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The influence of planting density on the oil and fatty acids content of soybean in hydroponic and soil conditions of the Ararat Valley

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ABSTRACT

Background: Plant seed oils are major renewable resources from nature that can be used in multiple applications. Oils and fats are a vital component of human diets. About 1/3 of the daily energy requirements of a healthy diet come from fats. Each branch of the food industry has its own demands for the fatty acid composition of seed oils. High amounts of polyunsaturated fatty acids are a requirement of healthy diets. However, food manufacturers need highly saturated fatty acids because they need oil that is resistant to high temperatures and oxidation. Soybean oil is second only behind palm oil as the most commercially abundant plant oil globally. Soybean has been recognized for its agricultural importance and their various positive effects on human health.

Objective: to identify the quantitative changes of soybean fatty acids depending on the planting density and growing conditions in the Ararat Valley of Armenia.

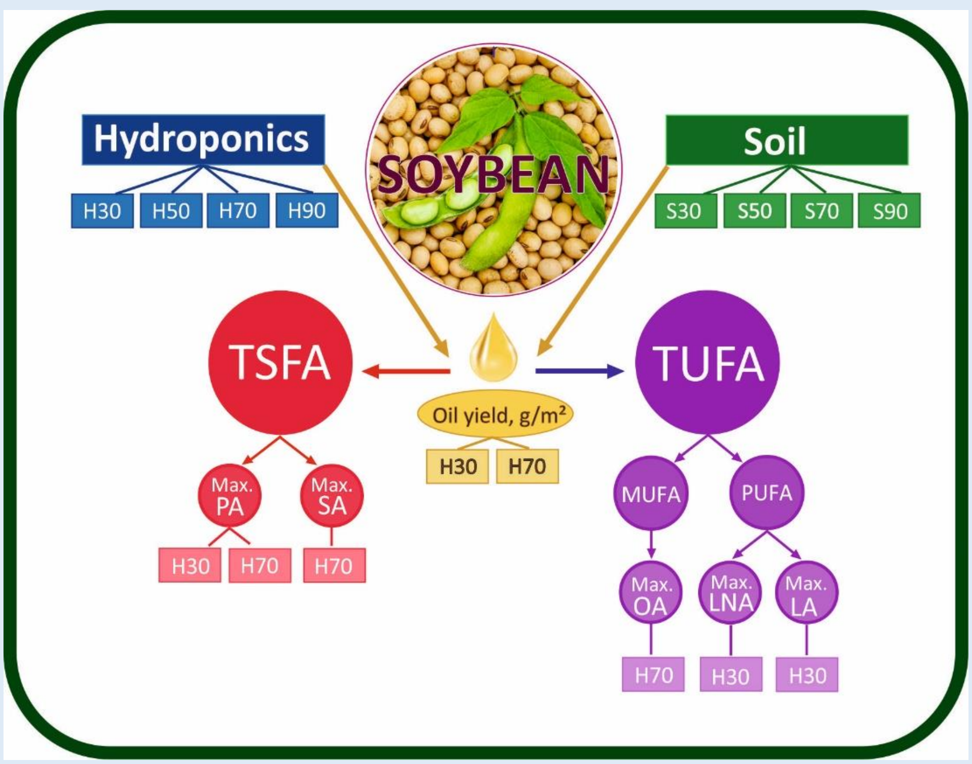
Methods: Soybean was grown in hydroponic and soil conditions of the Ararat Valley. The seeds were sown in the middle of April. Experiments were carried out in hydroponic equipment and soil with different planting densities (30, 50, 70 and

90 plants/m^2). The oil content was measured by using the pulsed nuclear magnetic resonance method. The composition of fatty acids in seed oil was analyzed by chromatographic method on "Chromatec Crystal 5000" GC.

