TABLE V

Baking Quality

Sample code[a]	Loaf volume (cm^3)	Appearance	Crumb structure[b]	Crumb color[c]	Baking absorption (%)
1	825	8.0	6.8-o	6.2 dy	62.0
2	820	7.8	7.0-o	6.5 dy	63.0
3	820	8.0	6.8-o	6.5 dy	63.0
4	815	7.8	6.8-o	6.2 dy	63.0
5	820	7.8	6.8-o	6.2 dy	62.0
6	830	7.8	6.8-o	6.5 dy	63.0
7	835	7.8	6.8-o	6.2 dy	63.0
8	820	8.0	6.8-o	6.2 dy	63.0

[a]Refer to Table I for the meaning of the sample code.

[b]o = Open.

[c]d = Dull, y = yellow.

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The Relationship between Rapeseed Chlorophyll, Rapeseed Oil Chlorophyll and Percentage Green Seeds[1]

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ABSTRACT

Oils with high levels of chrlorphyll have become a major problem in the Canadian crushing industry. It was not possible to compare visually the color of samples of rapeseed oil from various crushing plants in Western Canada with the nickel sulfate standard used as a trade standard. Comparison was easy using samples of oil prepared from seed in the laboratory. The difficulty in comparison was probably caused by conversion of green-colored chlorophyll to russet-colored pheophytin in the crushing process. An "apparent chlorophyll" standard with a maximum of 20 ppm (measured by AOCS Cc 13d 55) is recommended. The "percentage green seed" count used in the Canadian grading system was found to correlate poorly ($r^2 < 0.5$) with the chlorophyll level in the seed or oil. A maximal chlorophyll level of 12 ppm was found to be allowable in the top grade of seed. It is recommended that a rapid, accurate and inexpensive procedure for chlorophyll measurement be developed to supplement the grading system.

[1]Contribution No. 478 from the Grain Research Laboratory, Canadian Grain Commission, 1404 - 303 Main St., Winnipeg, Manitoba, Canada R3C 3G9.

INTRODUCTION

In the Canadian grain grading system, grain samples are assigned grades according to the number and type of damaged seeds and the amount and kind of admixture found on visual inspection. In the case of rapeseed or canola, damage factors include immature seeds, heated seeds, and frosted and otherwise weathered seeds. Despite the relatively stringent restrictions on the number of damaged seeds allowed in the top grades (Table I), about 90% of the rapeseed inspected annually arrives at shipping points as No. 1 Canada Rapeseed.

Canola is a trademark of the Canola Council of Canada and refers to seed and products from varieties of *B. napus* and *B. campestris* which are low in erucic acid and glucosinolates. Although about 75% of Canada's rapeseed production has been converted to canola, there is no effort to segregate canola from rapeseed in export channels. Since canola crushers use about two thirds of the seed produced and the carryover of rapeseed from previous years is large, canola does not regularly appear in Canadian export cargoes. This study uses samples of both canola and rapeseed.

TABLE I

Tolerance Maximum (%) for Grading Factors in Rapeseed (1)

	Damage			Admixtures of foreign material				
Grade	Heated	Distinctly green	Total damage[a]	Sclerontinia	Ergot	Stones	Total conspicuous	Total inconspicuous[b]
No. 1 Canada	0.1	2	3	0.1	0.05	0.05	1	5
No. 2 Canada	0.5	6	10	0.2	0.05	0.05	1	5
No. 3 Canada	2	20	20	0.3	0.05	0.05	1	5

[a]Damage includes distinctly shrunken or shriveled seeds as from frost, discolored as from mold, completely rimed, or excessively weathered, sprouted, distinctly green, or heated. Broken seeds not assessed as dockage which are otherwise sound are considered sound.

[b]Includes common wild mustard and/or domestic brown or Oriental mustard seed.

Immaturity, or greeness, is the major degrading factor in Canadian rapeseed. The presence of immature seeds in a sample is assessed by examining a minimum of five "100 seed crush strips." The strips are prepared by transferring 100 seeds to the sticky side of a piece of masking tape using a special applicator. The seeds are then crushed using a roller. Greeness is assessed subjectively by the inspector without the use of a color standard, although the use of official grade standard samples is encouraged. Only distinctly green seeds are classed as damaged although seeds with a light green tinge are considered as part of the overall color of the sample and samples with large numbers of light green seeds are graded lower on the basis of "poor natural color" (1).

