Can chemical analysis profiles be used to identify rapeseed samples?

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INTRODUCTION:

The Royal Canadian Mounted Police was contacted regarding stolen grains; they were able to recover canola samples from producer bins and from loads delivered at various grains elevators.
The chemical analyses of the samples provided by the Royal Canadian Mounted Police showed that two samples were statistically chemically similar. However, no study has shown how and if chemical similarity of canola samples could be used to assess if the samples are form one or different locations.
Knowing that the same grain varieties are grown at several locations in Canada, DNA evidence is far from sufficient to say that two grain samples are from the same bin. Canola quality parameters such as oil, protein, chlorophyll, glucosinolate contents and fatty acid compositions are governed by the genetic - the variety – but also the environment – weather and soil conditions.
The aim of the project was to assess whether chemical profiles from quality analysis of rapeseed could be used to correctly classify samples of unknown identity (could two samples be from the same source?). A program for the Base SAS system was developed for grouping random samples based on pair-wise comparison to every observation in the database across each quality parameter. The variance in matching random samples to known identities based on similarity in their parameter values was also estimated.
Matches were randomly taken and NIR spectra of the matches were compared to confirm chemical similarity.

MATERIAL AND METHODS:

Material: Canola samples from Canadian Grain Commission harvest survey obtained from 2000 to 2010 were used for this study. Each sample has a single identification number specific of year and location. Variety identification was provided by the producer; if no variety identification is provided the variety is recorded as unknown. Information is sometimes provided by the producer such as herbicide tolerance, the variety is then recorded as the herbicide tolerance. Grade was assigned to each sample after cleaning by trained Grain Inspector according to. Official Grain Grading Guide for Canola and Rapeseed (Chapter 10) that can be found at: http://grainscanada.gc.ca/oggg-gocg/10/oggg-gocg-10-eng.htm.

Method:

NIR analysis: Samples were analyzed for oil, protein, chlorophyll, total glucosinolates, oleic acid, linoleic acid, α-linolenic acid, total saturates contents and iodine value using a NIRSystems 6500 scanning near-infrared spectrometer (FOSS NIRSystems Inc., Silver Spring, MD). The reflectance spectra of the canola samples (log 1/R) were recorded at 2 nm intervals from 400 to 2500 nm with the NIR monochromator using NSAS software v 3.53. The NIR Systems 6500 whole seed analyzer was calibrated with the following reference methods: (a) oil content is ISO 10565:1992(E) Oilseeds calibrated using "Oilseeds-Determination of Oil Content-Solvent Extraction (Reference Method)" described in *F.O.S.F.A. International, Technical Manual, Part Two, Standard Contractual Methods (2005),* (b) Protein content is determined by AOCS Ba 4e-93, 1995, 1997 & 2009, (c) Chlorophyll content is determined by ISO 10519:1997(E), (d) Glucosinolate content is determined by ISO 9167–3: 2007 (E), (e) Fatty acid composition is determined ISO 5508:1990 (E) and (f) Iodine value is determined by AOCS Cd 1c-85, 1995, 1997 & 2009.
All results are expressed on a 8.5% moisture basis excepet for chlorophyll which is reported on tel quel basis.
Database: The database was developed, recorded parameters were: sample identification, grade, year, location (province, crop district, lattitude and longitude), class (*B. napus*, *B. rapa* or *C. juncea*), sub-class (low erucic acid, high erucic acid, low linolenic acid), oil, protein, chlorophyll, glucosinolate, oleic acid, linolenic acid and total saturates contents as well a iodine value.
Statistical analysis: Analyses were done using SAS software[1]. The selection parameters were: (a) grade, variety, class and subclass should be identical. The ranges allowed for the chemical

[1] The data analysis for this paper was generated using SAS software, Version 9.1.3 of the SAS System for Windows. Copyright © 2002-2003 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

characteristics were based on standard deviations previously calculated: oil content ± 0.3%, protein content ± 0.2%, total glucosinolate content ± 0.5 µmol/g, chlorophyll content ± 1.0 mg/kg, oleic acid content ± 0.2%, linolenic acid content ± 0.2%, total saturated acid content ± 0.2% and iodine value ± 0.40 units.

RESULTS – DISCUSSION

The database contains 22277 lines of analyses. Table 1 presented the parameters used for the pair-wise comparison. The variable without freedom level (must be identical) were: (a) grade, identified by visual inspection and based on seed defect altering grain quality; (b) class also identified upon visual inspection and (c) variety determined by genetic analysis and usually known from producer. Using these variables, there were from 5 to 6433 pairs of matching results. The chemical composition of the samples was then used to further pair matching samples. These chemical parameters were judged similar when within a measurable deviation from compared results. The variations allowed on each chemical parameter were obtained from a previous ANOVA suggesting the two samples were chemically similar. These standard deviations included the error due to the analysis plus the error due to sampling.

