



# Effects of Fertilization on the Fatty Acid Content of Oil Flax

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## Abstract

The paper presents the results of a two-year experimental research of two varieties of flax oil and population in two locations (Čojluk and Ostrožnica, Bosnia and Herzegovina), with the aim of determining the variability of seed yield, oil content and fatty acids composition in oil. The experiment was set in field conditions by using random block schedule with four repetitions. The common technology of flax cultivation is applied, without chemicals, but with different treatments of fertilization: T1 - control, T2 - fertilizer, T3 - organic fertilizer, T4 - bacterial fertilizer and T5 - organic + bacterial fertilizer. Two varieties of flax were used for the research: Mikael, Biltstar and population Vrtoče. We determined statistically significant differences in seed yield, oil content, and fatty acid composition, in relation to year of research, location and fertilization treatments. The presence of five most significant fatty acids was processed, C16:0, C18:0, C18:1, C18:2 and C18:3, and whose content and interaction in the seed significantly depend on fertilization treatments.

**Keywords:** flax; treatments; fertilization; fatty acid composition; yield

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## 1. Introduction

Flax (*Linum usitatissimum L.*) first appeared in the Middle East 10,000 years ago (Allaby *et al.*, 2005), and spread to the territory of Egypt, the Mediterranean, Central and Western Asia. Flax seeds were considered by-product that is rejected, and multi-cell flax fibers were used for processing in yarns according to traditional technologies (Cierpurcha *et al.*, 2004). Lately, in all countries of the world, yarn plants/oil crops, including flax, are experiencing their renaissance (Solomon and Frank, 2005). Today, the flax is grown in more than thirty countries around the world (Dimić, 2005). It used to be grown mainly for medical purposes and for the production of fibers, and today it is mainly grown for production of linseed oil, whether it is for chemical or food industry (Bhatty, 1993; Đorđević and Dinić, 2011).

Highly appreciated flax oil contains from 45% to 58% alpha linoleic, about 22% of linoleic acid and it is a true exception among vegetable oils. Besides being a rich source of omega-3 fatty acids (linoleic acid) that have multiple effects on human health, flax seeds are very rich in other healthful substances. The use of flax in the human nutrition helps protect the cardiovascular system, as the cardiovascular diseases are responsible for 53.9% of all deaths of the Bosnia and Herzegovina population (Velić *et al.*, 2012). The main problem arises as a result of the imbalance between omega-3 and omega-6 fatty acids, whose ratio in the nutrition of the population from 15 to 20:1 leads to congestion of blood vessels. Flax is an ideal plant culture, which by regular use in nutrition, directly in the form of seeds or as omega product, can correct this ratio closer to the ideal 1:2 to 1:3. This balancing can be successfully implemented through the inclusion of linseed in the human and animal nutrition (Mollet and Rowland, 2002). In the north western part of Bosnia and Herzegovina, the flax cultivation has been established and developed only in the area of municipality of Bosanski Petrovac, due to its climate and soil conditions.

Since the flax can be used in numerous ways, due to its multiple roles, characteristics and nutritional value in promoting health, we believe that it has ensured a better future and a deserved place in plant production.



## 2. The Aim of Experimental Research

The aim of research is to determine seed yield of two foreign and one indigenous population of flax oil, as well as the influence of different fertilization treatments on the yield and quality of seeds in agro-ecological conditions of the northwestern part of Bosnia and Herzegovina. The hypothesis is that the fertilization with organic and microbiological fertilizers changes the fatty acid composition of flax seed and to achieve the best performance in terms of physical and chemical characteristics of the flax oil. After hand harvesting, seed analysis will be performed and fatty acid composition of the seeds will be determined. It is particularly important to determine what kind of impact the fertilizer formulations have on the ratio of omega-6 (C18:2) and omega-3 (C18:3) fatty acids.

## 3. Methods and Materials

In order to research the issues, the experiments are set up in the open field, in the north western part of Bosnia and Herzegovina, municipality Bosanska Krupa, locations Čojluk and Ostrožnica. The experiments have been conducted in field conditions, according to the random block method with four repetitions, and all experimental studies were conducted in 2013 and 2014.

Before the experiment was made, the chemical analysis of soil has been carried out. Basic soil cultivation (ploughing at a depth of 30 cm) was done in autumn and additional processing (milling) in the first week of April next year.