The green color in immature rapeseed is caused by the photosynthetic pigment chlorophyll (2). Chlorophyll has become a major problem in the Canadian crushing industry as it is often extracted into the oil in such quantities that it is difficult to remove by conventional bleaching processes (3). Besides giving a product with an undesirable color, chlorophyll has been implicated as a prooxidant in the formation of oxidative rancidity in oils (4).

The system just described has been used successfully to grade rapeseed since the early 1960s. Recently, however, rapeseed processors have been complaining that rapeseed with low percentages of green seed has been giving oils which were extremely dark in color (5). In order to provide initial background to the problem, this study was carried out to establish the relationship between seed chlorophyll and oil color and to examine the relationship between seed and oil grading systems.

MATERIALS AND METHODS

Samples of rapeseed were received from grain companies through the Grain Research Laboratory's annual new-crop survey. Samples of canola oil and seed were obtained from Western Canadian canola crushing plants.

Chlorophyll Determinations

Many spectrophotometric procedures which measure chlorophyll actually measure the group of compounds which have absorption maxima near 670 nm. These compounds include both chlorophylls "a" and "b" and their respective pheophytins. In this study, the term "apparent chlorophyll" will be used where it is possible that a mixture of chlorophylls and pheophytins is being measured.

Chlorophyll in seed samples was determined using the reflective spectrophotometric method of Daun (6). Chlorophyll in oil samples was determined in oils samples using the AOCS Official Method Cc 13d 55 as modified by Kelly and Yuen (3). Spectrophotometric measurements on liquid samples, including chlorophyll analyses, were made on a Beckman Acta M VI spectrophotometer equipped with a 10-mm flow cell (4 mm path width).

Color comparisons were made using a Hellige Comparator. Wratten filters of various colors and strengths were used to correct background color.

RESULTS AND DISCUSSION

The Canadian General Standards Board (CGSB) specification for crude rapeseed oil (7) requires that the oil color be compared to that of a nickel sulfate solution (Standard A). Using the Hellige color comparator and Wratten filters to adjust for the yellow color of the oil, it was not possible to compare the color of any of the oils from crushing plants to the nickel sulfate standard (Table II). Even oils which had extremely high levels of apparent chlorophyll, as determined by spectrophotometric measurement, had a color less than than the nickel sulfate solution. Quality control personnel at Western Canadian crushing plants confirmed this situation.

Samples of oil with a range of chlorophyll were prepared by extracting fresh samples of rapeseed with a mixture of heptane and ethanol (9 1) (8). These samples exhibited a wide range of green color which were easy to compare with the standard nickel solution using the Hellige comparator (Table II) Oils containing less than 25 ppm chlorophyll were found to be less green than Standard A.

Examination of the absorption spectra of the oil used in the experiments just described showed that laboratory extracted oils had a major absorption maximum at about 665 nm whereas oils from crushing plants had a maximum

TABLE II

Comparison of Oil Colors of Laboratory and Crushing Plant Extracted Oils with CGSB Standard A for Green Color[a]

Sample	"Chlorophyll" (ppm)[b]	Lighter than standard A	Darker than standard A
Lab extract 1	18	X	
→ 2	20	X	
→ 3	25	X	
→ 4	26		X
→ 5	28		X
→ 6	39		X
Crushing plant 1	15	X	
→ 2	18	X	
→ 3	23	X	
→ 4	27	X	
→ 5	37	X	
→ 6	42	X	

[a]Oils compared using a Hellige Comparator and a No. 12 (yellow) Wratten gelatin filter.

[b]Chlorophyll measured as in ref. 3.

in the range 667-668 nm (Fig. 1). Furthermore, treatment of the laboratory-extracted oils with acetic acid and heat resulted in brown colored oils with an increase in the wavelength of absorption maximum and a decrease in its intensity (measured as apparent chlorophyll [Table III]). This shift to higher wavelengths and decreasing absorptivity suggests conversion of chlorophylls to pheophytins (9).

Figure 2 shows that the amount of chlorophyll extracted into the oil correlates well with the amount in the seed. If a correction is made for the lower absorptivity of pheophytin, it can be seen that almost all of the chlorophyll in the seed is extracted into the oil.