Protein content narrowed the numbers of matching pairs. However, protein content allowed to find only one pair of matching HEAR samples, probably because the database contained few samples (235). This HEAR pair was chemically similar when looking at the other chemical parameters. Only one pair of HEAR samples was found similar based on class, sub-class, variety, grade and protein; this represented 0.85% of the samples.

When oil content and glucosinolate content were added, the number of possibility was greatly reduced and some matching pairs were identified but it was not possible to completely match some of the *B. napus* samples (LEAR and LowLin). Partial fatty acid composition (oleic acid, linolenic acid and saturates contents) was able to finally separate all the marching pairs.

Only five pairs of canola samples of similar quality and known identified variety were found. This means that only 0.045% of the samples of known variety from our database were also chemically similar. All these pair samples were grown the same year. The growing locations were very close to each other except for one pair which was grown in different provinces.

We also found that 33 pairs of chemically similar samples, however the variety was reported unknown for at least one of the sample in the pair. In some case, the producer did not remember the variety name to record it or only the herbicide tolerance was reported (insufficient to identify the variety). These samples were considered of identical variety by the program; however in reality the variety could be different. This represented 0.30% of the samples from the database. Of there 33 pairs, nine pairs were grown the same year, ten pairs were grown one year apart. Then, two, two, seven and three pairs were grown two, three, four and more than four years apart. Our results showed that a known variety is not grown for more than four year. Canadian canola industry usually considers that that the life expectancy of a known variety is about four years maximum. Therefore we could hypothesized that canola variety identified as unknown and grown more than four years apart are not from the same variety. Therefore, only another 30 pairs of samples could be added to the pervious five pairs of perfectly marching results. We could consider that the program found 35 pairs of canola samples of similar quality and identical class, sub-class, grade and variety.

The NIR scan 2nd derivatives were used to look at the matching pairs, an example is shown in Figure 1. The difference between the scans of the matching pairs was due to the moisture content of the samples, since this parameter could vary greatly and is independent of the sample.

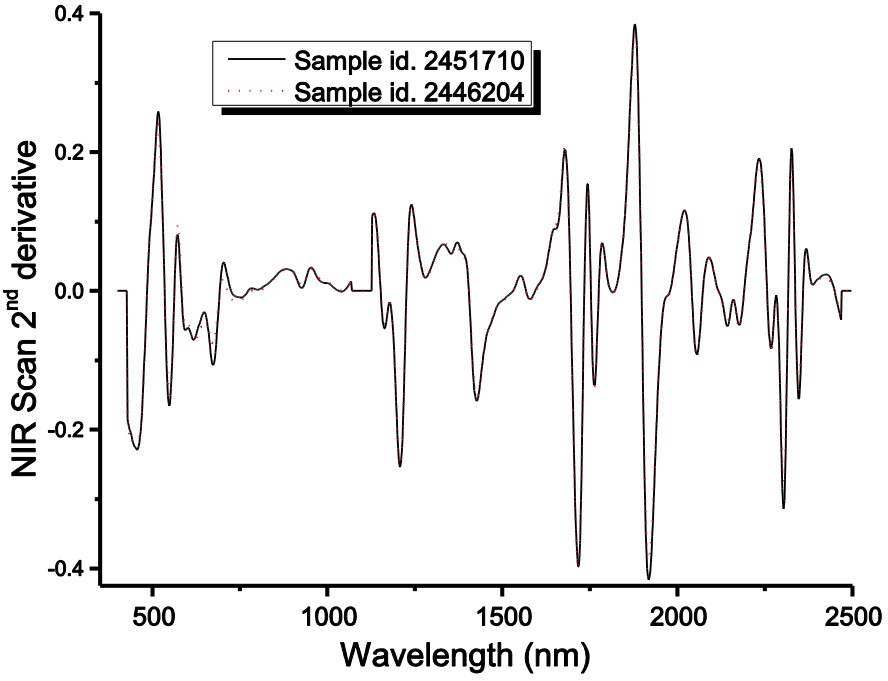
This suggested that at best 0.30% of the canola samples grown in Canada are identical based on class, subclass, variety and chemical composition (oil, protein, glucosinolate, chlorophyll, oleic acid, linolenic acid, saturates and iodine value).

Table 1: Database parameters used for the pair-wise comparison. Data collected from 2000 to 2010 Canadian Grain Commission Harvest Survey.

