Energies **2012**, *5*, 3788-3802; doi:10.3390/en5103788

OPEN ACCESS

energies

ISSN 1996-1073

www.mdpi.com/journal/energies

Article

Study of Pea Accessions for Development of an Oilseed Pea

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Received: 25 August 2012; in revised form: 7 September 2012 / Accepted: 14 September 2012 / Published: 27 September 2012

Abstract: Global interest in stable energy resources coupled with growing demand for bio-oils in various conventional and arising industries has renewed the importance of vegetable oil production. To address this global interest, oilseed production has been increased in recent decades by different approaches, such as extending the cultivation area of oil crops, or breeding and growing genetically modified plants. In this study, pea (*Pisum sativum* L.) accessions were screened for lipid content using a rapid extraction method. This method quantifies lipid concentration in pea seeds and was developed by assessing and comparing the results of existing extraction methods used for canola and soybean, the top two Canadian oilseeds. Seeds of 151 field pea accessions were grown to maturity in 2009 and 2010 at McGill University (Quebec, Canada). Overall, lipid concentration in pea seeds ranged from 0.9 to 5.0%. Among several seed characteristics, only seed shape (wrinkled verses round) had a significant effect on the total lipid production in the seeds. Peas are a valuable source of protein and starch, but the lipid concentration in their seeds has been undervalued. This research supports the idea of developing a novel dual-purpose oilseed pea that emulates the protein and oil production in soybean seeds while being conveniently adapted to a colder climate.

Keywords: extraction; field pea; genetic diversity; lipid; oilseed; screening

1. Introduction

The energy crisis in the 1970s, coupled with the fast diminishing energy reserves aroused strong interest in renewable energy sources, such as biofuel [1]. Moreover, 30% of daily calories in the human diet are supplied by edible oil [2], which accounts for 80% of the total vegetable oil production in the world. Bio-lipid products used in oleo-chemical industries is another growing domain for vegetable oil, which accounts for 14% of the total vegetable oil production [3]. Bio-products, such as biofuels, bio-lubricants [4] and bio-surfactants [5], have a major advantage over petrochemical-based products in that they are biodegraded more quickly and disappear from the environment faster [3]. Taken together, the application of vegetable oils in food and non-food industries has increased during the last few decades and has led to a global increase in oilseed production, from 56 million tonnes in 1990 to 88 million tonnes in 2000 [6]. The top three world oilseed crops are soybean at 261.5 million tonnes on 102.4 Mha, canola at 59.07 million tonnes on 31.7 Mha and cottonseed at 42.389 million tonnes (harvest area data not available) [7].

Pea seeds are primarily produced for protein and starch, typically containing on average 23% protein and 55% starch [8–10]. Only a few papers have been published on the lipid concentration in pea seeds. Earlier research has reported lipid contents ranging from 9.7% to 35%, respectively [11,12], while later research has reported lipid contents in field pea seeds ranging from 1% to 4% [13–15]. Existing reports using a water-based *n*-butanol extraction method showed that the most commonly found fatty acids in peas are linoleic acid in small and medium, and palmitic acid in larger seed accessions [16]. Linolenic acid is present in low concentrations in all sizes of peas [16]. Other grain legumes have been studied for chemical composition, with common bean, chickpea and lentil containing 2.5%, 6.7% and 2.2%, respectively [17]. The same report [17] reported 2.3% lipid content in pea seed. By increasing the lipid concentration in pea seeds through breeding and genetic engineering, a new value can be added to the crop, which may bring economic benefits to the growers if the product can be used in various industries for food, feed and biofuel [18].

Lipid extraction methods vary in efficiency depending on the physical or chemical compatibility of the sample with a solvent. Lipids have a range of hydrophobicity, which is caused by the molecular variation in their structure. Triacylglycerol (TAG) and sterols are non-polar, whereas free fatty acids (FFAs), phospholipids and sphingolipids are slightly polar [3,19,20]. Polar lipids are more soluble in polar solvents, while non-polar lipids can be better dissolved in non-polar solvents. For a more efficient lipid extraction, the polarity of a selected solvent should be in agreement with the overall polarity of the lipid molecules [21]. Therefore, the choice of extraction method needs to be performed by consulting and comparing accepted methods in oilseeds [22,23]. Although hexane, petroleum ether and diethyl ether are the most common solvents used in the oilseeds industry [24–26], various lipid solvents are exploited in research to quantify the lipid concentration in oilseeds, such as chloroform and methanol [27], hexane [28], tetrachloroethylene [29], petroleum benzene [30] and 2-propanol [31] for canola, and ethanol, isopropanol, acetone, *iso*-hexane, heptane and trichloroethylene [32] for

soybean. In the context of this research, a selection of lipid extraction methods was examined on canola and soybean. The results were compared to previous published research to validate the experimental conditions and procedures. Comparison between the results of the selected methods on pea led to the selection of the most convenient method for screening the lipid concentration in pea accessions.