There is some controversy in the literature whether the apparent chlorophyll in rapeseed oil is in the form of pheophytin or chlorophyll. Early work by Neiwiadomski et al. (2) and by Box and Boekenoogen (10) indicated that pheophytins predominated in oils from crushing plants. Appelqvist (11) found that, for freshly extracted oils from Swedish crushing plants, chlorophyll predominated. He suggested that results in the earlier studies were due to changes taking place in those oils, which had been stored for long periods (up to 6 months) before analyses. Samples analyzed in this study varied in age from a few days to 2 weeks before being analyzed. Except for the period of postal travel, the samples were stored in the dark at 4 C to minimize any changes. It would appear that, if chlorophyll is present in Canadian oil immediately after extraction, it is rapidly converted to pheophytin. Also, pheophytin probably predominates in any oil which is handled by a refinery.

It is probably the russet color of the pheophytins which makes it impossible to establish a correspondence between the CGSB Standard Color A and the color of oils extracted from crushing plants. The adoption of a maximal amount of apparent chlorophyll (or pheophytin) as measured by AOCS standard procedure or a modification of this procedure would probably solve this problem. Such a method has been suggested by Kelly and Yuen (3). The group of compounds which have an absorption maximum in the range of 660-670 nm could be referred to as apparent chlorophyll with the understanding that pheophytin, rather than chlorophyll, probably is being measured. A better solution would be to treat all samples with acetic acid and heat and report the results as pheophytin. A correction to the factor used in the calculation would then be required. An upper limit of 20 ppm pheophytin (measured by AOCS Cc 13d-55 with no change in factor) is probably suitable as a replacement for Standard A.

If 25 ppm chlorophyll represents the upper limit for top grade oil based on comparisons of seed extracts with Standard A, then the upper limit for chlorophyll in top grade seed is only 10 ppm (allowing for 40% oil in the seed). Allowing for the average oil content being somewhat greater than 40% and for the effect of averaging of small lots, sampling and methodology error, the maximal upper limit for chlorophyll in the seed should be ca. 12 ppm. In Sweden, the maximal allowable value for seed chlorophyll is 30 ppm as measured in the oil by heptane/ethanol extraction (4). Assuming 40% oil in the seed, this corresponds to 12 ppm chlorophyll in the seed.

In order to find out how the Canadian Grading System is meeting this proposed standard, several sets of samples of rapeseed with a wide range in chlorophyll content by Canadian Grain Commission Inspectors and by Inspectors from a Western Canadian Canola crushing plant. The results from these studies (Fig. 3, Table IV) show that if 12 ppm is the maximal level of chlorophyll allowable, then only ca. 1.5% green seeds should be allowed in the sample rather than 2% allowed at present. Furthermore, the low correlation (r^2) indicates that only ca. 50% of the changes in chlorophyll content can be explained by changes in percentage green seed.

Further examination of the data (Table V) indicates that in one set of samples from an area with a severe chlorophyll problem, 60% of the samples with even 1% or less of green seeds exceeded the 12-ppm chlorophyll limit. For samples collected from throughout Western Canada, ca. 30% with 1% or less green seed had greater than 12 ppm chlorophyll.

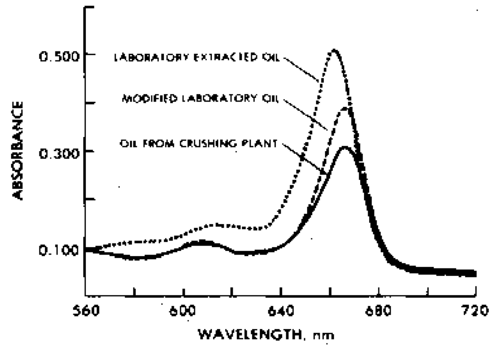

FIG. 1. Absorption spectra of oils extracted from seed in the laboratory, modified by treatment with acetic acid, and an oil extracted from a Western Canadian crushing plant (all diluted 1:10 with CCl_4).

TABLE III

Chlorophyll and Pheophytin in Samples of Oil from Crushing Plants and from Laboratory Extractions

	Chlorophyll in oil (ppm)[a]			
	Crushing plant oil		Laboratory extracted oil	
Sample	As is	Treated	As is	Treated
1	5	5	7	4
2	12	12	16	11
3	17	17	23	10
4	20	20	33	20
5	26	26	44	30
6	27	26	40	34
7	36	36	45	34
8	57	58	64	41

[a]Measured as in ref. 3.