Results: Oil content ranged from 19.6% to 24.4% in all variants. The maximum content was registered in soil with 30 plant/m^2 planting density. Linoleic acid content ranged from 46.35 to 51.28% of the total fatty acid content, regardless of planting density and growing media. The content of linolenic acid ranged from 4.13 to 5.11% in all variants. The maximum content of **ω**-3 and the minimal content of **ω**-9 was recorded in hydroponics at 30 plants/m^2 planting density. The highest percentage of stearic acid was observed in the soil variant with a planting density of 30 plants/m^2. Interestingly, the variants did not differ in the total content of the 5 main fatty acids (98.84-98.95%). Growing medium had no significant effect on the total saturated fatty acids content; in all variants it ranged from 14.29% to 14.83%. Total unsaturated fatty acids content ranged from 85.2% to 85.7%. The hydroponic variant with a planting density of 30 plants/m^2 had the lowest ratio of **ω**-6 and **ω**-3 fatty acids (10:1).

Conclusion: Planting density and growing media had a significant influence on the content of monounsaturated and polyunsaturated fatty acids. Under hydroponic conditions the lowest content of monounsaturated and the highest content of polyunsaturated fatty acids were observed at the lowest planting density (30 plants/m^2), which can be used as a functional food for daily consumption.

Keywords: *Glycine max* (L.) Merr., soilless culture, ω-3, ω-6, ω-9, saturated acids.



INTRODUCTION

Oils and fats are an important component of the human diet. Fats provide essential fatty acids (EFA) and promote the absorption of fat-soluble vitamins. Dietary fats are divided into 4 categories: monounsaturated fats, polyunsaturated fats, saturated fats and trans fats [1]. Traditionally, edible oils from crops such as soybean, canola, sunflower and corn are the richest sources of EFA [2].

Soybean - Glycine max (L.) Merr. is an annual plant that typically reaches a height of 20-180 cm. It usually produces pods with two or three seeds. It is a subtropical crop but can also be grown in tropical and temperate regions at temperatures up to 50 °C [3].

Soybean is widely cultivated in many parts of the world [4]. Soybean was first domesticated in central China 5000 years ago for food and medicinal purposes. Since the 1950s, soybean production has shifted from Asia to the United States, Argentina and Brazil, which now account for 80% of global soybean production. The global soybean sector is currently valued at 155 billion USD and is projected to reach 278 billion USD by 2031. 20% of soy production is consumed as edible oils and human food products [5].

Global soybean production in 2024/25 is forecast to reach 422 million tons, up 6% from 2023/24. Global oilseed production in 2024/25 is forecast to reach a new world record of 687 million tons, up 4% from 2023/24 [6].

Soybean has become known for its agricultural and nutritional values, as well as its effect on improving human health due to its functional characteristics. Soybeans are an important source of protein and oil; they contain a large number of healthy biologically active substances [7]. Soybean seed lipids function as energy storage for plants; they are part of membranes, signaling molecules and protection against pathogens [8].

Soybean oil is a major dietary fat in the human diet, it is second only to palm oil as the most widely consumed commercially available vegetable oil in the world [9]. Soybean oil contains five prominent fatty acids (FA): palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) and linolenic acid (LNA) [4, 8, 10]. Soybean oil is high in unsaturated fats, such as OA, LA and LNA. The high level of LA and OA indicates the role of soybean as a functional food in the prevention of cancer, diabetes and cardiovascular diseases [4, 11-12].

LA and LNA are ω-6 and ω-3 EFA that must be obtained through dietary intake as a functional food [13]. The lack of these FA has been linked with an increased risk of cardiovascular disease [2, 9, 14-15]. Numerous studies have found a positive correlation between EFA and infant development, optimal brain and vision functioning, and the reduction of arthritis, hypertension, diabetes mellitus and neurological disorders [16]. The soybean oil also has a free radical scavenging capability of approximately 163 g/mL [17].

The demand for soilless farming systems is growing every year all over the world. This method allows for control of water and nutrients, which results in higher plant yields. Hydroponic methods are especially effective and useful in areas where the soil and climate are not suitable for growing crops using traditional farming methods. As populations grow and arable land shrinks, hydroponics may gradually replace traditional farming. The different inorganic substrates such as gravel, sand, perlite, volcanic slag, etc. are widely used in soilless culture. The good qualities of the substrates that are used in soilless culture are good aeration and drainage, good water-holding ability, resisting decomposition, and good cation exchangeability and pH [18].