2. Experimental Section

2.1. Plant Material

Seeds of 174 different pea accessions (*Pisum sativum* L.) were acquired from Plant Gene Resources of Canada (Saskatoon, SK, Canada) and the pea collection of the U.S. Department of Agriculture (Pullman, WA, USA). The accessions were randomly selected based on the country of origin and plant characteristics, such as cotyledon color and flower color, to provide as much variability as possible in the selections. Seeds were grown in 2009 and 2010 at a field site, 25 by 40 m plot with loamy clay soil texture, located on the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Quebec, Canada (Lat: 45°24'29" Long: −73°56'10"). The plot was tilled twice before planting each year, with no fertilization applied to the soil. Six seeds were planted per accession in a row at a spacing of 10 cm between each seed with 40 cm between each accession, no spatial replication was performed. In 2009, seeds were planted on May 20, and harvested on August 30. In 2010, seeds were planted on May 2, and harvested on August 30. A field weather station recorded rainfall for the 2010 growing season at 389.2 mm. The average maximum daily temperature for the 2010 growing season was 28.5 °C, whereas the minimum daily average temperature was 18.7 °C. The average daily crop heat unit for 2010 was 28.6, while the total crop heat unit for the 2010 growing season was 4141.9. Local weather data is not available for 2009. Weeds were controlled by hand and a small gas rototiller was used for soil tilling. Plant characteristics including flower color, seed coat color, cotyledon color, and seed shape were visually compared and documented, and plant height was measured and averaged among plants of the same accession. Number of germinated and mature plants per accession varied from 1 to 6 plants, with 100 seeds weighed to obtain the seed mass. Accessions without germination or seed production were excluded from further analysis. Seeds of field pea (cv. Cutlass) and canola (*Brassica napus* L., cv. Roper) were obtained from plants grown in 2009 on the Lefsrud farm (Viking, Alberta, Canada). Seeds of soybean (*Glycine max*, cv. Champion) were obtained from plants grown in 2009 at the Belcan Agro Centre (Sainte-Marthe, Quebec, Canada). The seeds from all three locations were dried in the pods in paper bags, in an oven at 60 °C for 48 h. After drying, the seeds were ground by a Black and Decker coffee grinder (CBG100S, Richmond Hill, Ontario, Canada) for 1–2 min, until a fine powder was obtained.

2.2. Chemicals

1-Butanol (Certified ACS), hexanes (Certified ACS), 2-propanol (Certified ACS Plus), methanol (Certified ACS), chloroform (approx. 0.75% ethanol as preservative/Certified ACS), cyclohexane (Certified ACS), petroleum ether (Certified ACS), were purchased from Fisher Scientific (Ottawa, Ontario, Canada).

2.3. Instrumentation

Plastic centrifuge tubes (50 mL), plastic pipettes (15 mL) and glass pipettes (15 mL) were acquired from Fisher Scientific. Test tubes were weighed by an analytical balance (±1 mg; APX-153; Denver Instrument, Bohemia, NY, USA). Other instruments used in our experiments, such as tube rotator (VWR, H005302, Mississauga, Ontario, Canada), Fisher centrifuge, Fisher vortex mixer (Standard 120V), nitrogen evaporator (NEVAP-111, Berlin, MA, USA), and Soxhlet extractor (VELP scientifica, SER-148, Italy) were accessed in the McGill University laboratories.

2.4. Methods Used for Gravimetric Determination of Total Lipid Concentration

The field pea (cv. Cutlass), soybean (cv. Champion), and canola (cv. Roper) samples from the Lefsrud Farm and Belcan Agro Centre were tested for their lipid concentration with five extraction procedures: butanol; hexane/isopropanol; chloroform/methanol; and Soxhlet with petroleum ether or with hexane, to determine the best method. The McGill University grown peas were then only analysed for lipid concentration with the butanol extraction method, as it was determined to be the best method for lipid extraction.