After the processing of the soil has been done, the experimental area was scaled and marked with wooden posts and ropes. The area of one experimental plot covers 10 m<sup>2</sup>, while the total experimental area, along with paths covers 635 m<sup>2</sup>. Prior to the sowing, the soil was treated with the appropriate amount of fertilizer. The fertilizer is manually inserted into each randomly selected plot in the amount of 4 kg/plot and NPK in the amount of 0.250 kg/plot. Microbiological fertilizer is applied in the early morning hours, as high temperature and high solar radiation adversely affect the bacteria. Two kinds of oil flax (*Linum usitatissimum* L.), Mikael and Biltstar (Faculty of Agriculture, University of Zagreb, Croatia) and population Vrtoče (Agricultural Institute of the Republic of Srpska, Bosnia and Herzegovina) were used as research material. In order to explore the influence of fertilization on yield and quality of the seed, five treatments were used for experiment: T1 - control (without application of fertilizer), T2 - mineral fertilizer, T3 - bacterial fertilizer (azotofiksin), T4 - organic fertilization (cattle manure) and T5 – organic + bacterial fertilizer. Sowing in both years of research was carried out manually in the first half of April, in continuous rows at the distance of 25 cm between rows and at a depth of 2 cm (1 m<sup>2</sup>/1200 germinated seeds). During the vegetation, the common technology of flax cultivation was applied, as well as the measures of care that are comprised of regular destruction of weeds and crust by mechanical means, without the use of chemicals, e.g. herbicides.

The harvest in both years of research was carried out during the month of August. The yield of the seed has been determined after manually harvested three internal rows, without first and last plants in the plots, and seed yield of flax oil is calculated in kg/ha at 12% moisture. In the prepared soil samples the following laboratory analyses were carried out: Texture soil class - According to ISSS, (Baize, 1993); The active and substitution acidity of pH in H<sub>2</sub>O and 1 M KCl - potentiometric; The content of CaCO<sub>3</sub> (5) - volumetric; The easily available phosphorus and potassium (mg/100 g) - Al Egner Riehm method; Humus (%) by the Kotcman method; The content of total N (%) - calculated from humus content; Cd - digestion with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> has been read on IPC.

Analyses of the fatty acid composition of the selected seed samples were determined in the laboratory EMONA (Slovenia) and laboratories BIMAL (Brčko, Bosnia and Herzegovina), (EN ISO 5508: 1990 and ISO 5509: 1990). For the analysis of the total oil content in the flax seed, FOSS Camera Control unit 2055 was used, while the resulting fatty acid esters (FAME) were determined using capillary gas chromatography apparatus Agilent Technologies 6890 N.

The obtained experimental data were processed by mathematical-statistical methods and the statistical software XLSTAT 2010 and PAST 2013 were applied.



#### 4. Results and Discussion

Prior to the start of the experiment, the samples of soil were taken for the chemical analysis, and the results are shown in Table 1.

Table 1: Results of chemical analysis of soil, locations Čojluk and Ostrožnica (Agricultural Institute Bihać)

Location	Type	Depth/cm	pH		%			mg/100 g soil		µg/kg
			H <sub>2</sub> O	KCl	Humus	CaCO <sub>3</sub>	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Cd
Čojluk	Loam	0-30	6.65	6.15	4.95	0.00	0.32	3.82	22.78	304.65
Ostrožnica	Loam	0-30	6.51	6.75	1.35	0.03	0.19	4.72	19.78	296.04

Based on the content of humus in the upper layers of examined soil, type clay, at the location of Čojluk we conclude that this is a very high-quality land with high humus content of 4.95%, slightly acidic reaction (pH 6.15), good quantity with nitrogen (0.32 mg/100 g of soil), medium quantity with potassium (22.78 mg/100 g of soil), but rather poor soil in terms of phosphorus content, as it amounts only 3.82 mg/100 g of soil. Another experimental plot, at the location of Ostrožnica, after the chemical analysis, proved to be a land of poor quality when it comes to all the tested parameters (percentage of humus, the amount of nitrogen and potassium), but much better quality in terms of content of phosphorus, which amounted to 4.72 mg/100 g of soil. Based on the results of submitted samples, the concentration of heavy metals in the soil does not exceed maximum permissible values (Official Gazette FBiH 52/09). The measured value of 304.65 µg/kg (Čojluk) and 296.04 µg/kg (Ostrožnica) show that this is clean and high quality soil.

Comparing the total yield of three examined varieties of flax oil, in two years of research and two different locations, we have to conclusion that a variety Biltstar flax proved to be the most suitable for cultivation in the north western part of Bosnia and Herzegovina, because the average two-year yield was 1,123 kg/ha, then variety Mikael 971 kg/ha and population Vrtoče 765 kg/ha (Table 2).

Further analysis of data shows there is a very significant statistical difference in yield of three cultivars of flax, and the results are shown in table 2.

