(13), *H. syriacus* (13), *H. panduriformis* (14), *H. trionum* (15), *H. diversifolius* (15), *H. sabdariffa* (16), *H. cannabinus* (17), *H. moscheutos* (17) and *H. syriacus* (17).

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Effect of Frost Damage on the Quality of Canola *(B. napus)*

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ABSTRACT

Samples of frost-damaged rapeseed from the 1982 Western Canadian Crop were tested for oil content, protein content, fat acidity, chlorophyll content, fatty acid composition, glucosinolate content, conductivity and germination. These quality factors were related to two frost-related damage factors, green seeds and external "frost-damage," used in the Canadian grain grading system. The green seed factor was positively correlated with chlorophyll, free fatty acids and conductivity, and a negative correlation was found with linolenic acid, iodine value and germination. The frost-damage factor was positively correlated with conductivity, free fatty acids and palmitic acid and negatively correlated with linolenic acid, iodine value, oil content and germination. The effects of frost damage were explained by assuming that the seed maturation process was halted due to freezing.

INTRODUCTION

A severe frost on August 27, 1982 damaged much of the rapeseed and canola[1] growing in the eastern and central Canadian prairies. The extent of the frost damage and the monetary costs involved will be described in a separate report (Clear, K.M., J.K. Daun and J.T. Mills, In Preparation). An estimated 38% of the crop, or about 1,160,000 tons of seed, graded No. 3 Canada or lower as a result of the frost, compared with an average of only 4% of the crop in these grades over the previous five years. Frost damage of this extent is extremely rare in Western Canada, but isolated instances of frost-damaged seeds do occur almost every year. The 1982 crop of rapeseed offered an opportunity to evaluate the quality of a large number of frost-damaged samples under field conditions. The storageability (1) and morphology of frost-damaged seed (2) have been reported elsewhere. This study compared quality parameters (oil content, protein content, fatty acid composition, chlorophyll, free fatty acids, glucosinolates, germination and conductivity) with visual degrading factors associated with frost damage for samples of rapeseed taken after the 1982 frost.

MATERIALS AND METHODS

Sources of Samples

Samples were obtained from 60 bins containing rapeseed harvested from the 1982 crop and located on farms within a 200 km radius of Winnipeg (1). Information on the binned seed including variety name, dates of swathing, combining and binning were obtained from the producer. The bins were sampled in early November, 1982 and two samples (where possible), each of 450 g, were removed by probe (deep bin cup no. 232, Seedburo, Chicago, Illinois) at depths of 2 m and 45 cm either vertically through the top surface or at an angle of about 45° through the side port. The samples were transported in double plastic bags, and stored at −15 C until tested individually.

In order to minimize the effect of species and type in the farm bin study, *B. campestris* varieties (10 samples) and the *B. napus* (non-canola) variety Midas (4 samples) were eliminated from the study. Also eliminated were samples severely infested with wild mustard (11 samples) and samples which showed extensive bin-heating (4 samples). The remaining 50 samples included 40 of *B. napus* cv. Regent and 10 of *B. napus* cv. Altex.

Samples of pooled commercial lots also were obtained from grain firms and crushing plants as a part of the Grain Research Laboratory's annual new crop survey. These samples were graded by Canadian Grain Commission grain

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[1] Canola is a trade mark of the Canola Council of Canada (3) and refers to seeds of *Brassica campestris* L. and *B. napus* L. with low levels of erucic acid in the oil (5%) and low levels of glucosinolates in the oil-free meal (30 μM/g of the four main alkenyl glucosinolates). More than 90% of the Western Canadian rapeseed and canola growing area was planted with canola varieties in 1982. The Canada Grain Act does not recognize canola as separate from rapeseed since, for visual grading purposes, it is impossible to differentiate between canola and rapeseed.

inspectors (4) and composited by grade and growing area before testing.

Grading of Samples

Frost-damage was a major degrading factor for rapeseed harvested in 1982. The percentage of green seeds and total damage were assessed by Canadian Grain Commission grain inspectors (4), and frost damage was calculated as the difference between % green seeds and total damage. Actually, both "frost damage" and "% green seeds" were considered as resulting from frost damage as they are both manifestations of immaturity. The "% green seeds" referred to seeds which were distinctly green in appearance when crushed, while "frost damage" included mainly discolored and misshaped seeds. Damage factors other than "frost-damage" and "% green seeds" were negligible in the samples selected for the study.

