

Chemical Characteristics of *Linum usitatissimum* Sub. Species *Humile* and *Usitatissimum*

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(Received: 5 April 2010;

Accepted: 5 October 2010)

AJC-9161

Flax seed species is oil seed that has oil percentage with a good quality of ω -3 essential fatty acids. *Linum usitatissimum* (crop species of *Linum* genus) has two sub species. The aim of this study is to introduce better sub species for agricultural purposes. Important characteristic of this species including: 1000-seed weight, seed weight/plant weight, oil content, protein content and fatty acid components were measured. Oil and protein content based on dry seed was measured using the extraction by Ether and Lowry method. Sub. sp *humile* had more 1000 seed weight, seed weight/plant weight, oil content and, sub. Sp *usitatissimum* had more protein content. Analysis of variation revealed the existence of significant variation at 1 and 5 % level of significance for oil and protein content, respectively. The amounts of the five fatty acids namely, palmitic acid, stearic acid, oleic acid, linolenic acid and linoleic acid were detected and measured by gas chromatography. Analysis of variance showed the existence of significant variation for all five fatty acids at 1 % level of significance, too. Two studied sub species were different in measured characteristic, completely. Mostly investigated characteristics belonged to sub. Sp. *humile*, except protein content.

Key Words: *Linum*, Oil content, Protein content, 1000-Seed weight, Fatty acid.

INTRODUCTION

Flax is plant of disturbed land habitats¹. Its production goes back to ancient history. Its remnants were found in stone age dwellings in Switzerland and ancient Egyptians made fine from flax fiber. Two types of flax are grown, seed flax for the oil in its seed and fiber flax for the fiber in its stem². We investigate seed flax. The seed is flat and oval with a pointed tip. It is a little larger than a sesame seed and measures about 2.5 mm \times 5.0 mm \times 1.5 mm³. Flax is rich in fat, protein and dietary fiber⁴. Flax contains mixture of fatty acids. It is rich in polyunsaturated fatty acid, particularly alphanolenic acid (ALA), the essential ω -3 fatty acid and linoleic acid (LA) and the essential ω -6 fatty acid. These two polyunsaturated fatty acids are essential for human. They must be obtained from the fats and oil in food because our bodies cannot make them. Flax seed oil is the world richest source of ω -3 fatty acids at a whopping 57 % (over two times the amount of ω -3 fatty acids as fish oil)⁵. An analysis of Canadian flax averaged 41 % fat, 20 % protein, 28 % total dietary fiber 7.7 % moisture and 3.4 % ash which is the mineral rich residue left after samples are burned⁴. Sub species of *Linum usitatissimum* are different apparently. For determination of chemical components and comparison of them, also to select of better sub species for agricultural purposes, this project was performed.

EXPERIMENTAL

This study was performed in plant genetic resources Department of Agricultural and Natural Resources Research Center of West Azerbaijan. Seed samples of *Linum usitatissimum* sub species, including sub species *usitatissimum* and *humile* were selected from agricultural researches center of Urmia. The 1000 seed weight and ratio of seed weight to plant weight were measured with a digital balance in three replications⁶. All seed samples were powdered and placed in 72 °C oven for 24 h to be dried⁷. The extraction by Ether and Lowry method was used for measure of total oil content⁸. One gram of each samples were transferred in test tubes and 10 mL ether were added them, twice. Each time tubes were placed in 40 °C oven for 12 h and above solutions were transferred in balanced tubes. Tubes were placed in 40 °C oven for 4 h so that its ether was evaporated. Weight difference of tubes before and after experience was used for oil content. For measure of total protein the Lowry method was used⁹. 0.005 g from dried samples were transferred in test tubes and 4 mL from below extraction buffer was added: (1) tris (0.2 N) 50 mL, (2) HCl (0.2 N) 26.8 mL, (3) sucrose 17.2 g, (4) ascorbic acid 1 g. Samples were centrifuged in 8000 rate. 1 mL from above solution was added to C solution that prepared as follows;

A solution: Na₂CO₃ 2 g, K.Na tartarte 0.02 g, NaOH 0.4 g.

B solution: CuSO₄·5H₂O 0.5 g and distilled water 100 mL.

C solution: A solution (50 mL) + B solution (1 mL) after 10 min, folin indicator that was diluted the ratio of 1-9 before, was added. Tubes were placed in dark location for 0.5 h. Light absorption of samples was measured by spectrophotometer in wavelength of 660 nm. First, set was been zero with standard solution. Protein content was obtained from formula: protein content (%) = $[420A - 6.9 \times 4]/50$. Fatty acids were determined by gas chromatograph after the preparation of their methyl esters. Esterification was accomplished by addition of 3 mL *n*-heptane in a test tube. The tubes were vortexed for 5 min until the glycerol a supernatant. The amount of 0.2 μ L from each sample was used for analysis. The gas chromatograph (Dany, Italy) model GC-1000 equipped with a flame ionization detector and interface DS-1000 integrator attached to a column for the separation of methyl esters was 30 m long with 0.33 mm inner diameter. The column temperature was set from 100-220 °C with an increment of 30 °C/min for 3 min and following an 8 min stop at 180 °C, it was again raised at rate of 10 °C/min until the final temperature was reached. The injector and detector temperatures were set at 220 °C.