Table 2: The yield of the seeds based on fertilization treatment in the two years of research

Treatments	Value	Student's T	pr>F
T1	250,000	1,449	0,178
T2	383,333	1,571	0,147
T3	753,333	3,087	<b>0,011*</b>
T4	653,333	2,678	<b>0,023*</b>
T5	1116,667	4,576	<b>0,001**</b>

The high statistical significance was determined at fertilization treatments T3, T4 and T5 in all three cultivars of flax included in research. The two-year average, however, shows that significantly the highest yield was achieved by cultivar Biltstar, fertilization treatment T5 (1900 kg), followed by T4 treatment (1600 kg), and T3 treatment (1200 kg). The lowest yield, in all cultivars was recorded in the treatment of T1 and T2, ranging from 310 kg to 700 kg. Summarizing the results and comparing them with the results of research by other authors (El-Azzouni & El-Banna, 2002; Hussein, 2007), we prove that using microbiological fertilizers can increase and improve the morphological characteristics of plants, in this case the oil flax, without the use of high quantities of mineral fertilizers which, in addition to significantly increasing the cost of production, lead to environmental pollution. The fatty acid composition of flax seed/oil is specific because it contains fatty acids that are rarely and in small quantities in other oils of plant origin, while numerous studies have shown that they have beneficial effect on human health (Almeida et al., 2009; Goyal et al., 2014; Uauy & Valenzuela, 2000).

After manual harvesting and drying of the seed, samples of various examined cultivars, fertilized with different treatments were processed in laboratories EMONA (Slovenia) and laboratories BIMAL (Brčko, Bosnia and Herzegovina), where the total amount of oil, expressed in percentages, was determined for all cultivars, populations and treatments, as well as fatty acid composition of the same.

The content of oil in the examined oil cultivars and the flax population for the entire study during two-year research was 45.52%, which is significantly higher share of oil in the seed, compared to the research conducted by Filipović et al., (2010), who explored the influence of agro ecological conditions on the productivity and quality of flax oil seeds in Serbia. The research included two cultivars of flax oil (Zlatko and Vistro), and the



average oil content in their two-year research was 41.30%, which is 4.22% lower amount of oil compared to our research.

Pali and Metha (2014) explored the fatty acid composition of 48 randomly selected cultivars of flax, and research was conducted in Raipur (India) during years 2012/2013. In all examined varieties, there was a significant difference in terms of the overall quantity of oil in the seed, where the oil content ranged from 33.00% to 42.27%. The highest oil content was recorded in the Deepika variety (42.27%) and the lowest in the Neela variety (33.90%), which is significantly lower than in studies conducted in the experimental part of this paper.

Bayrak *et al.*, (2010) examined 41 cultivar of flax from 5 countries (Turkey, Austria, Czech Republic, Hungary and Germany). The aim of the research was to determine the fatty acid composition of the seed, where the total amount of oil in the seed ranged from 23.28% (cultivar Saramerz) to 32.77% (cultivar Emerando). The obtained results are considerably lower than the recorded average quantity of oil in the examined seed of three varieties of flax seed in the NW part of Bosnia and Herzegovina.

In Pakistan, Muhammad *et al.*, (2013) examined the effect of extrusion on the content of total fat and fatty acid composition of twenty samples of flax seed, where they have recorded significantly lower values in terms of examined characteristics in these studies. The average oil content in the oil flax seed for the entire experiment during 2013 was 51.31%, while in 2014 it was only 39.74%, which is 11.57% lower amount of oil compared to the first year. This difference produced statistically significant variation ( $p < 0.01$ ), (Table 3).

Table 3: Oil flax yield based on cultivars/populations and treatment, multivariate test of significance

Sources	Test	Value	Fishers	Effect df	Error df	p
Variety/ Population	Wilks	0,000002	93,50	16	2,000000	0,010628*
Treatment	Wilks	0,000131	1,70	32	5,282998	0,002823**

Hypothesis of the study is that the combination of organic and bacterial fertilizers leads to improvement of physical and chemical characteristics of the examined cultivars and populations of flax oil. Special emphasis in research is given to the determination of the influence of the fertilizer formulations to the ratio of  $\omega$ -6 and  $\omega$ -3 fatty acids.

The analysis of the seed determined the presence of five main fatty acids: C16:0, C18:0, C18:1, C18:2 and C18:3. The total amount of unsaturated fatty acids in selected varieties and population was from 87,72% to 88.40% during for 2013, and from 88.82% to 89.10% during 2014, while the total amount of saturated fatty acids ranged from 10.52% to 11.08% during 2013, and from 9.22% to 9.68% during 2014 of the total amount of oil.