2.5. Determination of Total Lipid Concentration

2.5.1. Butanol Extraction Method

A summary of the butanol extraction reported by Murcia *et al.* [16] is provided. Ground seed sample (2 g) was added to screw-capped centrifugal plastic tubes of known mass in triplicate. A second tube with the same amount of sample was prepared as a control tube and was processed without the lipid extraction procedure (grinding and drying was applied) to limit errors created from varying initial moisture content in the seeds. *n*-Butanol (20 mL) was added to the test tubes that were placed in the tube rotator for 30 min, followed by 10 min of centrifugation at 3000 rpm. Two separate phases formed in the tube, the solid material in the lower layer, and a mixture of solvent and dissolved lipid in the top layer. The top layer was decanted off into a separate container with special attention to avoid sample loss. The experiment was continued by adding fresh solvent and the extraction steps were repeated twice. The test tubes were placed in the nitrogen evaporator for up to 30 min at 70 °C until the remaining solvent was completely evaporated. To ensure complete moisture removal, the test tubes along with the control were placed in the oven for 24 h at 95 °C, and were covered with caps after removal from the oven. The final mass of the tubes was recorded after leaving the tubes in a lab-made Drierite box to allow them to reach room temperature. The difference between initial and final mass of the control tube, which represents strictly the moisture loss during the drying period, was subtracted from the difference between initial and final test tube mass, which represents combined moisture and lipid loss during the drying and extraction period. This difference in mass loss represents the lipid content of the samples.

2.5.2. Hexane/Isopropanol

The hexane method was a modified version of that described by Ryan *et al.* [33]. Three ground samples (2 g) were weighed into three test tubes. Six mL of solvent (hexane/isopropanol 3:2, v:v) was added to the tubes and placed in the tube rotator for 1 h. It was then centrifuged for 10 min at 3000 rpm at which point the solvent layer was transferred into a second tube of known mass. The remaining pellet was washed twice with 4 mL of fresh solvent. Each wash was followed by a transfer of the solvent into the solvent tube after a 30 s of vortexing and 10 min of centrifugation at 3000 rpm. Contrary to the butanol extraction, the oil concentration was quantified by direct measurement of lipid left in the solvent tube after the solvent was evaporated under nitrogen stream at 60 °C for 3 h.

2.5.3. Chloroform/Methanol

The chloroform/methanol method is a modification of the Bligh and Dyer method which was developed for dry samples, as described by Manirakiza *et al.* [34]. Three replicates were prepared. In the first extraction, 8 mL of methanol and 4 mL of chloroform was added to the ground sample (2 g) in each of the test tubes. Tubes were vortexed for 2 min, and another 4 mL of chloroform was added to the sample. Distilled water (7.2 mL) was added to each tube, which was vortexed for 2 min, followed by 10 min in the centrifuge at 3000 rpm. The lower layer was transferred into an empty weighed tube (solvent tube) by a Pasteur pipette or a syringe.

The second extraction was started by adding 8 mL of methanol in chloroform (10% v/v) to the test tubes. The tubes were vortexed for 2 min, and centrifuged for 10 min at 3000 rpm. The upper layer was decanted off into the solvent tube. The solvent was evaporated off under nitrogen stream at 104 °C for 3 h. Total lipid concentration was calculated directly from the mass of the lipid recovered in the solvent tubes.

2.5.4. Soxhlet Extraction with Petroleum Ether or with Hexane

The Soxhlet extraction method was performed as described in the apparatus manual. A ground sample (5 g) was added to a cellulose thimble in triplicate. The Soxhlet apparatus was assembled with the thimbles and a solvent (petroleum ether or hexane). The Soxhlet extraction with petroleum ether solvent was performed on the ground sample with 30 min of immersion, 45 min of washing and 15 min of recovery at 130 °C. The Soxhlet extraction with hexane solvent was performed on the ground sample with 45 min of immersion, 45 min of washing and 15 min of recovery at 180 °C. The lipid concentration of the sample was directly measured by the mass of lipid recovered in the Soxhlet extraction beaker.

2.6. Statistical Analyses

Statistical analyses of data were performed using SAS 9.2, Version 6.1 for Windows operating system (SAS Institute Inc., Toronto, ON, Canada). The effect of sample types and extraction methods were analyzed as fixed effects in a mixed ANOVA model (multi-way classification), and the calculated *F*-ratios were compared with the tabulated F-value at $P < 0.05$ to determine the significant terms in the model.

The effect of accession, growing year, the interaction between accession and year, flower color, cotyledon color seed shape type, mass of 100 seeds and plant height were considered as possible regression combinations. The strongest correlation was found with the seed mass and plant height, thus all factors were fitted in a mixed Multi-way Classification model using seed mass and plant height as regression factors. The calculated *F*-ratios were compared with the tabulated *F*-value at $P < 0.05$ to determine the significance of the terms in the model. The least square means of significant factors were compared using Bonferroni comparison method for both the extraction and accession screening.

3. Results and Discussion

3.1. Method Validation

The selected extraction methods recovered a range of lipid concentration from 0.67% to 46.2% (dry mass) for field peas, soybeans and canola seeds (Table 1). Analysis of variance (ANOVA) of the results found the difference in species ($p < 0.0001$) and extraction method ($p = 0.0114$) to be statistically significant. Interaction effects were measured ($p < 0.0001$) and using the Bonferroni comparison test, a statistically significant interaction effect was measured.

