Effect of Sprouting on the Quality and Composition of Canola Seed and Oil

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ABSTRACT: Sprouting has been considered as a damage factor in grading canola. This project deals with the evaluation of the effect of sprouting on the quality and composition of canola seed and oil. Sprouted seeds had lower oil content than nonsprouted seeds as determined by exhaustive petroleum ether extraction. The difference, although statistically significant, was small, less than 0.1% oil at the maximum level of sprouting allowed in top–grade canola. There were no differences in chlorophyll contents or moisture contents between sound and sprouted seeds. Sprouted seeds had significantly higher levels of FFA and crude protein than sound seeds. Oxidation parameters (diene and aldehyde) were higher in oils from sound seeds than oils from sprouted seeds, but there was no statistically significant difference in PV. Sprouted seeds had higher levels of tocopherols and sucrose, but lower levels of raffinose, stachyose, and total sugars than sound seeds. There was no difference in overall FA composition of the oil between sound and sprouted seeds. The second extraction of the Federation of Oils Seeds and Fat Associations (FOSFA) extraction method, which allowed the extraction of more polar lipids, contained significantly more saturated FA. However, this was not significant in the overall FA composition of the oils because this fraction counted for about 2% of the total lipid content. The presence of sprouted seed had an effect on results for oil and crude protein determined by NIR as compared with results by FOSFA extraction, or pulsed NMR for oil and Dumas combustion for crude protein. Addition of sprouted seed samples to the NIR calibration set overcame this problem. These results suggested that sprouting did not have a highly damaging effect on the quality and composition of canola seed and oil when less than 10% of the seeds in a sample were sprouting.

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KEY WORDS: Aldehydes, canola, free fatty acid, peroxide value, sprouted seeds, tocopherol.

In 2000 an extended period of high humidity during the late summer and fall led to a significant amount of sprouted seed in the canola crop harvested in Manitoba, eastern Saskatchewan, and northern Alberta, Canada. Sprouting in canola may occur when mature seeds are subjected to high moisture combined with warm weather over a long period of time before or after harvesting. On visual inspection, the seeds may show rootlets of several millimeters that are easily detectable by untrained eyes. These rootlets are the initial roots or radicles developed during germination.

During germination, the TAG are metabolized to generate the energy necessary for seed development (1). This decrease in nonpolar lipids is associated with an increase in FFA and synthesis of polar lipids (2,3). For canola seed crushers, levels of FFA greater than 1% are undesirable since more refining is needed to process the seeds, thereby increasing the cost of production.

Work in Canada (4) and Sweden (5) showed that sprouted seed was associated with lower oil contents and higher FFA and chlorophyll than sound seeds. The decrease in oil content and the increase in FFA have led to sprouting being characterized as damage in canola seed. Available information is limited, however, on the effect that the damage has on the quality of canola and its oil and meal. The objectives of this study were to establish a better definition of sprouting damage and therefore grading guidelines for sprouted canola samples. Concurrently, different secondary methods to determine oil and protein were investigated to understand the effect of the sprout damage on the accuracy of the results from the rapid secondary methods, i.e., NIR and NMR spectroscopy.

MATERIALS AND METHODS

Materials. Nine samples of canola seeds were selected from the 2000 CGC harvest survey based on different levels of sprout damage. Each sample was hand-separated into sprouted and nonsprouted portions using the criteria for sprouting in the *Canadian Grain Commission Official Grain Grading Guide* (6); the sprouted seed in a 10-g of sample was expressed as a percentage (w/w). Sprout damage ranged from 3 to 17%. A laboratory-generated sample was prepared by placing sound seed on wet paper towels at room temperature. After 48 h the seeds had developed radicles similar to those in the field-sprouted sample.

Methods. (i) Moisture. Moisture was measured gravimetrically using the AOCS Official Method Ai 2-75 (7) at 103°C.

(ii) Protein. Protein content was measured using the AOCS Official Method Ba 4e-93 and a LECO FP-428 Nitrogen (St. Joseph, MI).

