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## A Comparison of Important Physical and Chemical Characteristics of *Linum usitatissimum* Sub. Species

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**Abstract:** *Linum* species is oil seed that has oil percentage with a good quality in point of view omega-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 selected and measured. Weight measurements performed by balance with 0/0001 (gr) sensitivity. Oil and protein content based on dry seed was measured using the extraction by Ether and Lowry methods. Sub. sp *humile* had more 1000-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, Stearic, Oleic, Linolenic acid and Linoleic were detected and measured by gas chromatography. Analysis of variance revealed 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. The most amounts of 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 habitats (Traits, 1994). 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 stem (Berqlund and Zollinger, 2002). We investigate seed flax. The seed itself in flat and oval with a pointed tip. It is a little larger than a sesame seed and measures about 2.5×5.0×1.5mm (Morris, 2004). Flax is rich in fat, protein and dietary fiber (Anonymous, 2001). Flax contains mixture of fatty acids. It rich in polyunsaturated fatty acid, particularly alpha-linolenic acid (ALA), the essential omega-3 fatty acid and linoleic acid (LA), the essential omega-6 fatty acid. These two polyunsaturated fatty acid are essential for human. They must be obtained from the fats and oil in food because our bodies cannot make them (Morris, 2004). Flax seed oil is the world richest source of omega-3 fatty acids at a whopping 57% (over two times the amount of omega-3 fatty acids as fish oil) (Anonymous, 2002). 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 burned (Anonymous, 2001). 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.

### Materials and Methods

This study was performed in Biology department of Urmia University. Seed samples of *Linum usitatissimum* sub species, including sub species *usitstissimum* 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 replications (Raney and Diederichsen, 2002). All seed samples were powdered and placed in 72°C oven for 24 hrs to be dried (Dini and Carapetian, 2006). The extraction with ether method was used for measure of total oil content (Leiboritz *et al.*, 1987). One gram of each samples were transferred in test tubes and 10ml ether were added them, twice. Each time tubes were placed in 40°C oven for 12 hrs and above solutions were transferred in balanced tubes. Tubes were placed in 40°C oven for 4 hrs so that its ether was evaporation. Weight difference of tubes before and after experience was used for oil content.

For measure of total protein the Lowry method was used (Lowry *et al.*, 1951). 0/005 gram from dried samples were transferred in test tubes and 4ml from below extraction buffer was added : 1. Tris (0/2N) 50ml, 2. HCl (0/2N) 26/8ml, 3. Sucrose 17/2gr, 4. Ascorbic acid 1gr. Samples were centrifuged in 8000 rate. 1ml from above solution was added to C solution that prepared as follows;

**A solution:** NaCO<sub>3</sub> 2gr, K.Na tartaric 0/02gr, NaOH 0/4gr and distilled water 100ml.

## Ranjzad et al.: *Linum usitatissimum* Sub. Species

Table 1: Comparison of measured parameters in sub species of *Linum usitatissimum*

<i>L. usitatissimum</i> sub species	Oil content (%)	protein content (%)	1000-seed weight (gr)	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

Table 2: Comparison of mean amounts of the five fatty acids in sub species of *Linum usitatissimum*

<i>L. usitatissimum</i> sub species	Linolenic acid (%)	Linoleic acid (%)	Stearic acid (%)	Oleic acid (%)	Palmitic acid (%)
Sub. sp <i>humile</i>	65/13	28/39	10/63	43/18	13/23
Sub. sp <i>usitatissimum</i>	45/36	13/69	4/15	24/60	6/36

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

\* and\*\* significant at 5% and 1%, respectively.

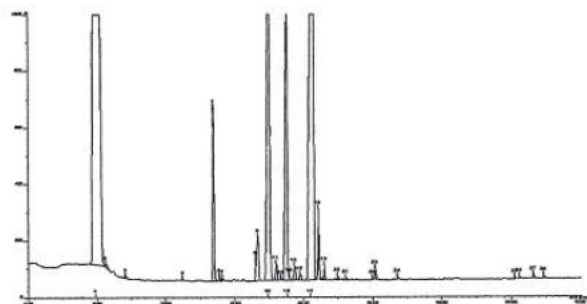


Fig 1: Chromatographical of Sub sp. *usitatissimum*

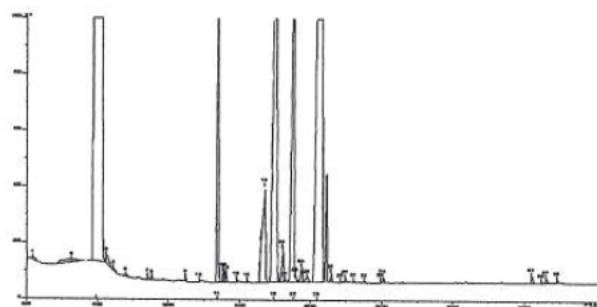


Fig 2: Chromatographical of Sub species *humile*

**B solution:**  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0/5gr and distilled water 100ml.

**C solution:** A solution (50ml)+B solution(1ml)

After 10min, Folin indicator that was diluted the ratio of 1 to 9 before, was added. Tubes were placed in darkness location for 30min. Light absorption of samples was measured by Spectrophotometer in wave length of 660nm. First, set was been Zero with standard solution. Protein content was obtained from below formula:

$$\text{Protein content (\%)} = [420A - 6.9 \times 4] / 50$$

Fatty acids were determination by gas chromatograph after the preparation of their methyl esters. This section of project was performed in Artemia researches center of Urmia University. Esterification was accomplished by addition of 3 ml n-heptan in a test tube. The tubes were vortexed for five min until the glycerol a supernatant. The amount of 0.2 ul 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 to 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 (gr) 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 cases (Diederichsen et al., 2002). 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 amounts of the five fatty acids of Palmic, Stearic, Oleic, Linoleic and Linolenic in sub species *humile* and sub sp. *usitatissimum* were found to be 13/23, 10/63, 44/83, 28/39, 65/13, and 6/36, 4/15, 24/60, 14/69, 45/36, respectively (Fig. 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 usitatissimum*. In a study of the world collection of Flax introductions, the range of fatty acids measured were Palmitic; 2/9-6/9, Stearic; 2/2-8/6, Oleic; 12/9-32/3, Linoleic; 7/5-24/7, Linolenic; 40/8-66/7 (Raney and Diederichsen, 2002). The results of table 2 also indicate the seed protein content of sub species is significantly different from each other. The obtained low C.V 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 cases (Bhatty, 1995).

Our results indicate a minimal difference in chemical composition of *L. usitatissimum* seed as compared with other 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 (Omega 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 omega 3 fatty acid. So this sub species is better than Sub species *usitatissimum* and we offer it for economical crop.

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