Table 1. Average lipid concentration (% dry mass) of field pea, soybean and canola seeds scored by using different extraction methods.

Method *	Field pea (*Pisum sativum* L., cv. Cutlass)	Soybean (*Glycine max*, cv. Champion)	Canola (*Brassica napus* L., cv. Roper)
1-Butanol	1.2 ± 0.21 ^{b}	13.9 ± 1.9 ^{ab}	41.8 ± 3.2 ^{bc}
2-Hexane/isopropanol	1.6 ± 0.04 ^{c}	15.8 ± 0.4 ^{ab}	34.8 ± 0.3 ^{a}
3-Bligh & Dyer	2.0 ± 0.02 ^{d}	15.8 ± 0.2 ^{ab}	41.0 ± 0.8 ^{b}
4-Soxhlet (PE)	0.7 ± 0.03 ^{a}	13.3 ± 0.2 ^{a}	40.5 ± 0.3 ^{b}
5-Soxhlet (hexane)	0.9 ± 0.05 ^{ab}	16.6 ± 0.3 ^{b}	46.0 ± 0.2 ^{c}

* Values followed by the same letter in the same column do not differ significantly at $P < 0.05$ (Bonferroni test).

The average lipid concentration was 1.3 ± 0.5% for field pea (cv. Cutlass), 15.1 ± 1.6% for soybean (cv. Champion), and 40.8 ± 3.9% for canola (cv. Roper). The selected methodologies were confirmed to yield a result within the reported range of lipid concentration by previous research on the three crops (1%–4% in field pea 13%–22% in soybean and 35%–45% in canola) [33,35–37].

The statistically significant difference between the methods was due to the variation in the solvents' chemical compatibility to solubilize various lipid molecules [38]. An observed orange color in the chloroform/methanol method extracts compared with yellow color from the other methods refers to the chloroform's capability of extracting carotenoids [39]. The larger amount of lipid extracted from canola and soybean by the Soxhlet extraction with hexane in contrast with petroleum ether was due to a better solubility of non-polar lipids in hexane [24]. This indicates that there is a larger fraction of the lipid concentration in the seeds which is non-polar, thus supporting previous studies on field pea [35], soybean [40], and canola [27].

Our experiments on field pea cv. Cutlass showed that a binary solvent system of hexane/isopropanol was able to extract a higher amount of lipid than a single solvent of hexane used in the Soxhlet method [41]. However, the result was opposite for canola. The higher level of recovered oil from canola seeds by hexane in Soxhlet extractor was due to its greater lipid solubility in a hot solvent [41]. But, the lower result of the same method on field pea may be a device limitation indicating a minimum lipid concentration required for an efficient extraction.

A difficulty was experienced during the hexane/isopropanol extraction to completely separate the solvent from the pellet, which initially caused an underestimation of the total lipid concentration in the samples. In order to separate the mixture of lipid and solvent from the remaining pellet more efficiently, an assembled vacuum filter was used during the isolation steps. However, the applied filtration was not successful since parts of the lipid concentration were immobilized on the sides of the flask as well as the funnel and paper filter. To avoid this loss, an additional centrifuging step was found to be an efficient approach to purify the final extract. This step was repeated once or twice until the amount of solid material remaining in the solvent became negligible. A similar challenge occurred in the butanol extraction to separate the pellet from the solvent without losing the solid material. It was found that the sample loss can be effectively reduced by carefully drawing off the upper level of the fluid with a slow and gradual inclination of the tube.

The results show that among the selected methods there were significant differences in method, with Soxhlet (hexane) as the most efficient method for the soybean and canola. For peas the top method was Bligh and Dyer followed by hexane/isopropanol, and butanol. The butanol and the hexane/isopropanol methods were the most convenient and fast lipid screening methods to be employed for this study. Hexane/isopropanol was selected as the primary extraction procedure for the pea screening due to industry acceptance, speed of screening and results from this experiment.

3.2. Lipid Concentration Variation in Field Pea Accessions

From the 174 acquired accessions, the lipid extraction results were collected from only the 151 accessions that germinated, were grown to maturity and produced sufficient seeds for the experiment (Table 2). The mean lipid concentration in field pea seeds was estimated at 2.6 ± 0.1 and 2.4 ± 0.1 from plants grown in 2010 and 2009, respectively. Statistical analyses revealed a significant difference between accessions ($p < 0.0001$), the growing years ($p = 0.0002$) and the interaction between the two factors ($p < 0.0001$).