(iii) Oil content. Solvent extraction was performed according to AOCS Official Method Am 2-93 (9) using a Soxtec extraction unit (FOSS-Tecator, FOSS North America, Eden

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TABLE 1
Effect of Sprout Damage on the Quality of Canola Seed[a]

Canola sample	Original sprout (%)	Oil (%)					Crude protein (%)					Crude protein in defatted meal (%)					Chlorophyll (mg/kg)		
					Loss at[b]					Increase at[b]					Increase at[b]				
		Sound	Sprouted	Diff.	3%	10%	Sound	Sprouted	Diff.	3%	10%	Sound	Sprouted	Diff.	3%	10%	Sound	Sprouted	Diff.
1	8.5	42.0	39.6	2.5	0.07	0.25	26.6	28.0	–1.4	0.04	0.14	45.9	46.3	–0.4	0.01	0.04	6.71	6.37	0.34
2	8.1	46.2	45.0	1.2	0.04	0.12	25.6	26.6	–1.1	0.03	0.11	47.5	48.4	–0.9	0.03	0.09	6.12	5.35	0.77
3	10.3	45.1	43.1	2.0	0.06	0.20	26.2	27.4	–1.2	0.04	0.12	47.7	48.2	–0.5	0.01	0.05	10.18	8.65	1.53
4	13.2	44.3	42.4	1.9	0.06	0.19	26.5	27.6	–1.1	0.03	0.11	47.6	47.9	–0.3	0.01	0.03	11.57	9.69	1.89
5	17.0	49.5	47.4	2.1	0.06	0.21	21.1	22.4	–1.3	0.04	0.13	41.8	42.6	–0.8	0.02	0.08	5.22	3.92	1.30
6	3.0	47.1	42.4	4.7	0.14	0.47	23.2	26.3	–3.1	0.09	0.31	43.9	45.7	–1.8	0.05	0.18	7.67	12.22	–4.55
7	3.6	44.2	43.9	0.3	0.01	0.03	23.9	26.7	–2.8	0.08	0.28	42.9	47.6	–4.7	0.14	0.47	9.16	15.35	–6.19
8	12.3	45.6	42.7	2.9	0.09	0.29	25.7	28.1	–2.4	0.07	0.24	47.3	49.0	–1.7	0.05	0.17	9.59	10.05	–0.46
9	9.6	46.0	43.9	2.2	0.07	0.22	24.8	26.3	–1.5	0.04	0.15	46.0	46.8	–0.8	0.02	0.08	8.48	8.08	0.40
Check	0[c]	45.6	42.7	2.9	0.09	0.29	26.7	26.7	0.0	0.00	0.00	46.6	49.1	–2.6	0.08	0.26	11.23	3.92	7.31
Mean		45.6	43.3	2.3[d]	0.07	0.23	25.0	26.6	–1.6[d]	0.05	0.16	45.7	47.2	–1.4[d]	0.04	0.14	8.59	8.59	8.36
St. dev.		2.0	2.0	1.2			1.8	1.6	0.9			2.1	2.0	1.4			2.1	3.7	3.7

[a]Comparison between sound and sprouted seeds and calculation of the effect the sprout damage would have with maximum damage for the two top canola grades as in 2000.
[b]Difference due to sprouting expected at 3 and 10% (the maximum damage tolerances for No. 1 and No. 2 Canada canola at the time of the study) based on the results for that sample.
[c]The sprouted seed in the check sample was prepared by sprouting a subsample as described (in the Materials and Methods section.
[d]Significant at 95% confidence level.

Prairie, MN). The samples were immersed in boiling petroleum ether according to the Soxtec procedure (FOSS-Tecator), but otherwise the method was followed as described. Oil content was also determined by NMR spectrometry according to AOCS Recommended Practice Ak 4-95 (10) calibrated with standards tested by the solvent extraction procedure.

(iv) Chlorophyll content. The ISO Method 10519 (11) was modified to accommodate a small sample size. Ground seeds (0.3 g) were extracted with 5 mL of isooctane/ethanol (3:1, vol/vol). The results were expressed in mg chlorophyll/kg of seed or ppm of chlorophyll.