Quality Analyses

Chlorophyll contents (ppm) were determined by reflectance spectroscopy (5) with results corrected to correspond to the new Grain Research Laboratory extraction procedure. The new procedure involves calibration with pure chlorophyll and gives somewhat higher results than older methods (Tkachuk, R., and V.J. Mellish, In Preparation). Chlorophyll contents of smaller samples were determined by the new extraction procedure. Total oil content was determined by broad-band NMR (6) or on smaller samples by grinding in a coffee mill followed by extraction (6 hr) with petroleum ether on a Goldfisch apparatus. Free fatty acids were determined by titration of samples of oil extracted from ground seed (as above) (7). Protein content was determined by a Kjeldahl procedure (8).

Fatty acid composition (9) and glucosinolates (10) were determined by gas liquid chromatography. Conductivity of the canola steep water was determined using a CDM-2 conductivity meter (11). Conductivity measured the concentration of electrolytes in water which contained a known amount of seed for a specific time period and was related to the extent of ion-leakage through the cell membranes. Seed germination was determined by the method of Wallace and Sinha (12).

Statistics (means, standard deviations, Student's "t" tests and correlation coefficients) were determined using procedures as described by Snedecor and Cochran (13).

RESULTS AND DISCUSSION

A survey of producers in Southern Manitoba had indicated that the mean planting date for *B. napus* varieties of canola in 1982 was May 24 and plantings continued until at least June 25. The severe frost of August 27 occurred just 95 days after the mean planting date and only 63 days after the latest reported planting date. *B. napus* varieties were expected to mature in 95 to 98 days in Southern Manitoba (14, 15), while flowering and seed formation were expected to occur after 50-60 days of growth. At the time of the frost, at least one-half of the canola crop had not reached full maturity and a portion of the crop had only just begun to develop seeds. Where the freezing temperatures killed the plants, seed development stopped. As the development of seeds in a single field of rapeseed may be spread over different stages for up to two weeks, wide ranges of damage were noted in individual samples of seed (2).

1982 Crop Survey

A first approximation of the overall effect that frost damage had on quality was obtained by assessing samples graded "sample account damage" or "sample account green" from the 1982 crop survey. Samples which contained more than 20% combined damage were classed as "sample grade." Composite samples of "green" and "damaged" samples from the 1982 crop survey were compared with top quality seed (No. 1 Canada rapeseed (1 CR), less than 3% total damage) from the same growing area (Table I).

The oil contents of the damaged and green seeds appeared to be lower than the oil content of the top quality seed. Also, the chlorophyll content and the free fatty acid levels of the sample grade seed were much higher than the No. 1 Canada seed. There appeared to be slightly lower levels of glucosinolates in the damaged seeds, and the sample grade seeds also had lower iodine values (possibly related to a higher level of palmitic acid C16:0). A lower level of protein was noted in the "damaged" seeds.

Five samples of seed from the 1982 harvest survey were hand-separated into apparently sound (round, no outside discoloration) and damaged (misshaped, discolored) fractions (Table II). A comparison of the two fractions showed similar, statistically significant, quality differences between the No. 1 grade and the sample grade composites.

Farm-Bin Samples

The samples taken from the farmers' bins were assessed for quality factors on an individual basis. Table III shows the mean, standard deviation and range of values for these samples. Significant correlations (Table IV) were observed between the quality factors and the damage assessment, which further supports many of the observations made

TABLE I

Quality of Severely Damaged Rapeseed and Top Quality Rapeseed from the Frost-Affected Regions of the 1982 Crop in Western Canada

	No. 1 Canada	Sample Acct. Green	Sample Acct. Green
No. of samples in composite	680	140	97
Oil content[a] (%)	43.4	40.9	40.2
Protein content[b], N X 6.25 (%)	39.0	39.8	37.6
Chlorophyll (ppm)	9	75	72
Free fatty acids (%)	0.2	1.3	1.7
Glucosinolates[b] (µM/g)	22	18	19
Fatty acid composition			
C16:0 (% of total fatty acids)	4.1	4.1	4.5
C18:3 (% of total fatty acids)	10.5	9.9	10.0
Iodine value[c]	119	112	114

[a] 8.5% moisture basis.

[b] Oil-free, 8.5% moisture basis.