The result of the butanol extraction method showed the average lipid concentration in pea accessions ranged from 0.3% (accession 112340 in 2009) to 6.3% (accession 29569 in 2009). The combined two year data ranged from 0.9% (accession 22713) to 5.0% (accession 31656). Specific fatty acid composition was not analyzed. However, Murcia *et al.* [16] characterized the fatty acid composition in field pea seeds and reported that the most commonly found fatty acids in peas are linoleic acid in small and medium, and palmitic acid in larger seed accessions. Linolenic acid was the least common fatty acid found in field peas.

Table 2. Average lipid concentration in different pea accessions. Peas were grown in St. Anne de Bellevue, QC, Macdonald Campus of McGill University, in 2009 and 2010. Missing values are not reported.

Plant ID		Accession number	Lipid content average*	Standard deviation	Plant characteristics				100 seed mass
					Flower color	Height	Seed color	Seed surface	
1	31656	-	5.0 [a]	0.96	White	-	yellow	wrinkled	19.1
2	112369	-	4.6 [ab]	0.33	White	130	grey-green	smooth	10.9
3	29486	-	4.1 [abc]	0.35	Color	125	grey	medium	7.1
4	29612	-	4.1 [abcd]	0.31	-	-	black	smooth	13.8
5	29579	-	3.7 [abcde]	0.52	Color	125	grey	smooth	12.3
6	112322	-	3.7 [abcde]	0.49	Color	70	green	wrinkled	22.5
7	29569	-	3.6 [abcdef]	3.14	Color	100	green	wrinkled	28
8	43016	-	3.6 [abcdefg]	0.29	White	85	green	wrinkled	22.8
9	45760	-	3.5 [abcdefgh]	0.34	White	80	mix	wrinkled	22.9
10	Dakota (Early Dwarf)	-	3.5 [abcdefgh]	0.35	-	-	green	wrinkled	15.4
11	Frosty	-	3.3 [abcdefgh]	0.42	White	70	yellow	medium	22.6
12	ILCA 5117	PI 505146	3.3 [abcdefgh]	0.35	Color	110	red	medium	25.3
13	Dual (early-season)	-	3.2 [abcdefghi]	0.1	White	85	white	medium	18.8
14	29526	-	3.1 [abcdefghi]	0.22	White	120	white	smooth-	17.3
15	29531	-	3.1 [abcdefghi]	0.56	White	-	white green	smooth	25.9
16	29602	-	3.1 [abcdefghi]	0.54	Color	125	green	medium	15.8
17	112338	-	3.1 [abcdefghi]	0.18	Color	90	green	smooth	9.4
18	112349	-	3.1 [abcdefghi]	0.04	-	-	green	smooth	18.4
19	Mendel	-	3.1 [abcdefghi]	1.03	White	-	green	smooth	8.5
20	22722	PI 343990	3.0 [abcdefghi]	0.25	-	100	grey	wrinkled	16.4
21	29514	-	3.0 [abcdefghi]	0.46	Color	130	mix	smooth	17.4
22	29546	-	3.0 [abcdefghi]	0.32	Color	135	green	medium	5.8
23	29577	-	3.0 [abcdefghi]	0.04	Color	100	green	smooth	13.6
24	45761	-	3.0 [abcdefghi]	0.29	White	-	yellow	wrinkled	27.8
25	46702	-	3.0 [abcdefghi]	0.17	White	85	yellow	medium	17
26	112356	-	3.0 [abcdefghi]	0.12	White	-	green	wrinkled	19.2
27	29590	-	2.9 [abcdefghi]	0.26	White	125	green	smooth	14.5
28	AWP 517923	PI 517923	2.9 [abcdefghi]	1.06	Color	45	green	smooth	21.6
29	Dual	-	2.9 [abcdefghi]	0.68	Color	110	white	wrinkled	17.6
30	Wando	-	2.9 [abcdefghi]	0.57	White	70	green	wrinkled	23.8
31	31655	-	2.8 [abcdefghi]	0.59	White	75	yellow	wrinkled	19.6
32	35751	-	2.8 [abcdefghi]	0.72	White	80	green	wrinkled	23.2
33	43015	-	2.8 [abcdefghi]	0.68	White	140	green	medium	12.2
34	45762	-	2.8 [abcdefghi]	0.51	White	90	grey	medium	14.5
35	45763	-	2.8 [abcdefghi]	0.51	White	80	yellow	wrinkled	19.9
36	AA38	PI 269762	2.7 [abcdefghi]	0.63	Color	150	green	wrinkled	19.7
37	29535	-	2.7 [bcdefghi]	0.2	Color	110	green	smooth	14.9
38	29540	-	2.7 [bcdefghi]	0.59	White	70	yellow	smooth	30.8