(v) NIR spectroscopy. The NIR reflectance spectra of the canola (log 1/*R*) were recorded at 2-nm intervals from 400 to 2500 nm with a NIRSystems 6500 scanning monochromator (FOSS NIRSystems Inc., Silver Spring, MD) using NSAS software v 3.53. The NIR Systems 6500 whole–seed analyzer was calibrated with the following reference methods: (i) oil content by the AOCS extraction method previously described, (ii) protein content according to the Dumas method previously described, and (iii) chlorophyll content by AOCS Official Method Ak 2–92 (11)

(vi) FA Composition of the extracted oil. Methanolic base derivatization. Extracted oil (0.05 ± 0.005 g) was weighed into a 16/125 mm borosilicate glass test tube followed by the addition 0.5 mL of the internal standard (triheptadecanoin; 2 mg/mL in toluene) and 5 mL of isooctane. The tubes were then mixed, and 0.5 mL of methanolic base was added. After incubation (30 min) at room temperature, two drops of bromothymol blue (0.1%, wt/vol in methanol) were added, and the solution was neutralized by sequentially adding 0.4 mL HCl (1 M) and 0.6 mL of sodium carbonate (0.15 M). After each addition, the tubes were mixed on a vortex mixer, and deionized water (7 mL) was then added to the mixture. The tubes were left to sit for 1 h or until the top layer was clear. The top layer was then transferred to a GC vial.

(vii) FA composition of the extracted oil. Acid–catalyzed derivatization. Extracted oil (0.025 ± 0.005 g) was weighed into a 5-mL Reacti-vial™ (Pierce, Rockford, IL), and 0.5 mL of C_{17} standard solution (2.5 mg/mL dissolved in toluene) and 50 µL of 2,2–dimethoxypropane were added. One milliliter of 2% sulfuric acid in methanol (prepared as required) was added to perform the derivatization. The capped vials were placed in a block heater (50°C) overnight. After cooling, 2 mL of 2% sodium bicarbonate and 1.2 mL of isooctane were added with mixing. Once clear, the top layer was transferred into a GC vial.

(viii) GC analysis. Separations of FAME were carried out using a Hewlett-Packard 5890 gas chromatograph, equipped with an FID and controlled by a Hewlett-Packard Chemstation (Agilent Technologies Canada, Mississauga, Ontario). All samples (1 µL) were injected into a 15 m × 0.32 mm open tubular fused–silica capillary column with a 0.25 mm SUPELCOWAX 10 coating (Supelco, Bellefonte, PA). Hydrogen was used as the carrier gas. Injector and detector temperatures were 280 and 300°C, respectively, and the temperature program started at 125°C for 2 min followed by two temperature gradients: 125 to 175°C at 25°C/min, then 175 to 220°C at 4°C/min and hold

TABLE 2
Effect of Sprout Damage on the Quality of Oil from Canola Seed[a]

Canola sample	Original prout (%)	FFA (%)					PV (meq/kg)			Diene value (absorbance units)			Aldehydes (nmol/g)		
					Loss at[b]										
		Sound	Sprouted	Diff.	3%	10%	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.
1	8.5	0.2	0.8	–0.5	0.02	0.05	4	6	–2	166	158	8	127	159	–32
2	8.1	0.2	0.4	–0.2	0.01	0.02	12	6	6	222	189	33	385	140	245
3	10.3	0.3	0.6	–0.4	0.01	0.04	8	7	1	243	149	94	212	156	56
4	13.2	0.5	0.6	–0.1	0.00	0.01	11	5	5	201	166	35	365	172	193
5	17.0	0.2	0.7	–0.4	0.01	0.04	8	5	2	189	150	39	193	137	56
6	3.0	0.2	1.4	–1.1	0.03	0.11	2	10	–8	ND	ND	ND	130	232	–102
7	3.6	0.5	0.7	–0.2	0.00	0.02	11	7	3	193	203	–10	517	221	296
8	12.3	0.3	0.7	–0.4	0.01	0.04	7	5	2	221	181	40	189	128	61
9	9.6	0.6	0.6	0.0	0.00	0.00	14	7	7	254	199	55	559	175	384
Check	0[c]	0.5	0.1	0.4	–0.01	–0.04	11	5	6	188	201	–13	359	169	190
Mean		0.4	0.7	–0.3[d]	0.01	0.03	9	6	2	209	177	31[d]	304	169	135[d]
St. dev.		0.2	0.3	0.4			4	2	5	29	22	33	156	34	152

