted from differences in seed quality.

Sensory scores from part 2 (oil stored 15 wk at 4°C) did not differ from part 1 (stored 7 d at room temperature) except for a significant increase in bitter flavor (Table 2). Although the increase in mean bitter score may indicate oil deterioration during the 15-wk storage, it is more likely that the bitter melon reference was not stable during frozen storage. This explanation is supported by the observations that overall quality remained consistent—and even increased slightly in several samples—and that the frozen commercial sample showed a similar increased bitter score. FFA remained very low in part 2, with all values ≤0.12%. PV showed a significant increase in part 2 (Table 2); however, PV remained low (≤1 mequiv/kg), again with the exception of sample 5, which increased to a PV of 8.3 mequiv/kg. From the sensory comparisons, together with the FFA and PV comparisons in Table 2, it was concluded that samples remained very stable during the 15-wk storage.

FFA and PV were not significantly correlated with any sensory characteristic after 15 wk of storage (Table 3), based on linear least squares analysis of mean data from the five samples. This was not surprising, given the low values for FFA and PV. However, all sensory characteristics were significantly interrelated, especially bitter flavor with overall quality (Table 3). Surprisingly, nutty flavor at 15 wk was positively correlated with painty and bitter flavor. All three flavors were negatively correlated with overall quality. Although nutty flavor may be a desirable attribute, nutty flavor intensified in parallel with other, negative sensory attributes.

The pronounced negative correlations of overall quality with nutty, painty, and bitter flavor led to a deeper look into these relationships. The linear least squares analysis was repeated, but with one difference; rather than use 1 point—the mean score from 11 panelists—for each of the 5 samples, a total of 55 points were used (5 samples × 11 panelists). This approach is illustrated in Figure 2. The correlations for overall quality vs. nutty, painty, and bitter flavor were highly significant by this approach, with the significance of F being 3×10^{-3}, 4×10^{-10}, and 1×10^{-16}, respectively. Figure 2 revealed the panelists' differing perceptions of bitter flavor in the oil. For example, two-way ANOVA showed that four panelists gave an average score of 6.1 to 6.9 (panelist groups A and AB in Table 4) and that another three panelists (group D in Table 4) gave an

TABLE 1
Mean Sensory Scores on a 10-Point Scale (0 = low, 10 = high), PV, and FFA Content for Four Screw-Pressed Flaxseed Oils and One Commercial Sample[a]

Sample no.	Nutty	Painty	Bitter	Overall quality	PV (mequiv/kg)	FFA (%)
3	3.3^{A}	2.3^{C}	$3.9^{B,C}$	4.9^{B}	0.8^{B}	0.05^{C}
4	3.8^{A}	3.7^{B}	4.6^{B}	2.9^{C}	0.4^{C}	0.10^{B}
5	3.6^{A}	5.2^{A}	6.3^{A}	2.0^{D}	7.5^{A}	0.10^{B}
7	2.4^{B}	0.8^{D}	1.6^{D}	7.1^{A}	0.4^{C}	0.05^{C}
Commercial	3.9^{A}	$3.0^{B,C}$	3.3^{C}	4.3^{B}	0.2^{C}	0.12^{A}

[a]Mean values represent averages over two storage periods (7 d at room temperature and 15 wk at 4°C); values in the same column with different LSD groupings (uppercase superscript letters) are statistically different ($P \leq 0.05$).

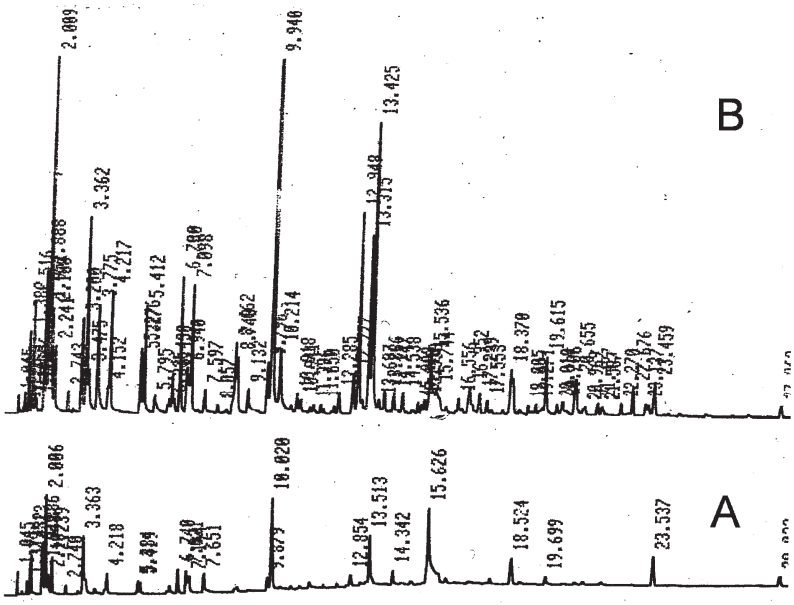

FIG. 1. Headspace volatile profile of flaxseed oil samples 7 (A) and 5 (B), after 7 d of storage at room temperature, as determined by solid-phase microextraction. The peak with retention time 15.6 min was an internal standard, 2,4-hexadienal.