There are several studies about the content of total unsaturated and saturated fatty acids in the seed of various cultivars of flax, and whose content ranges from 87% to 91%, and from 9% to 12% (Bhatty, 1995; Choo *et al.*, 2007; El-Beltagi *et al.*, 2007.; El-Beltagi *et al.*, 2011). The correlation value between the total amount of oil in the seed and the most important fatty acid in both years of research are shown in Table 4.

Table 4: The coefficient of correlation between the total oil content and fatty acids in the flax seeds

	Oil % Ć	Oil % O	C16:0 Ć	C16:0 O	C18:0 Ć	C18:0 O	C18:1 Ć	C18:1 O	C18:2 Ć	C18:2 O	C18:3 Ć	C18:3 O
Oil % Ć	1											
Oil % O	0.303	1										
C16:0 Ć	-0.473	<b>-0.765</b>	1									
C16:0 O	-0.273	-0.447	<b>0.829</b>	1								
C18:0 Ć	0.280	<b>0.852</b>	<b>-0.917</b>	<b>-0.775</b>	1							
C18:0 O	0.429	<b>0.764</b>	<b>-0.982</b>	<b>-0.856</b>	<b>0.946</b>	1						
C18:1 Ć	<b>-0.607</b>	<b>-0.599</b>	<b>0.937</b>	<b>0.846</b>	<b>-0.815</b>	<b>-0.932</b>	1					
C18:1 O	-0.356	<b>-0.945</b>	<b>0.719</b>	0.396	<b>-0.827</b>	<b>-0.741</b>	<b>0.609</b>	1				
C18:2 Ć	-0.129	0.814	<b>-0.538</b>	-0.402	<b>0.794</b>	<b>0.599</b>	-0.360	<b>-0.811</b>	1			
C18:2 O	0.489	0.664	<b>-0.956</b>	<b>-0.875</b>	<b>0.893</b>	<b>0.976</b>	<b>-0.938</b>	<b>-0.655</b>	0.496	1		
C18:3 Ć	<b>0.636</b>	0.002	<b>-0.529</b>	<b>-0.551</b>	0.215	0.482	<b>-0.714</b>	-0.005	-0.365	<b>0.573</b>	1	
C18:3 O	0.187	<b>0.772</b>	-0.310	0.042	0.472	0.318	-0.189	<b>-0.837</b>	<b>0.716</b>	0.198	-0.336	1

*Bolded values show a significant statistical difference in the level of significance 0.05*



The correlation between the value of the total oil content and ratio of the most important fatty acids shows significant statistical differences at the significance level of 0.05. In the first year of research, a high and positive correlation was found between the total oil and the content of linoleic acid ( $r=0.636^{***}$ ). The change in the presence of linoleic acid and its total amount of oil in flax seed was affected by number of factors, and the highest statistical difference was observed in the effect of fertilization treatment on the fatty acid composition, the hypothesis of the study was confirmed because its percentage distribution in the seed increased with the help of T5 treatment.

In the second year of research, location Ostrožnica, due to poor quality of soil, fertilization treatments had a greater impact on the fatty acid composition of the soil, a positive correlation between the total oil content and stearic acid was recorded ( $r=0.764^*$  i  $r=0.852^*$ ).

It is evident from the results that during 2014, the yield of seed with significantly lower total amount of oil was achieved at location Ostrožnica, but statistically significant increase in the stearic acid has been noticed, compared to the previous year of research on location Čojluk. In addition, there was a high and positive correlated in the content of linoleic acid ( $r=0.772^{***}$ ), where significantly higher values were recorded compared to the fertilization treatments. The content of linoleic acid was influenced by climatic factors, variety/population, but the most statistically significant differences were observed in different fertilization treatments which led to an increase in the content of linoleic acid from 6.69% to 11.61%.

A negative correlation ( $r = -0.765^*$ ) was observed in the content of palmitic acid and the content of oleic acid ( $r = -0.945^*$ ).

The two most important determined acids are C18:2 and C18:3, which are essential, or so-called, vital important acids that the body cannot produce, but must be imported only through food. As previously indicated, an excessive amount of omega-6 (C18:2) in the body leads to inflammation and damage the walls of various blood vessels, due to excess fat, blood begins to coagulate to form a clot, leading to blockage of blood vessels and the appearance of various health disorders, while omega-3 (C18:3) reduce inflammation and thin the blood, so it is preferred that their proportion, in human body, be as close as possible to a ratio of 1:1.