[a]Comparison between sound and sprouted seeds and calculation of the effect the sprout damage would have with maximum damage for the two top canola grades.
[b]Difference due to sprouting expected at 3 and 10% (the maximum damage tolerances for No. 1 and No. 2 Canada canola at the time of the study) based on the results for that sample.
[c]The sprouted seed in the check sample was prepared by sprouting a subsample as described in the Materials and Methods section.
[d]Significant at 95% confidence level. ND, no data.

at 220°C for 4 min, giving a total run time of 15.5 min. The percentage of TAG was calculated according to the procedure outlined in AOAC method 996.06 (12).

(ix) Measurement of oxidation status. Aldehyde values and PV were performed using SafTest Inc. reagents and methodology (13). Diene values were determined by their absorbance at 232 nm, according to ISO 3656:2002 (14).

(x) Tocopherols. Tocopherols were measured by HPLC. The oil samples were diluted with hexane to a concentration of about 5 mg/mL, and 10 μL was injected for analysis. Separation was performed on Shimadzu HPLC model 10AD (Shimadzu, Columbia, MD) equipped with a fluorescence detector (RF–10AXL; Shimadzu). Excitation and emission wavelengths were 290 and 330 nm, respectively. Tocopherols and tocotrienols (chromanols) were separated on normal-phase silica column (5 μm, 3.2 mm i.d. × 25 cm length; Prodigy, Phenomenex, Torrance, CA) using a solution of *tert*-butylmethyl ether (5%, vol/vol) in hexane as eluant (flow rate 0.75 mL/min). Quantification of the various E vitamins was done using individual vitamin E isomers (Merck, Dortmund, Germany) as external standards (15).

(xi) Oligosaccharide content. Oligosaccharide content was measured by HPLC. The samples were defatted using the Soxtec extraction unit. Meal (1 ± 0.01 g) was extracted with 80% aqueous ethanol (20 mL) at 70°C for 30 min with lactose as internal standard. After centrifugation (9,000 × *g* for 15 min at 15°C), an aliquot of the supernatant was further submitted to a second centrifugation (18,000 × *g* at 15°C) for 5 min. After dilution with deionized water, an aliquot of sample (20–50 μL) sample was analyzed. Separation was performed on a Dionex HPLC equipped with an AS3500 autosampler and an ED 40 pulse amperometric detector. Sugars were eluted from an anion exchange chromatography column (4 mm i.d. × 25 cm length, PA1 analytical; Dionex Corporation, Sunnyvale, CA) combined with a Carbopac PA1 guard column (4 mm i.d. × 50 mm length) using a solution of sodium hydroxide (150 mM) as eluant (flow rate of 1 mL/min).

Statistical analysis. The NMR results are the mean of five analyses. NIR data are the average of four values obtained by 100 scans each. All other analyses were run in duplicate, and when the results showed variations higher than 10%, the analyses were repeated. Microsoft Excel was used for the data analysis. The analyses performed were independent *t*-tests of two populations with a significance level of 0.05.

RESULTS AND DISCUSSION

Oil, protein, and chlorophyll content. According to the CGC's Grain Grading Guide, canola samples are segregated into three grades according to the damage visually distinguishable in the seeds. Various percentages of green, frozen, heated seed are allowed into the samples assigning a grade to the seed sample. For example, canola grade 1 allows 2% distinctly green seeds and 3% of other damage. Sound seed had higher oil contents (Table 1) than sprouted seeds. Although the average difference was significant, the estimated loss of oil at the maximum damage allowed in the top two grades of canola seed was relatively small (0.07 and 0.23%, respectively), suggesting that, on the basis of oil content, the levels selected are not too large and the level of damage allowed in the top grade might be increased somewhat without serious consequences.