Table 2. *Cont.*

	Plant ID	Accession number	Lipid content average*	Standard deviation	Plant characteristics				
					Flower color	Height	Seed color	Seed surface	100 seed mass
39	29542	-	2.7 bcdefghi	0.56	Color	120	green	smooth	9.5
40	29595	-	2.7 bcdefghi	0.3	White	120	green	smooth	11.8
41	29610	-	2.7 bcdefghi	0.58	White	125	green	smooth	18.9
42	35748	-	2.7 bcdefghi	0.46	White	110	yellow	smooth	22.2
43	112337	-	2.7 bcdefghi	0.35	White	115	mix	smooth	20.8
44	112344	-	2.7 bcdefghi	0.66	-	125	green	medium	21.6
45	ILCA 5041	PI 505082	2.7 bcdefghi	0.17	Color	110	-	-	-
46	ILCA 5089	PI 505122	2.7 bcdefghi	0.67	Color	110	grey	medium	7.8
47	29525	-	2.6 bcdefghi	0.3	Color	110	grey	Medium	14.7
48	29575	-	2.6 bcdefghi	0.28	Color	130	black	medium	14.3
49	29600	-	2.6 bcdefghi	0.18	White	-	yellow	wrinkled	12.5
50	29608	-	2.6 bcdefghi	0.43	White	135	yellow	wrinkled	19.8
51	112310	-	2.6 bcdefghi	0.07	Color	125	brown green	smooth	16.5
52	112343	-	2.6 bcdefghi	0.51	Color	-	brown	medium	15.6
53	112355	-	2.6 bcdefghi	0.22	White	120	yellow	smooth	23
54	Thomas Lacton (early)	-	2.6 bcdefghi	1.16	White	-	yellow	medium	17.8
55	29548	-	2.5 bcdefghi	0.69	White	130	yellow	smooth	10.4
56	33551	-	2.5 bcdefghi	0.09	White	135	yellow	smooth	16.6
57	42819	-	2.5 bcdefghi	0.11	White	120	white green	smooth	26.6
58	46718	-	2.5 bcdefghi	0.11	Color	120	black	smooth	18.8
59	112324	-	2.5 bcdefghi	0.11	White	125	green	wrinkled	25.1
60	112373	-	2.5 bcdefghi	0.24	White	100	yellow	smooth	26.7
61	112385	-	2.5 bcdefghi	0.04	White	65	green	medium	18.6
62	299448	-	2.5 bcdefghi	0.07	White	-	green	smooth	25.5
63	Canstar	-	2.5 bcdefghi	0.43	White	70	yellow	smooth	22.3
64	Galena (mid-season)	-	2.5 bcdefghi	0.21	White	55	green	smooth	15
65	ILCA 5077	PI 505112	2.5 bcdefghi	0.85	Color	130	-	-	-
66	YI	PI 391630	2.5 bcdefghi	0.2	White	125	yellow	smooth	7
67	22718	PI 343987	2.4 bcdefghi	0.11	White	60	green	smooth	22.4
68	29547	-	2.4 bcdefghi	0.47	White	155	white	smooth	13.4
69	46716	-	2.4 bcdefghi	0.32	White	105	yellow	smooth	23.7
70	112363	-	2.4 bcdefghi	0.46	Color	135	green	medium	9.1
71	Big Pea	PI 262189	2.4 bcdefghi	0.64	White	120	yellow	smooth	30.5
72	Galena		2.4 bcdefghi	0.26	White	70	white	wrinkled	23
73	Oregon Sugar II	-	2.4 bcdefghi	0.47	White	75	green	smooth	26
74	76	-	2.3 cdefghi	0.18	Color	35	brown	smooth-	10.68
75	29434	-	2.3 cdefghi	0.35	White	130	green	smooth	21.5
76	29500	-	2.3 cdefghi	0.39	White	40	green	smooth	15.8