Variable	Min	Max	Min	Max	Min	Max
Class	*B. napus*		*B. rapa, B. napus & B. juncea B*		*B. napus*	
Subclass	HEAR		LEAR		LowLin	
N subcalss	235		20468		2221	
N Variety	3		287		65	
N per variety	39	138	1	1115	1	193
N Unknown variety	58		6436		278	
Oil (%, 8.5% moisture)	38.0	50.0	31.4	52.2	13.1	51.5
Protein ((%, 8.5% moisture)	17.3	29.2	14.5	31.5	13.8	30.5
Glucosinolates (umol/g seed)	3.1	18.1	0.0	38.2	1.0	28.2
Chlorophyll (mg/kg)	0.0	128.3	0.0	211.6	0.0	161.0
Oleic acid (% in oil)	9.9	38.4	0.0	74.7	57.6	79.3
α-linolenic acid (% in the oil)	6.7	12.7	0.0	16.6	0.2	12.8
Total Saturates (% in the oil)	4.5	5.9	0.0	9.5	5.4	8.2
Iodine Value (units)	96.6	112.2	0.0	132.0	90.7	114.8

HEAR = high erucic acid
LEAR = low erucic acid
LowLin = low linolenic acid

Figure 1: Example of NIR scan 2nd derivatives of a matching pair of samples.



White mustard (*Sinapis alba* L.) breeding for oil and meal quality

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Abstract

Key words: white (syn. yellow) mustard (*Sinapis alba* L. syn. *Brassica hirta* Moench.), oleic, linolic, linolenic and erucic acids, glucosinolates, sinalbin, zeroerucic variety (0), double low variety (00, canola type).

The results of Polish breeding works aiming at the development of white mustard (*Sinapis alba* L.) cultivars with low content of erucic acids and glucosinolates in seeds are presented in this paper. Zeroerucic variety Bamberka was licensed in 2006 and double improved (canola type) variety POH-209 is now under official testing necessary for licensing.

Introduction

White mustard (*Sinapis alba* L.), one of the spring oil crops, is characterized by stable yield and especially by its good resistance to temporary droughts, very frequent in Polish climatic conditions. Its economic importance is still increasing due to its many possible uses. It can be used for seed production, for sowing as after crop, as plant important in crop rotation for Heterodera schachtii control, also as a honey yielding plant. White mustard cultivated as after crop works as phytosanitary treatment and can be used as a pasture crop. Its seeds have pale yellow color, what is connected with lower fiber and higher protein contents as compared with rapeseed (Ochodzki, Piotrowska 1997). Amino acid composition of mustard protein is very suitable for feeding purposes but high glucosinolate content does not allow the use of its meal or pressing cake for animal feeding. The seeds of traditional varieties of white mustard also contain in oil high content of harmful erucic acid (about 40%). Research and breeding works to change the chemical composition of seeds of white mustard have been conducted in Department of Genetics and Breeding of Oilseed Crops at our Institute in Poznan.

Materials and Methods

New strains of white mustard characterized by low erucic acid content in seed oil or by low glucosinolate content in seeds have been bred in preliminary works. These strains were obtained from hybrids between different varieties or strains by individual selection connected with inbreeding (Krzymanski J. et al. 1990, 1991, Pietka T. et al. 1998, 2004).

Next the crosses between single improved strains allowed for the selection of double improved strains with both desired qualities. Many new double improved strains of white mustard were selected from obtained segregating populations. These materials were improved for yielding ability and oil content in seeds by inter-crossing and individual selection or strain selection based on field trials. These field trials were conducted in differentiated environments what allowed to improve also other agronomic values of double low strains.

Results

As the first step new zero erucic variety with high glucosinolate content was bred. New variety was licensed under name Bamberka by Research Center for Cultivar Testing (RCCT-COBORU) in 2006 (Pietka T. et al 2007). Seeds of this variety can be used as a spice and for table mustard production. Oil produced from seeds of this variety has composition similar to double improved rapeseed oil and can be used as edible oil or for technical purposes. This oil has very good dietary value, a little better than rapeseed oil because it contains more omega-3 acids and has better omega-6 to omega-3 ratio (1:1) (table 1).

Table 1
Characteristics of zeroerucic white mustard of Bamberka variety as compared to conventional variety Nakielska
(field trials conducted at Borowo and Malyszyn stations in 2003)

Trait	Nakielska	Bamberka
Beginning of flowering – day after sowing	53,0	49,5
End of flowering - day after sowing	71,5	68,5
Ripeness to harvest - day after sowing	129,0	125,0
Plant height (cm)	116,0	88,0
1000 seeds weight (g)	7,4	6,7
Yield seeds (dt ha^{-1})	15,25	13,12
Oil content (% s.m.)	26,8	28,9
Total glucosinolate (μM g^{-1} seeds)	143,5	136,7
Sinalbin content (μM g^{-1} seeds)	138,4	132,5
Fatty acid content: - (%)		
- palmitic	2,6	4,3
- stearic	1,1	2,1
- oleic	29,5	63,4
- linoleic	9,7	12,3
- linolenic	9,9	14,5
- eicosenoic	11,6	2,6
- erucic	35,7	<1,5