Seeds need energy to develop during germination. In oilseeds, nonpolar lipids, the TAG, are the source of this energy. The first step of the metabolism of nonpolar lipids is the hydrolysis of the reserve TAG by lipolytic enzymes and the release of FFA. The released FFA are then catabolized by β-oxidation to produce the required energy in the form of ATP. The degradation of plant storage lipids is in the sequence of TAG,

TABLE 3
Effect of Sprout Damage on the Tocol Content (mg/kg) of Oil from Canola Seed[a]

Canola sample	Original sprout (%)	α–Tocopherol			β-Tocopherol			Plasmachromanol–8			γ-Tocopherol			δ–Tocopherol			Total tocopherol		
		Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.
1	8.5	296	319	–23	0	3	–3	20	40	–20	628	649	–20	13	25	–11	969	1036	–67
2	8.1	239	363	–124	0	4	–4	12	41	–29	369	591	–222	17	17	1	635	1015	–380
3	10.3	300	412	–112	2	5	–3	17	48	–31	379	581	–202	25	20	5	715	1065	–350
4	13.2	288	356	–68	1	3	–1	34	52	–18	358	607	–249	15	23	–8	698	1041	–343
5	17.0	319	385	–66	0	5	–5	6	27	–21	587	586	2	18	24	–6	932	1026	–94
6	3.0	298	331	–33	0	4	–4	14	33	–19	579	632	–53	18	29	–12	913	1029	–116
7	3.6	278	264	14	0	5	–5	15	31	–16	578	634	–56	19	40	–21	899	975	–76
8	12.3	305	402	–97	0	4	–4	20	50	–30	579	563	16	22	19	4	919	1037	–118
9	9.6	ND	ND	ND	0	4	–4	ND	ND	ND	44	580	–536	28	24	5	ND	ND	ND
Check	0[b]	203	690	–487	1	12	–11	ND	ND	ND	591	305	286	16	14	2	812	1049	–237
Mean		281	391	–111	0	5	–4[c]	17	40	–23[c]	469	573	–103	19	24	–4	832	1030	–198[c]
St. dev.		37	121	148	1	3	3	8	9	6	183	98	216	5	7	9	122	25	130

[a]Comparison between sound and sprouted seeds.
[b]The sprouted seed in the check sample was prepared by sprouting a subsample as described in the Materials and Methods section.
[c]Significant at 95% confidence level. ND, no data.

DAG, MAG, and FFA. It was shown in flaxseeds that TAG were the major compounds involved in the catabolism that provides substrate for oxidation during germination (2,16). Saturated and unsaturated FA are used without discrimination during germination in cottonseed, as shown by the constant relative FA composition of the oil (3).

There was a slight increase in crude protein in sprouted seeds (Table 1) in both the seed and the defatted meal. In cottonseed, the decrease in oil content during germination was associated with an increase in the protein content, similar to that observed in this study (3). This was likely due to the *de novo* synthesis of enzymes to ensure new plant development, but it may also be related to the inverse relationship between oil and protein in canola seeds.

There was no difference in chlorophyll content between sound and sprouted seeds (Table 1). This is contrary to a report by Appelqvist and Lööf (5) but may be a reflection of differences in the degree of sprouting. Obviously, if the sample has sprouted to the point where cotyledons are developing, chlorophyll will increase.