High and positive correlation ( $r=0.716^{***}$ ) was determined between C18:3 and C18:2 ratio on location Ostrožnica, and ( $r=0.573^{**}$ ) on location Čojluk, which means that we have changed fatty acid composition of flax seed using different fertilization treatments, increasing content of C18:3, and reducing the content of C18:2. With all the three varieties, C18:3 increased in the following percentages: 6.69% (Mikael), 7.55% (Biltstar) and a stunning 11.61% (Vrtoče population), while the C18:2 content was simultaneously reduced by 1.53% (Mikael), 1.93% (Biltstar) and 3.25% (population Vrtoče).

## 5. Conclusion

The highest statistical significance was determined using fertilization treatment with combination of organic and bacterial fertilizer (T5). With this treatment, the fatty acid profile of the examined oil flax seed was improved at both locations. High and positive correlation ( $r=0.716^{***}$ ) was determined between C18:3 and C18:2 at location Ostrožnica, and ( $r=0.573^{**}$ ) at the location Čojluk, which means that we have changed the fatty acid composition of flax seed by using fertilization treatment T5, increasing the content of C18:3, and reducing the content of C18:2.

In all examined cultivars and population, the positive effect of fertilization on the content of the aforementioned acids was determined, and is most pronounced in the treatment T5, which led to an increase in C18:3 in the following percentages: 6.69% (Mikael), 7.55% (Biltstar) and 11.61% (Vrtoče population), while at the same time C18:2 content was reduced to: 1.53% (Mikael), 1.93% (Biltstar) and 3.24% (Vrtoče population). With the help of T5 treatment, there was a significant increase in yield for all examined cultivars and population, in the following proportions: Mikael >41.17%, Biltstar >34.50% and population Vrtoče >34.60%, as well as the increase in the quantity of oil and more favourable ratio of omega-6 and omega-3 fatty acids.



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## **A Brief Author Biography**

**Vildana Jogić** – I was born on 24<sup>th</sup> of May 1981 in Bosanska Krupa, Bosnia and Herzegovina. I completed primary school in Cazin and Gymnasium in Bosanska Krupa. In 2004 I finished Biotechnical Faculty, University of Bihać, and graduated at the same faculty in 7<sup>th</sup> of December. I was awarded with the title of "Silver Badge" at the Biotechnical Faculty, University of Bihać. In 2009 I finished master studies in Agricultural Faculty of the University of Banja Luka. In 2015 I completed my doctoral studies under the title: Analysis of the impact of mineral, organic and microbial fertilizers on physicochemical characteristics of the oil flax. During academic year 2004/2005 I was employed as an external associate-assistant professor at the Biotechnical Faculty the field of Plant production, and on academic 2015/2016 I became an employee of the Biotechnical Faculty of the University of Bihać. I have published 37 scientific papers. I am also participant of a Project Erasmus+. Currently working as a Professor in the field of agricultural sciences.

**Subha Dzafić** – I was born on 14<sup>th</sup> of September 1991 in Ključ, Bosnia and Herzegovina. I completed primary school in Gornja Sanica and Gymnasium in Ključ. In 2014 I finished Faculty of Natural Sciences and Mathematics, University of Sarajevo, Department of Biology, Department of Biochemistry and Physiology. I graduated at the same faculty in 2015. I was twice excellently awarded with the title of "Silver Badge" at the University of Sarajevo, with an average grade 9.39 and master degree with an average of 9.90. During academic year 2015/2016 I was employed as an external associate - assistant professor at the Biotechnical Faculty, University of Bihać, in the field of Natural Science. On academic 2016/2017 I became an employee of the Biotechnical Faculty of the University of Bihać. I have published 10 scientific papers and participant of a Bilateral Project. I am currently working as a Senior Assistant in the field of natural science.

**Jelena Nikitović** – I was born on 24<sup>th</sup> of May 1981 in Bihać, Bosnia and Herzegovina. My education starts with primary school in Bihać and secondary school in Banja Luka. In January 2005 I graduated on Faculty of Agriculture, University of Banja Luka. I become MSc in Agricultural Sciences on the same Faculty in March 2013. Now, I am a PhD Candidate in the field of Preservation of Genetic Resources. My working experience begins as volunteer in City Hall of Banja Luka, Department of Inspection during 2006 and Associate in Agriculture Institute of Republic of Srpska, Department of Industrial Plants in 2010. From November 2015, I have been working as Associate at Biodiversity Centre of Institute for Genetic Resources, University of Banja Luka. Additionally, I am working as Senior Assistant at Faculty of Agriculture, University of Banja Luka, narrow scientific field Preservation of Genetic Resources.