[c] Calculated from fatty acid composition.

TABLE II

Comparison of Visually Damaged and Visually Undamaged Seeds Hand-Separated from Frost-Damaged Seed Samples

Sample	Oil content (dry basis)	Protein content (dry basis)		Chlorophyll content in seed (ppm)	Free fatty acid (% in oil as oleic)	Iodine value	Palmitic acid (% of total fatty acids)
		Seed	Oil-free meal				
Visually undamaged seed							
1	46.4	22.8	42.5	26	1.5	112	4.5
2	45.5	25.8	47.3	26	1.2	115	4.5
3	46.1	24.2	44.9	20	0.8	112	4.5
4	42.9	24.2	42.4	NS^a	1.7	111	5.4
5	46.8	22.6	42.5	80	1.1	110	4.5
Mean (std. dev.)	45.54 (1.55)	23.92 (1.29)	43.92 (2.16)	38 (28)	1.26 (0.35)	112 (1.87)	4.68 (0.36)
Visually damaged seed							
1	37.6	22.3	35.7	51	4.7	109	5.9
2	37.9	23.5	37.7	51	6.3	110	6.0
3	36.4	22.4	35.2	44	3.2	108	6.0
4	27.0	21.1	29.0	NS^a	5.2	106	7.8
5	35.9	22.3	35.2	80	3.9	110	6.7
Mean (std. dev.)	34.96 (4.52)	22.38 (0.86)	34.56 (3.27)	57 (16)	4.66 (1.19)	109 (1.67)	6.48 (0.80)
Paired "t"^{b}	7.37	3.35	8.01	−3.00	−7.33	3.67	−8.68
Mean diff.	10.6	1.28	9.4	−19	−3.4	3.4	−1.8

[a] NS = insufficient seed for analysis.

[b] Hypothesis 1. $\mu_1 = \mu_2$, reject 1 and accept 2 if (a) t 2.571 (n=5) for $\alpha = 0.05$. Hypothesis 2. $\mu_1 = \mu_2$, reject 1 and accept 2 if (b) t 2.776 (n=4) for $\alpha = 0.05$.

TABLE III

Mean and Ranges for Quality Factors Assessed on 50 Samples of *B. napus* Canola from Farmers' Bins, 1982 Crop

Factor	Mean		Std. dev.		Min.		Max.	
	n=50	n=46^a	n=50	n=46^a	n=50	n=46^a	n=50	n=46^a
Total damage (%)	17.9	14.9	13.6	8.4	3	3.0	65.0	36.0
Frost damage (%)	6.3	4.6	7.3	3.7	0.2	0.2	42.2	14.0
Green seeds (%)	11.7	10.2	9.3	7.0	0.6	0.6	46.0	28.0
Chlorophyll (ppm)	52.0	34.0	19.2	12.0	21.0	14.0	98.0	60.0
C16:0 (% of total fatty acids)	4.4	4.4	0.3	0.3	3.8	3.8	5.3	5.3
C18:3 (% of total fatty acids)	10.1	10.2	0.9	0.8	8.6	8.6	12.1	12.1
Iodine value (from f.a. composition)	115.6	115.7	2.2	2.2	112.0	112.0	120.0	120.0
Seed protein (% dry basis, N × 6.25)	22.7	22.6	2.0	1.9	18.6	18.6	27.0	36.5
Meal protein (%, oil-free dry basis)	40.5	40.4	2.8	2.7	33.2	33.1	45.1	45.1
Free fatty acids (% as oleic)	1.06	0.9	0.8	0.6	0.1	0.1	4.6	2.2
Oil content (%, dry basis)	43.9	44.2	3.1	3.0	37.0	37.8	49.1	49.1
Glucosinolates (μM/g, oil-free, dry basis)	16.7	16	5.6	5.0	8.0	8.0	34.0	28.0
Conductivity	99.2	90.0	64.2	47.0	22.0	22.0	382.0	236.0
Germination (%)	84.7	87.0	12.2	9.0	36.0	64.0	98.0	98.0

[a] Four samples with largest total damage removed from analysis.

above. Examination of the distribution of % frost damage and % green seeds showed four samples had large amounts of damage and were outliers on the frequency histogram. The correlation matrices were repeated with these samples omitted to confirm the relationships. Quality factors which correlated significantly with both damage factors were subjected to multiple regression analysis with "green seeds" and "frost damage" as the independent variables (Table V).