Oil composition and quality. Sprouted seeds had higher levels of FFA than sound seeds (Table 2). The differences were small at the maximum damage tolerances for the top two grades of canola. The Canadian industry prefers canola with FFA contents lower than 1% to make oil with levels of FFA within the industry guidelines. In this study, at 100% sprouting, the level of FFA was well within the 1% level in all cases but one. Although it might be hypothesized that oil from sprouted seed would be more oxidized than oil from sound seed, the opposite was observed (Table 2). It should be noted that most of the oxidation observed in these experiments likely takes place during the extraction process (17). The "quality" of the seeds rests on the initial oxidation of its oil and the resistance of the oils to form oxidation products during the extraction. The improved oxidation status of oil from sprouted seeds might be due to increased tocopherols in those seeds (Table 3). There was an increased amount of all tocopherols in sprouted seed compared with sound seed. This suggests a *de novo* synthesis of these compounds as part of the germination metabolism or possibly the hydrolysis of tocopherol esters to produce more free tocopherols. Similar results—an increase of α- and γ-tocopherols—were observed during *Sorghum bicolor* seed imbibition (18).

It is likely that the increase in antioxidant content of the seed during germination is a necessary response to allow successful germination. During germination, seed metabolism is increased, resulting in the production of highly reactive radicals such as superoxide, hydrogen peroxide, and hydroxyl and lipid peroxide radicals. Therefore, the seed viability is directly related to its ability to respond to this oxidative stress.

There was no difference in the overall FA composition between the sound and sprouted seeds; all samples had FA compositions in the normal range for canola (data not shown). However, there was a statistically significant increase in the amount of saturated FA in the second hexane extraction of sprouted seeds (Table 4). This extract has been shown to contain about 2% of the total neutral lipids and significantly more

TABLE 4
Effect of Sprout Damage on the Saturated FA (% of total FA) of Oil from Canola Seed[a]

Canola sample	Original sprout (%)	Palmitic acid (C16:0)						Stearic acid (C18:0)					
		First extraction			Second extraction			First extraction			Second extraction		
		Sound	Sprouted	Diff.	Sound	Sound	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.
1	8.5	4.5	4.5	0.0	4.1	5.8	–1.7	2.7	2.7	0.0	2.5	2.8	–0.3
2	8.1	4.2	4.1	0.1	6.0	6.6	–0.6	2.2	2.2	0.1	2.1	2.4	–0.3
3	10.3	4.4	4.4	0.0	5.6	5.9	–0.3	2.1	2.1	–0.1	2.3	2.4	–0.1
4	13.2	4.3	4.2	0.1	5.7	6.4	–0.8	2.0	2.0	0.0	2.1	2.3	–0.2
5	17.0	4.2	4.2	0.0	5.4	6.2	–0.8	2.2	2.2	0.0	2.2	2.2	0.0
6	3.0	4.1	4.4	–0.3	5.9	6.6	–0.6	2.1	2.2	–0.1	2.0	2.4	–0.4
7	3.6	4.2	4.5	–0.3	5.5	6.2	–0.6	2.0	2.2	–0.2	2.0	2.5	–0.4
8	12.3	4.1	4.2	–0.1	6.9	7.1	–0.2	2.1	2.1	0.0	2.3	2.8	–0.5
9	9.6	4.2	4.1	0.1	6.2	6.2	0.0	2.2	2.1	0.0	2.2	2.3	–0.2
Check	0[b]	3.7	3.5	0.2	5.2	6.3	–1.1	2.1	2.0	0.1	2.4	2.8	–0.4
Mean		4.2	4.2	–0.02	5.7	6.3	–0.7[c]	2.2	2.2	0.0	2.2	2.5	–0.3[c]
St. dev.		0.2	0.3	0.2	0.7	0.4	0.5	0.2	0.2	0.1	0.2	0.2	0.2

[a]Comparison between sound and sprouted seeds. The first extraction with hexane represents about 98% of the total neutral lipids and the second represents the remainder, including some more polar and structural lipids (19).
[b]The sprouted seed in the check sample was prepared by sprouting a subsample as described in the Materials and Methods section.
[c]Significant at 95% confidence level.