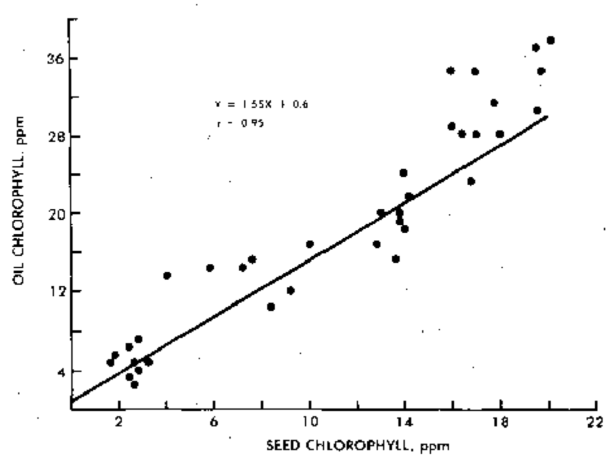

FIG. 2. Relationship between seed chlorophyll and oil chlorophyll in samples extracted at Western Canadian crushing plants. Seed chlorophyll determined by reflectance spectroscopy (6) and oil chlorophyll by spectrophotometry (3).

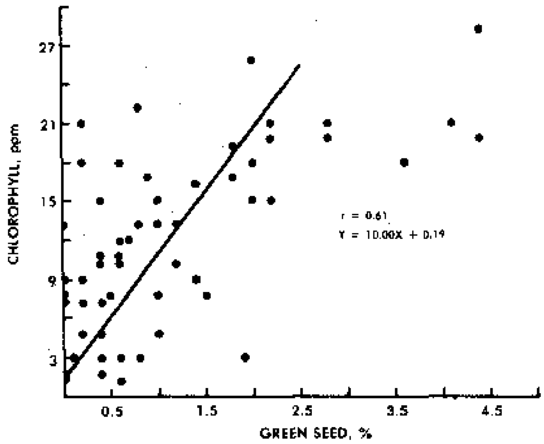

FIG. 3. Scater diagram showing the relationship between percentage of green seeds and seed chlorophyll in samples from the 1979 new crop survey of Western Canadian canola/rapeseed.

This situation obviously is not satisfactory for good quality control. One answer to the quality control portion of this problem is to adopt a procedure to measure the chlorophyll content of samples of rapeseed, rather than the percentage green seed. Such a procedure is used at present in Sweden (4) and might be adopted in Canada for use at canola crushing plants. General use of such a system in the Canadian grading system is unlikely, however, since extraction and spectrophotmetric techniques are too time consuming, expensive and hazardous to be used at primary grading points or even at government inspection offices. Development of an instrumental procedure using a reflectance technique similar to the rapidly developing near-infrared technology may eventually provide a satisfactory answer to this problem.

Quality control is only part of the problem. The more important problem is the large amount of Canadian canola/rapeseed which fails to meet the standard. Research is being done to relate agricultural parameters to chlorophyll content of the seed. Hopefully, this will result in improvements in agricultural practice and less immaturity in Canada's canola/rapeseed crop.

TABLE IV

Relationship between Percentage of Green Seeds and Chlorophyll Content of Samples of Rapeseed Collected and Tested at Different Times and Locations

Sample	Inspectors	No. of samples	Range chll.[a] (ppm)	Green (%)	Correlation	Green seeds at 12 ppm chll. (%)	Equation of line[b]
1979 New crop survey	Can. Grain Com.	56	1-28	0-4.5	0.61	1.2	Y = 0.10X − 0.02
1980 New crop survey	Can. Grain Com.	46	0-34	0.4-13	0.67	1.8	Y = 0.18X − 0.37
1979-80 Crushing plant samples	Can. Grain Com.	115	1-24	0-7.8	0.65	1.4	Y = 0.12X − 0.06
1980 Farm samples	Canola Crushing Plant	291	4-48	0-20	0.67	1.4	Y = 0.20X − 1.00

[a]Seed chlorophyll measured as in ref. 6.
[b]Y = % green, X = chlorophyll.

TABLE V

Distribution of Percentage of Green Seeds and Chlorophyll in Samples from New Crop Surveys and Crushing Plant Surveys

Survey	Green seeds seeds	Total no. of samples	Total samples (%)	Samples > 12 ppm chll. (%)
A	1	112	53	60
	2	38	18	79
	>2	63	30	87
B	1	73	63	30
	2	30	26	83
	>2	12	10	92
C	1	59	77	34
	2	10	13	90
	>2	8	10	100
D	1	25	54	24
	2	13	28	54
	>2	8	17	75

A—Samples from a single crushing plant: fall 1980.
B—Samples from 7 Western Canadian crushing plants 1979-80.
C—1979 New crop survey.
D—1980 New crop survey.

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