Chlorophyll

Chlorophyll content itself is useful as a measure of seed maturity. Chlorophyll has been shown to decrease rapidly as the moisture content of developing rapeseed decreases from 60% to 40% (21). Increased chlorophyll was noted in the sample grade rapeseed samples (Table I) and in the hand-picked damaged seeds (Table II). In the samples from farm bins, however, chlorophyll was found only to be significantly correlated with the green seeds in the sample, and frost damage did not contribute significantly to the multiple regression (Table V). For an individual sample, it is possible that the contribution to chlorophyll from green seeds far outweighed the contribution from the smaller damaged seeds.

Oil Content

Most of the effects of frost damage on rapeseed quality can be explained if frost damaged seeds are considered as immature seeds. Several authors (16, 17, 18) have noted that the oil accumulation in rapeseed occurred primarily between 14 and 30 days after flowering. Shrunken and misshapen seeds had extremely low oil contents (Table II), which suggested that these seeds were killed by the frost before the major deposition of oil had been completed. This is further supported by the increased level of palmitic acid (C16:0) in the oils from these samples. Fowler and Downey (16) noted that low erucic acid *B. napus* rapeseed

TABLE IV

Correlation (r) Between Visible Damage Factors and Quality Factors for Samples of Frost-Damaged *B. napus*[a]

	Total damage		Green seeds		Frost damage		Chlorophyll	
Quality factor	n=50	n=46	n=50	n=46	n=50	n=46	n=50	n=46
Chlorophyll	0.72**	0.90**	0.89**	0.94**	0.20	0.27	–	–
C16:0	0.57**	0.43**	0.40**	0.18	0.45**	0.63**	0.40**	0.29*
C18:3	−0.35*	−0.51**	−0.42**	−0.39**	−0.12	−0.41**	−0.53**	−0.52**
Iodine value	−0.45**	−0.61**	−0.51**	−0.49**	−0.20	−0.45**	−0.62**	−0.59**
Seed protein	0.29*	0.20	0.23	0.20	0.32*	0.09	0.11	0.14
Free fatty acids	0.77**	0.83**	0.65**	0.61**	0.62**	0.71**	0.54	0.65**
Oil content	−0.45**	−0.29*	−0.22	−0.08	−0.55**	−0.50**	−0.19	−0.12
Glucosinolates	0.03	0.03	0.23	0.28	−0.14	−0.20	−0.21	−0.17
Conductivity	0.82**	0.86**	0.87**	0.80**	0.43**	0.44**	0.80**	0.82**
Germination	−0.76**	−0.69**	−0.81**	−0.72**	−0.37**	−0.22	−0.71	−0.71**

[a]Test for r=0.

*Signficant at 5%.

**Significant at 1%.

had similar levels of (5-7%) palmitic acid at 21-28 days after pollination. At the same time the developing seed had 50-88% of its possible total oil content and 53-76% of potential maximum dry matter accumulation. The hand-picked damaged seed (Table II) had 68-83% as much oil content as visually undamaged seeds, and damaged seed had an average palmitic acid content of 6.5%. Similar results were found in the farm bin studies where both oil content and palmitic acid were correlated with frost-damaged seeds (Table IV) and were also significantly correlated (r=—0.53, n=50; r=—0.50, n=47) to one another. There was no significant correlation between oil content and green seeds, however, and in the multiple regression analysis (Table V) the regression coefficient for green seeds with oil content and palmitic acid was not significantly greater than zero, which suggests that most of the oil has been laid down in the seed before greenness beomes a grading factor.

Protein

In developing rapeseed, protein content is established at an earlier time than oil content and does not increase as rapidly near maturation (17, 18). For hand segregated seeds (Table II) there were significant differences between sound and damaged seed in both seed protein and meal protein, but the reduction in protein was not very large in the comparison between 1 CR and the sample grade rapeseed (Table I). In the study of samples from farm bins (Table IV), there was a significant correlation between seed protein and total damage and frost damage but no correlation with green seed or between meal protein and any damage factors. Relationships between meal protein and damage may have been hidden by the inverse relationship between seed protein and oil content. Also, by the time a seed has matured sufficiently for its total chlorophyll to contribute to overall sample chlorophyll, most of its protein had likely been laid down.