TABLE 5
Effect of Sprout Damage on the Carbohydrate Content (mg/kg) of Canola Seed[a]

Canola sample	Original sprout (%)	Glucose			Sucrose			Raffinose			Stachyose			Total		
		Sound	Sprouted	Diff.	Sound	Sound	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.	Sound	Sprouted	Diff.
1	8.5	0.10	0.15	–0.05	7.1	7.2	0.0	0.42	0.05	0.37	1.2	0.1	1.1	8.9	7.5	1.4
2	8.1	0.10	0.14	–0.04	7.1	7.4	–0.3	0.29	0.05	0.24	1.5	0.2	1.3	8.9	7.7	1.2
3	10.3	0.88	0.14	0.74	7.2	7.4	–0.1	0.00	0.06	–0.06	0.0	0.1	–0.1	8.1	7.7	0.4
4	13.2	0.12	0.75	–0.63	7.0	7.5	–0.5	0.29	0.03	0.26	1.4	0.0	1.4	8.8	7.8	1.1
5	17.0	0.12	0.14	–0.02	7.0	7.2	–0.2	0.28	0.06	0.22	1.2	0.1	1.1	8.6	7.5	1.1
6	3.0	0.13	0.15	–0.02	6.6	7.6	–1.0	0.42	0.15	0.27	1.9	0.5	1.4	9.1	8.4	0.7
7	3.6	0.13	0.21	–0.08	7.2	7.8	–0.5	0.41	0.11	0.30	1.7	0.4	1.3	9.5	8.5	1.0
8	12.3	0.13	0.16	–0.03	7.2	7.3	–0.2	0.33	0.02	0.31	1.4	0.3	1.1	9.1	7.8	1.2
9	9.6	0.12	0.13	–0.01	7.1	7.3	–0.2	0.36	0.10	0.26	1.7	0.4	1.3	9.2	7.8	1.4
Check	0[b]	0.11	0.75	–0.64	5.6	7.5	–2.0	0.51	0.03	0.48	2.2	0.0	2.2	8.4	8.3	0.1
Mean		0.2	0.3	–0.08	6.9	7.4	–0.5[c]	0.3	0.07	0.3[c]	1.4	0.2	1.2[c]	8.9	7.9	1.0[c]
St. dev.		0.2	0.3	0.38	0.5	0.2	0.6	0.1	0.04	0.1	0.6	0.2	0.6	0.4	0.4	0.4

[a]Comparison between sound and sprouted seeds.
[b]The sprouted seed in the check sample was prepared by sprouting a subsample as described in the Materials and Methods section.
[c]Significant at 95% confidence level.

TABLE 6
Comparison of Differences Between Sprouted and Sound Seeds for Different Analytical Methods[a]

Canola sample	Sprout (%)	Oil content difference (%)			Protein content difference (%)		Chlorophyll content difference (ppm)		TAG content difference (%)	
		Extraction	Pulsed NMR	NIR	Dumas	NIR	Modified AOCS	NIR	Acid-catalyzed	Base-catalyzed
1	8.45	2.46	3.12	–0.11	–1.36	1.33	0.34	3.00	0.33	2.02
2	8.05	1.23	1.03	0.11	–1.08	0.36	0.77	–3.00	0.15	0.88
3	10.30	1.95	1.32	–0.45	–1.19	0.71	1.53	2.00	–0.82	0.54
4	13.20	1.89	1.81	0.67	–1.07	0.04	1.89	–3.00	–0.31	1.46
5	16.96	2.10	2.15	1.23	–1.29	–0.42	1.30	–2.00	1.0	8.89
6	2.95	4.73	4.87	2.56	–3.09	0.16	–4.55	–1.00	–0.13	2.57
7	3.58	0.28	4.72	1.08	–2.78	–0.51	–6.19	–6.00	0.75	2.52
8	12.27	2.90	3.25	0.30	–2.35	0.18	–0.46	7.00	1.53	2.76
9	9.62	2.17	1.72	0.39	–1.45	0.27	0.40	8.00	–0.39	2.67
Check	0.00	2.91	2.06	0.87	–0.04	1.2	7.31	11.00	–0.47	1.54
Mean		2.26	2.61	0.67[b]	–1.57	0.33[b]	0.23	1.60	0.16	2.59[b]
St. dev.		1.17	1.35	0.85	0.91	0.61	3.66	5.58	0.74	2.35

[a]The differences using the extraction method for oil, the Dumas method for protein, and the acid catalyzed method for TAG content were considered reference methods for purposes of comparison.
[b]A paired *t*-test showed the secondary method difference to be significantly different from the reference method difference at greater than a 95% level of confidence.

structural and polar lipids than the first extract made up primarily of storage TAG (19).