Next research works were conducted to obtain a variety which could be used as valuable oil/protein crop. On the way of recombination breeding of many new strains of double improved quality were produced. After three years of field experiments conducted in four locations new variety PN 820/08 was selected from this material. This variety was accepted to official testing by RCCT (COBORU) under the name POH-209. This variety is characterized by very low erucic acid content in oil (below 1,5%) and very low content of alkenyl glucosinolate in seeds (below 15 μM g^{-1} of seeds) and lack of sinalbin which is the main glucosinolate of white mustard. Quality of oil is similar to Bamberka variety (Table 2).

Table 2
Characteristics of double low white mustard of POH-209 variety as compared to conventional variety Nakielska and zeroerucic variety of Bamberka
(field trials conducted at Lagiewniki and Karzniczka stations in 2009)

Trait	Nakielska	Bamberka	POH-209
Beginning of flowering - days after sowing	151,0	146,5	148,5
End of flowering - days after sowing	178,0	174,0	175,0
Ripeness to harvest - days after sowing	225,5	225,5	225,5
Plant height (cm)	166,8	142,5	148,0
1000 seeds weight (g)	7,2	5,7	6,3
Yield seeds (dt ha^{-1})	22,4	17,3	20,2
Oil content (% s.m.)	27,8	29,6	30,2
Erucic acid content (%)	36,6	1,3	1,3
Glucosinolates - (μM g^{-1} seeds):			
- glucotropeolin	0,1	0,0	1,3
- sinalbin	156,2	132,5	0,0
- gluconapin	0,0	0,0	0,8
- glucobrassicanapin	0,0	0,0	0,0
- progoitrin	4,7	3,2	12,7
- napoleiferin	0,1	0,1	0,5
- brassicin	0,2	0,3	2,8
- 4-hydroxybrassicin	0,4	0,6	3,7
Total of glucosinolates	161,7	136,7	20,4
Total of alkenyl glucosinolates	4,8	3,3	13,9

Conclusions

The presented breeding works allow for changing white mustard to valuable oil/protein crop. Low yielding linked with drastic changes in chemical composition was overcome in new variety. POH-209 variety is characterized by practically zero erucic acid content in seeds oil and very low glucosinolate content in seeds and the lack of sinalbin the main glucosinolate of white mustard (Pietka T. et al. 2010).

Seeds of POH-209 variety can be a source of protein not only for feeding animals but also may be used in food production. Seed oil has composition which ideally fulfills the needs of human nutrition. It can play a good role in the prevention of sclerosis and diseases of the circulatory system. This oil should be distinguished because of:
- high oleic acid content lowering cholesterol level in blood,
- optimal content of linolic and linolenic acid content (essential polyunsaturated fatty acids) ,
- desired ratio of omega-6 to omega-3 fatty acids (1:1),
- very low content of not desired saturated fatty acid.

References

Krzymański J., Piętka T., Ratajska I., Byczyńska B., Krótka K. 1990. Selekcja gorczycy białej o niskiej zawartości glukozynolanów. Rośliny Oleiste. Wyniki Badań. IHAR Radzików, część I: 115-121.

Krzymański J., Piętka T., Ratajska I., Byczyńska B., Krótka K. 1991. Development of low glucosinolate White Mustard (*Sinapis alba* L. syn. *Brassica hirta*). Proc. VIII[th] International Rapeseed Congress. 9-11.07. 1991. Saskatoon, Canada, 5: 1545-1548.

Ochodzki P., Piotrowska A. 1997. Zmienność składu chemicznego odtłuszczonych nasion rzepaku o niskiej zawartości włókna. Rośliny Oleiste - Oilseed Crops, XVIII (2): 511-524.

Piętka T., Krzymański J., Michalski K., Krótka K. 1998. Postępy prac nad tworzeniem gorczycy białej podwójnie ulepszonej. Rośliny Oleiste - Oilseed Crops, XIX (2): 455-462.

Piętka T., Krótka K., Krzymański J. 2004. Gorczyca biała podwójnie ulepszona – alternatywna jara roślina oleista. Rośliny Oleiste - Oilseed Crops, XXV (2): 403-413.

Piętka T., Krótka K., Krzymański J. 2007. Bamberka – zeroerukowa gorczyca biała. Rośliny Oleiste - Oilseed Crops, XXVIII (2): 119-124.

Piętka T., Krzymański J., Krótka K. 2010. Pierwsza podwójnie ulepszona odmiana gorczycy białej (*Sinapis alba* L.) *First double low variety of white mustard (Sinapis alba L.)*. Rośliny Oleiste - Oilseed Crops, XXIX (2): ??

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