Free Fatty Acids

Free fatty acids were found to be higher in damaged seeds in all three sets of samples in this study (Tables I, II, IV and V). Levels of free fatty acids were higher in oil extracted from immature seed than from fully mature seed (18, 20). Significant correlations were noted between chlorophyll and free fatty acid levels in rapeseed harvested in Sweden (21), and frost-damaged soybeans also had higher levels of chlorophyll and free fatty acids (22). In farm bin studies, free fatty acids were significantly correlated with both damage factors and chlorophyll content with approximately equal regression coefficients. For soybeans (22), freeze-damaged seed coats were ruptured, allowing mold penetration and growth, and increased free fatty acid levels were due to fungal lipase activity. In this study, apparently sound rapeseeds with high chlorophyll levels also had high levels of free fatty acids. Therefore, it is possible that some of the increased free fatty acid content was due to seed lipase activity stimulated by the frost conditions.

Glucosinolates

The glucosinolate level of developing rapeseed also increases as the seed nears maturity (23). This effect might account for the slightly decreased levels of glucosinolates found in the sample grade seed (Table I), although relatively large amounts of inconspicuous admixture also tend to dilute the glucosinolates in sample grade seed. Hydrolysis of intact glucosinolates by myrosinase released by frost damage to cell structure might also contribute to lower results. No significant correlation was observed between damage factors and glucosinolate levels (Table IV), but this is not surprising because environmental effects during the growing period have been shown to result in a four-fold variation in the glucosinolate content of canola (24).

Fatty Acid Composition

The changes in fatty acid composition during ripening, especially in linolenic acid and iodine value, are complex (16, 17, 20, 24), but iodine value and linolenic acid have been noted to increase in the later stages of maturity. Frost-damaged seed was found to contain more saturated fatty acids than fully mature seed (Tables I and II). The linolenic acid content of developing seeds has been noted to go through a minimum during maturation (17, 20). The correlation (Table IV) between linolenic acid or iodine value and frost damage improved when the severely damaged samples (i.e., very immature) were left out of the statistics.

Germination

The decrease in germination with increasing green seed count also corresponded with studies on maturing rapeseed where germination was found to increase with maturity. Germination in spring rapeseed has been noted as being particularly sensitive (19) to seed maturity.

Conductivity

The increase in ion leakage (conductivity) with seed damage was likely related to changes taking place in the hull during maturation. The content of aqueous-soluble material in rapeseed has been noted to decrease during maturation as the hull becomes less permeable due to the formation of insoluble tannins (2, 17).

TABLE V

Multiple Regression Statistics for Quality Factor as the Dependent Variable and Damage Factors as the Independent Variables[a]

Dependent variable (quality factor)	Independent variable (damage factor)									
	Green seeds				Frost-damaged seeds				Multiple correlation coefficient	
	Standardized partial regression coefficient[b]		Student's t[c]		Standardized partial regression coefficient[b]		Student's t[c]			
	n=50	n=46	n=50	n=46	n=50	n=46	n=50	n=46	n=50	n=46
Chlorophyll	1.28	1.59	13.77**	18.68**	−0.18	0.41	−1.50	2.54*	0.90	0.95
C16:0	0.010	0.0042	2.14*	0.77	0.017	0.054	2.76**	5.19**	0.53	0.64
C18:3	−0.040	−0.041	−3.04**	−2.60*	0.003	−0.081	0.17	−2.70**	0.42	0.53
Iodine value	−0.12	−0.14	−3.76**	−3.64**	−0.012	−0.23	−0.29	−3.21**	0.51	0.63
Free fatty acids	0.043	0.042	5.10**	7.04**	0.051	0.097	4.74**	8.66**	0.77	0.88
Oil content	−0.015	6.1×10^{-5}	−0.35	0.002	−0.23	−0.40	−4.12**	−3.77**	0.55	0.50
Conductivity	5.66	5.03	11.19**	9.76**	1.43	4.14	2.24*	4.27**	0.88	0.87
Germination	−1.02	−0.95	−8.87**	−6.66**	−0.19	−0.27	−1.28	−1.01	0.82	0.73

[a] Only quality factors with one or more significant correlations considered.

[b] This statistic ($b_i\,(\Sigma x_i^2/\Sigma y^2)$) has been used as a measure of the relative importance of the partial regression coefficients (β_i).

[c] Test for $b_i = 0$. *Reject at 5% level. **Reject at 1% level.

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