Table 2. *Cont.*

Plant ID		Accession number	Lipid content average*	Standard deviation	Plant characteristics				100 seed mass
					Flower color	Height	Seed color	Seed surface	
77	29562	-	2.3 cdefghi	1.83	Colour	100	grey	smooth	9.9
78	29566	-	2.3 cdefghi	0.31	White	-	grey	medium	7.3
79	29572	-	2.3 cdefghi	0.07	Color	-	green	smooth-	15.7
80	29588	-	2.3 cdefghi	0.3	Color	135	grey	medium	15.5
81	29606	-	2.3 cdefghi	0.17	White	120	yellow green	smooth	8.7
82	31210	-	2.3 cdefghi	1.55	Color	75	grey	medium	28.6
83	36164	-	2.3 cdefghi	0.32	Color	120	brown	medium	21.4
84	40608	-	2.3 cdefghi	0.03	Color	-	brown	smooth	24
85	112365	-	2.3 cdefghi	0.28	Color	130	brown	medium	11
86	112406	-	2.3 cdefghi	0.22	White	130	green	smooth	28.9
87	G 611 764	PI 179124	2.3 cdefghi	0.43	Color	130	green	medium	12.6
88	ILCA 3005	PI 505062	2.3 cdefghi	1.02	Color	120	green	medium	18.6
89	29527	-	2.2 cdefghi	0.11	-	-	yellow	smooth	17.4
90	29567	-	2.2 cdefghi	0.33	Color	-	green	wrinkled	26.2
91	29578	-	2.2 cdefghi	0.32	Color	125	green	medium	9.2
92	31653	-	2.2 cdefghi	0.68	Color	70	grey	medium	28.6
93	42818	-	2.2 cdefghi	0.37	White	120	green	wrinkled	27
94	112311	-	2.2 cdefghi	0.17	Color	130	green	medium	21
95	112329	-	2.2 cdefghi	0.24	White	120	yellow	smooth	32.1
96	112393	-	2.2 cdefghi	0.25	White	115	yellow	smooth	22.3
97	Green Small Pea	PI 471211	2.2 cdefghi	0.11	White	125	green	smooth	15
98	ILCA 5052	PI 505092	2.2 cdefghi	0.69	White	115	white	smooth	17.3
99	Red Small Pea	PI 471293	2.2 cdefghi	0.32	Color	115	green	medium	17.2
100	29453	-	2.1 cdefghi	0.42	White	130	green	smooth	20.4
101	29482	-	2.1 cdefghi	0.19	White	135	yellow	smooth	34.4
102	29501	-	2.1 cdefghi	0.71	White	135	green	smooth	12
103	29534	-	2.1 cdefghi	0.41	White	130	white	smooth	21.2
104	29555	-	2.1 cdefghi	0.48	Color	-	brown green	smooth	8.5
105	227313	-	2.1 cdefghi	0.71	Color	-	red	smooth	16.8
106	Agassiz	CN 113649	2.1 cdefghi	0.64	White	75	yellow	medium	20.7
107	ILCA 5072	PI 505108	2.1 cdefghi	0.01	Color	85	green	smooth	11.4
108	Lincoln (mid-season)	-	2.1 cdefghi	0.59	White	85	yellow	wrinkled	26.6
109	Oregon Sugar Snap II	-	2.1 cdefghi	0.48	White	65	yellow	medium	19.1
110	Super Sugar Snap	-	2.1 cdefghi	0.12	White	100	green	wrinkled	20.3
111	31660	-	2.0 cdefghi	0.21	White	-	green	medium	23.3
112	33555	-	2.0 cdefghi	0.21	White	125	yellow	smooth	31.5

Table 2. *Cont.*

	Plant ID	Accession number	Lipid content average*	Standard deviation	Plant characteristics				100 seed mass
					Flower color	Height	Seed color	Seed surface	
113	112306	-	2.0 ^{cdefghi}	0.41	Color	65	green	medium	21.1
114	112316	-	2.0 ^{cdefghi}	0.44	Color	100	mix	medium	10.1
115	112347	-	2.0 ^{cdefghi}	0.21	Color	135	red	wrinkled	8.6
116	112358	-	2.0 ^{cdefghi}	0.11	White	55	yellow	smooth	12.1
117	112405	-	2.0 ^{cdefghi}	0.27	White	110	yellow	smooth	23.1
118	505112	-	20 ^{cdefghi}	0.37	-	-	green	medium	17.5
119	Chinese Snow Pea	PI 279933	2.0 ^{cdefghi}	0.18	Color	120	green	medium	14.6
120	Dull White Pea	PI 471312	2.0 ^{cdefghi}	0.26	-	115	white	smooth	26.6
121	ILCA 5094	PI 505127	2.0 ^{cdefghi}	0.07	Color	130	brown	smooth	12.9
122	Maple Pea NZ	PI 236494	2.0 ^{cdefghi}	0.11	Color	115	brown	smooth	13.4
123	40609	-	1.9 ^{cdefghi}	0.46	Color	125	green	medium	17.7
124	112408	-	1.9 ^{cdefghi}	0.11	White	65	green	smooth	30.3
125	ILCA 5006	-	1.9 ^{cdefghi}	0.78	Color	100	grey	medium	18
126	Marx 609	-	1.9 ^{cdefghi}	0.17	Color	75	grey	medium	17.8
127	Stella	-	1.9 ^{cdefghi}	0.86	White	80	yellow	smooth	21.1
128	Thunderbird	-	1.9 ^{cdefghi}	0.3	White	100	yellow	smooth	23.1
129	22719	PI 343988	1.9 ^{defghi}	0.06	Color	100	-	-	-
130	29497	-	1.9 ^{defghi}	0.32	White	125	white	smooth	18.9
131	29508	-	1.8 ^{efghi}	0.07	White	100	white	smooth	21
132	29559	-	1.8 ^{efghi}	0.01	White	135	yellow green	smooth	7.9
133	29563	-	1.8 ^{efghi}	0.16	Color	-	grey	medium	4.7
134	29564	-	1.8 ^{efghi}	0.07	Color	100	brown	smooth	11.8
135	41188	-	1.8 ^{efghi}	0.17	White	135	yellow	smooth	15.4
136	112367	-	1.8 ^{efghi}	0.54	Color	-	grey	medium	9.9
137	ILCA 5115	PI 505144	1.8 ^{efghi}	0.22	White	130	green	medium	23.3
138	36165	-	1.7 ^{efghi}	0.29	White	155	yellow	smooth	21.9
139	29565	-	1.6 ^{efghi}	0.54	-	125	green	medium	12.9
140	29596	-	1.6 ^{efghi}	0.43	White	125	black	smooth	11.6
141	112351	-	1.6 ^{efghi}	0.15	White	90	green	smooth	24.6
142	505082	-	1.6 ^{efghi}	0.1	-	-	green	smooth	15.2
143	ILCA 5032	PI 505074	1.6 ^{efghi}	0.04	Color	130	green	smooth	7.2
144	46700	-	1.5 ^{efghi}	0.5	White	120	yellow	smooth	20.5
145	31657	-	1.4 ^{fghi}	0.95	Color	130	green brown	smooth	18.1
146	112302	-	1.4 ^{ghi}	0.07	-	30	green	medium	23.8
147	112330	-	1.3 ^{hi}	0.85	White	-	yellow	smooth	20.1
148	112340	-	1.3 ^{hi}	1.08	Color	-	red	medium	14.1
149	22713	PI 343985	0.9 ^{i}	0.85	Color	-	-	-	-
150	ILCA5075	PI 505111	0.9 ^{i}	0.04	Color	135	green	wrinkled	30.9
151	Reward	-	0.9 ^{i}	0.49	White	80	yellow	smooth	25