average of 1.4 to 2.7. Panelists' perceptions of bitterness may have influenced their scores for overall quality. This dichotomy within the panel was not anticipated; in future sensory analyses, a better approach may be to screen panelists for their sensitivity to bitter flavor in flaxseed oil. Also, these data suggest a need for an alternative high reference for bitter flavor.

Analysis of HV by SPME. The HV traces showed multiple peaks of varying heights, as already noted (Fig. 1). These peaks probably mainly represent various aldehydes, ketones, and other oxidative decomposition products associated with alpha-linolenic acid (7). Identification of individual peaks was not an objective of this study; however, the HV traces may contain a wealth of information useful for oil quality evaluation. Thus, this method of analysis of HV might provide a sensitive indicator of flaxseed oil quality.

The contrast in HV traces between samples 5 and 7 (Fig. 1) was not surprising given their very different sensory scores (Table 1). This suggested that the number and size of peaks

TABLE 2
Mean Sensory Scores on a 10-Point Scale (0 = low, 10 = high), PV, and FFA for Screw-Pressed Flaxseed Oil After Two Storage Periods[a]

Sample no.	Nutty	Painty	Bitter	Overall quality	PV (mequiv/kg)	FFA (%)
7 d, room temp.	3.4^{A}	2.8^{A}	2.9^{B}	4.3^{A}	1.6^{B}	0.08^{A}
15 wk, 4°C[b]	3.4^{A}	3.1^{A}	4.6^{A}	4.3^{A}	2.2^{A}	0.08^{A}

[a]Mean values represent averages over samples 3, 4, 5, and 7 and a commercial sample. Values in the same column with different LSD/groupings (uppercase superscript letters) are statistically different ($P \leq 0.05$).
[b]The commercial sample was stored frozen (–18°C) during this period.

TABLE 3
Correlation Coefficient (*r* values) Matrix for Sensory Characteristics, PV, and FFA for Four Flaxseed Oil Samples After Refrigeration for 15 wk Plus a Commercial Sample Stored Frozen

	FFA	PV	Nutty	Painty	Bitter
Nutty	0.51	0.77	—		
Painty	0.57	0.74	0.99^{b}	—	
Bitter	0.37	0.66	0.94^{a}	0.94^{a}	—
Overall quality	–0.45	–0.71	-0.96^{b}	-0.97^{b}	-0.99^{b}

[a]Significant at $P \leq 0.05$.
[b]Significant at $P \leq 0.01$.

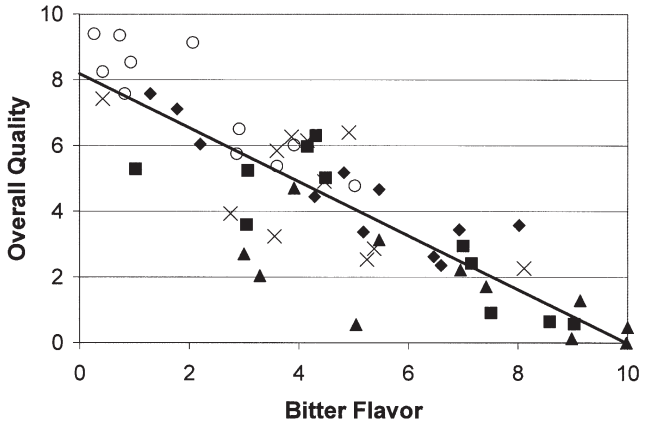

FIG. 2. Overall quality of flaxseed oil samples after 15 wk of refrigeration plotted vs. bitterness, with scores separated by panelist as well as sample. Points denote sample 3 (solid diamond), 4 (solid square), 5 (solid triangle), and 7 (open circle), plus a commercial sample (cross) that was frozen, not refrigerated. The diagonal line was fitted by the method of least squares (significance of $F = 10^{-16}$, $r = 0.85$). Each of the 55 points represents the mean of three replicates.

might serve as indicators of oxidative deterioration in flaxseed oil. However, the total peak area, minus the internal standard peak area (TPA − IS), was not significantly related with any sensory characteristic (Table 5). Many products of oxidation are not stable, thus their amounts may rise and fall over time. Nevertheless, by using the data from the five samples refrigerated for 15 wk, correlations with sensory scores were found for 3 of 11 peaks common to all 5 samples (Table 5). The peak that corresponded to a GC retention time of 4 min was significantly correlated with nutty, painty, and bitter flavors, and the 6.45 min peak with painty and bitter flavor. Although bitter flavor is not associated with volatile compounds, it may be that the volatile compounds are co-products with the agents that cause bitter flavor. Those agents may include hydroxy-FA (13).