RESULTS AND DISCUSSION

Using the Excel and SPSS computer software, the obtained results were subject to analysis of variance. Means were compared with the Duncan's multiple range test and correlation coefficients were calculated. The most average for oil content, 1000-seed weight and seed weight/plant weight belonged to Sub. sp *humile* with 40.38 %, 5.54 (g) and 0.97, respectively. But sub. Sp *usitatissimum* had more protein content with 11.47 % (Table-1). These results are in line with other reported cases⁶. The analysis of variance for seed oil indicated the existence of significant difference between two sub species. The variation for seed oil was 4.75 %. The average

TABLE-1
COMPARISON OF MEASURED PARAMETERS IN
SUB SPECIES OF *Linum usitatissimum*

<i>Linum usitatissimum</i> Sub species	Oil content (%)	Protein content (%)	1000-seed weight (g)	Seed weight/plant weight
Sub. Sp <i>humile</i>	40.38	10.60	5.54	0.97
Sub. Sp <i>usitatissimum</i>	35.63	11.47	3.90	0.50

amounts of the five fatty acids of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid in sub species *humile* and sub sp. *usitatissimum* were found to be 13.23, 10.63, 44.83, 28.39, 65.13, 6.36, 4.15, 24.60, 14.69 and 45.36, respectively (Figs. 1 and 2). Their variation at the 1 % level between the sub species is depicted in Table-2. The mean squares of Table-2 indicate the existence of significant variation at the 1 % in the amounts of all five fatty acids in the sub species of *Linum*

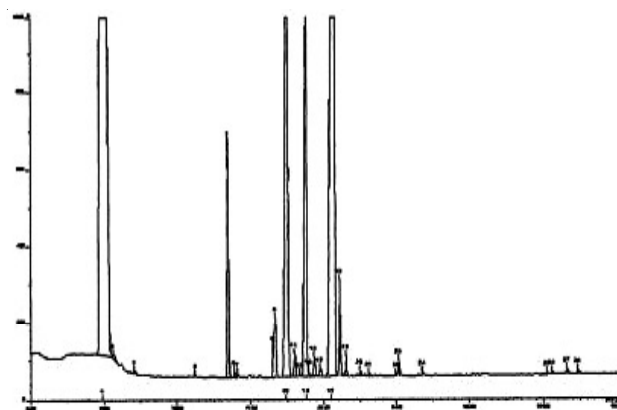


Fig. 1. Chromatographical of Sub sp. *Usitatissimum*

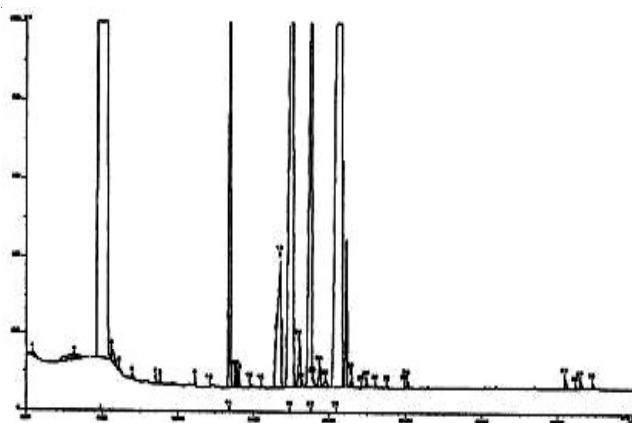


Fig. 2. Chromatographical of Sub species *humile*

TABLE-2
COMPARISON OF MEAN AMOUNTS OF THE FIVE FATTY ACIDS IN SUB SPECIES OF *Linum usitatissimum*

<i>Linum usitatissimum</i> (%)	Linolenic acid (%)	Linoleic acid (%)	Stearic acid (%)	Oleic acid (%)	Palmitic acid
Sub. Sp <i>humile</i>	65.30	28.39	10.63	43.18	13.23
Sub. Sp <i>usitatissimum</i>	45.36	13.69	4.15	24.60	6.36

usitatissimum. In a study of the world collection of flax introductions, the range of fatty acids measured were palmitic acid; 2.9-6.9, stearic acid; 2.2-8.6, oleic acid; 12.9-32.3, linoleic acid; 7.5-24.7, linolenic acid; 40.8-66.7⁶. The results of Table-2 also indicate the seed protein content of sub species is significantly different from each other. The obtained low CV value (Table-3) is a reflection of the accuracy in the performed experiment. The overall average for seed protein in two sub-species was 11.03 that it is rather similar different with other reported cases¹⁰. Present results indicate a minimal difference in chemical composition of *L. usitatissimum* seed as compared with other

TABLE-3
ANALYSIS OF VARIANCE FOR PARAMETERS IN SUB SPECIES OF *Linum usitatissimum*

Source of variation	d.f.	Mean squares						
		Oil	Protein	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Between groups	1	33.844**	1.316*	70.795**	62.986**	554.11**	324.13**	586.27**
Within groups	4	0.271	0.127	2.250	3.770	0.211	0.280	5.650
C.V.	-	0.06	0.5	0.38	0.51	0.30	23.0	0.19

reports. For crop purpose we need suitable and better physical and chemical characteristics. *Linum usitatissimum* is an oil seed. So high amount of 1000-seed weight, seed weight/plant weight and oil content distinct better sub species for crop. Oil of *Linum* seed has high percentage of linolenic acid (ω -3 fatty acid), that this fatty acid is necessary for human body. This research shows, in addition to above quality characteristics, Sub species *humile* has more amount of ω -3 fatty acid. So this sub species is better than sub species *usitatissimum* and we offer it for economical crop.

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