Carbohydrates. Whereas there was no significant difference in glucose, the sucrose content was higher in sprouted seeds than sound seeds, but the total raffinose and stachyose contents, along with the total sugars, were higher in sound seeds than in sprouted seeds (Table 5). Although sugars are minor components, they play an important role in *Brassica* seed viability. It was shown that sucrose was the only soluble sugar present in *Brassica* in all stages of seed development and that glucose, sucrose, raffinose, and stachyose amounts varied during seed development (20). However, the sugar content variations differ with the seed type; for example, sucrose levels increase in mung beans but decrease in soybeans and cottonseed during germination (21). In cottonseed, an increase in glucose and sucrose was associated with a decrease in raffinose and stachyose (22). No galactose, resulting from the hydrolysis of stachyose and raffinose, was found in the seeds during this study. It was suggested (22) that galactose may be rapidly metabolized into D-galacturonic acid to contribute to cell wall formation via the UDP derivative pathway.

Effect of sprout damage on the accuracy of analytical methods. Oil contents in this study were measured by extraction, pulsed NMR; and NIR; and crude protein content was determined by the Dumas method. In comparing the effect of sprouting on the results derived from these analytical methods, the differences in oil, chlorophyll, or crude protein content between sound and sprouted seeds were compared for each of the methods against the reference (extraction or Dumas) methods (Table 6). For oil content, extraction and pulsed NMR gave statistically

TABLE 7
Comparison of Differences Between Sprouted and Sound Seeds for Different Analytical Methods[a]

Canola sample	Modified CGC method Chlorophyll (mg/kg or ppm)				NIR Chlorophyll (mg/kg or ppm)		
	Sound	Sprouted	Diff.	Original	Sound	Sprouted	Diff.
1	6.71	6.37	0.34	9.50	8.00	5.00	3.00
2	6.12	5.35	0.77	4.57	3.00	6.00	–3.00
3	10.18	8.65	1.53	11.84	10.00	8.00	2.00
4	11.57	9.69	1.89	12.25	8.00	11.00	–3.00
5	5.22	3.92	1.30	5.08	1.00	3.00	–2.00
6	7.67	12.22	–4.55	8.18	6.00	7.00	–1.00
7	9.16	15.35	–6.19	10.71	6.00	12.00	–6.00
8	9.59	10.05	–0.46	9.11	10.00	3.00	7.00
9	8.48	8.08	0.40	7.98	11.00	3.00	8.00
Check	11.23	3.92	7.31		11.00	0.00	11.00
Mean	8.59	8.36	0.23	8.80	7.40	5.80	1.60
St. dev.	2.15	3.67	3.66	2.70	3.41	3.79	5.58

[a]The differences using the extraction method for oil, the Dumas method for protein, and the acid-catalyzed method for TAG content were considered reference methods for purposes of comparison. CGC, Canadian Grain Commission.

the same differences, but the difference obtained by NIR was significantly less. Similarly, the NIR method gave smaller differences for protein than the Dumas method. There was no statistical difference between NIR and the modified method. Once the NIR calibration was adjusted by including samples of sprouted seeds, the differences by NIR were the same as those from the other methods. This highlights the importance of ensuring that the sample and reference sets for the determination of components by NIR are compatible.

As a result of this research, the sprout damage tolerance in Canadian canola grades was increased. For top-quality canola, the tolerance was increased from 3 to 5%, as this research had demonstrated that the effect on oil content and on the quality of the extracted oil would be minimal since FFA were below 1% and there was an increase of tocopherol, ensuring a more stable oil. Secondary analytical methods must be applied, ensuring that samples and reference sets are compatible.

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