* Values followed by the same letter in the same column do not differ significantly at $p < 0.05$ (Bonferroni test).

Lipid concentration is dependent on plant accession, seed size [16] and seed shape [42], but no research has investigated the correlation between lipid concentration and cotyledon color, flower color, plant height or seed density in field pea seeds. However, given that such characteristics are easily measureable and could potentially correlate, they were included in the study. The majority of pea accessions (58%) evaluated possess colored flower as compared to white flower. The mature plants ranged in height from 30 to 155 cm with the average of 105 cm. A variety of cotyledon color was observed in the accession, yellow, green, and red, but a dominant proportion of seeds were in a spectrum, from yellow to green. The two types of seed shape were round or wrinkled with around 2/3 more round than wrinkled accessions. The analysis of variance revealed a significant difference in lipid content between the different classes of seed shape ($p = 0.001$) but cotyledon color, flower color, plant height and mass of 100 seeds had no effect on the total lipid production in pea seeds. There was a significant difference in lipid content between wrinkled seeds and round seeds ($p < 0.001$). Wrinkled seeds were found to have a greater lipid deposit (2.8 ± 0.1) as compared to round seeds (2.3 ± 0.1). This result is in agreement with Coxon and Davis [43] who reported that two mayor genes controlling lipid content were also associated with seed shape (wrinkled *vs.* round).

According to the literature, lipid concentration in field pea seeds usually ranges from 1 to 4% [13–15,44,45]. The results of the butanol extraction on the selected accessions were within the expectation of other research. A relatively high lipid concentration was previously reported in pea seeds by Letzelter *et al.* [11] and Bastianelli *et al.* [12] at 9.7% and 35%, respectively, however none of our results exceeded 8% lipid concentration. Experiments by Lezelter *et al.* [11] measured lipid content by photoacoustic detection, used in conjunction with multivariate partial least squares calibration whereas Bastianelli *et al.* [12] used a lipid extraction technique using petroleum ether after acid hydrolysis. Such existing extraction methods do not specifically consider appropriate moisture removal, which seems to be a data-altering factor in reported lipid concentrations.

4. Conclusions

The broad range of seed lipid concentration in pea cultivars and wild accessions ranged from 0.9 to 5.0% and revealed the potential of peas to be used to bio-synthesize and store lipid in the seeds. This characteristic, which has been overlooked in the past, could be enhanced by breeding and genetic engineering approaches, similar to what has been accomplished in canola and soybean. With such results in mind, pea seeds do have an oil production potential, but growing peas for lipid production is still in the early stages of research and development.

Acknowledgments

We would like to express our sincere thanks to Lefsrud Seed and Processor, Belcan Agro Centre, CRIBIQ and NSERC for the funding for this project. We also thank Phani Tej Raghav Narayanapurapu for his laboratory support.

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