Some squared correlations in Table 5 were very low, namely, peaks with retention times of 1.81, 1.88, 18.10, and 23.1 min. These may be associated with labile products of lipid oxidation. Other squared correlations were moderately high but below the threshold of significance ($P \leq 0.05$). Several combinations of peaks resulted in r^2 values that were higher than those associated with individual peaks, for example the 4 + 13 min peaks and the 1.46 + 4 min peaks (Table 5). All paired combinations of the 1.46, 1.93, 4, 6.45, 9.6, and 13 min peaks, plus select combinations of three and four peaks, were also checked; a number of correlations were significant, but all were lower than those for 4 + 13 min and 1.46 + 4 min.

Therefore, the Table 5 results supported our hypothesis that analysis of HV by SPME may be used to predict sensory scores for flaxseed. SPME as a tool for flaxseed oil quality analysis would be more sensitive than PV and FFA, and more objective and rapid than sensory evaluation with a trained panel. The approach used in this study should be expanded to include additional sensory characteristics and to identify other useful indicator peaks. Even though the peaks that were significantly correlated with flavors may not have directly influenced those flavors, they may still be useful indicators for studying the influence of seed storage, handling, and process conditions and oil storage conditions on flaxseed oil quality, and for screening new varieties of flaxseed.

TABLE 4
Mean Bitter Scores on a 10-Point Scale (0 = low, 10 = high) by Panelist and Sample for Screw-Pressed Flaxseed Oil After 15 wk of Refrigerated Storage[a]

	Sample					
Panelist	3	4	5	7	Commercial	Average[b]
1	6.9	9.0	10.0	0.7	4.9	$6.3^{A,B}$
2	8.0	3.1	5.0	2.1	4.2	4.5^{C}
3	6.6	4.3	7.4	2.9	3.9	$5.0^{B,C}$
4	5.2	7.2	9.0	3.6	5.4	$6.1^{A,B,C}$
5	5.5	7.0	9.1	5.0	8.1	6.9^{A}
6	1.8	4.2	3.3	0.8	3.6	2.7^{D}
7	2.2	1.0	3.0	0.4	0.4	1.4^{D}
8	1.3	3.0	3.9	0.3	2.8	2.3^{D}
9	4.3	7.5	5.5	2.9	3.6	$4.7^{B,C}$
10	4.8	4.5	7.0	3.9	4.5	$4.9^{B,C}$
11	6.5	8.6	10.0	0.9	5.2	$6.2^{A,B}$
Average[c]	$4.8^{B,C}$	5.4^{B}	6.7^{A}	2.1^{D}	4.2^{C}	

[a]Mean values represent three replicates/sample.
[b]Averages by panelist having different LSD groupings (uppercase superscript letters) in the same column are statistically different ($P \leq 0.05$).
[c]Averages by sample having different LSD groupings (uppercase superscript letters) in the same row are statistically different ($P \leq 0.05$).

TABLE 5
Relationship Between Headspace Volatiles and Sensory Characteristics, as Indicated by Squared Correlation (r^2 value), for Four Flaxseed Oil Samples After Refrigeration for 15 wk Plus a Commercial Sample Stored Frozen

RT[a] (min)	Nutty	Painty	Bitter
1.46	0.72	0.59	0.64
1.81	0.13	0.04	0.07
1.88	0.00	0.02	0.03
1.93	0.67	0.60	0.60
3.2	0.49	0.37	0.26
4.0	0.85[b]	0.92[b]	0.92[c]
6.45	0.76	0.82[b]	0.86[b]
9.6	0.62	0.52	0.48
13.0	0.79[b]	0.70	0.55
18.1	0.01	0.00	0.07
23.1	0.03	0.00	0.11
4.0 + 13.0	0.98[c]	0.96[c]	0.85[b]
1.46 + 4.0	0.93[c]	0.96[c]	0.97[c]
4.0 + 6.45	0.84[b]	0.91[b]	0.94[c]
TPA – IS[d]	0.76	0.68	0.44

[a]RT, peak retention time.
[b]Significant at $P \leq 0.05$.
[c]Significant at $P \leq 0.01$.
[d]TPA – IS denotes total peak area less the area of the internal standard.

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