Growing soybeans in hydroponic conditions can provide a sustainable source of grain and oil in adverse environmental conditions, such as the tens of thousands of salins in the Ararat Valley. Hydroponics technology allows to reduce of the use of water, mineral salts and human resources when growing plants on lands unsuitable for traditional farming. This is a more controlled system for increasing targeted primary and secondary metabolites. [19].

The study aimed to investigate the quantitative changes of soybean FA depending on the planting density and growing conditions in outdoor soilless and soil culture of the Ararat Valley. This research was carried out for the first time in Armenia.

MATERIALS AND METHODS

The seeds of "Menua" cultivar of soybean were used in this study (Pic. 1). This variety is not a genetically modified organism (GMO). The average weight of 1000 seeds was 143–213 g [19]. A mixture of volcanic slag and gravel in a 1:1 ratio was used as a hydroponic substrate for growing plants. The size of the substrate particles varies between 3-15 mm [20]. The seeds were sown in the second decade of April. The EBB & Flow hydroponic system was used for the nourishment of plants. The experiments were carried out on hydroponic equipment with a feeding area of 2 m^2 [21].

H30 H50 H70 H90

Picture 1. Hydroponic soybeans at different planting densities.

The planting density was 30, 50, 70 and 90 plants/m^2 for hydroponics (H30, H50, H70, H90) and soil (S30, S50, S70, S90). Hydroponic plants were nourished twice a day: during April-May with a concentration of 400-500 mg/L, and June-August with a concentration of 900-1000 mg/L of Davtyan's nutrient solution [22-23]. Soil plants were irrigated twice a week. In the soil culture (control variant) (4 x 0.5m^2) all accepted agrotechnical rules were observed (soil loosening, weeding, regular watering, fertilization, etc.).

Harvesting was done at the end of August, at the stage of technical maturity of the soybeans. The cultivation was extended for about 120 days. The seeds were dried under room conditions. An average sample was selected for biochemical analysis (oil and FA content).

Determination of oil content: The oil content was measured on the Nuclear Magnetic Resonance Spectrometer AMB 1006M by using the pulsed nuclear magnetic resonance method in accordance with the Interstate Standard GOST 8.597-2010 "State system for ensuring the uniformity of measurements. Oilseeds and oilseeds residues. Determination of oiliness and moisture content using pulsed nuclear magnetic resonance spectrometry" [24].

Preparation of methyl esters of FA: Preparation of methyl esters of FA was carried out in accordance with GOST 31665-2012 "Vegetable oils and animal fats. Preparation of methyl esters of fatty acids" [25].

In a 100 cm^3 flask, 1 g sample of soybean oil was weighed, then 13 cm^3 of a methanol solution of hydrogen chloride was added and the resulting mixture was boiled for 10 min. After boiling, the flask was cooled under running water and 25 cm^3 of distilled water was added. Then the content of the flask was transferred to a 100 cm^3 separatory funnel and extracted with heptane (hexane) twice (10 cm^3 each). The combined extracts were washed with distilled water in 7 cm^3 portions until the acid was completely removed.

Identification of FA: Identification of FA was carried out in accordance with the Interstate Standard GOST 30623-2018 "Vegetable oils and blended fat products. Falsification detection method" [26]. The composition of FA in soybean oil was analyzed by chromatographic method on a "Chromatec Crystal 5000" GC with an automatic liquid sample dispenser DAZh-2M on a capillary column SolGelWax 30 m × 0.25 mm × 0.25 µm.

Field experiments were replicated 4 times. All biochemical analyses were conducted in triplicates and the data were expressed as means ± standard deviation. GraphPad Prism 8 and Microsoft Excel 2016 statistical programs were used to analyze all data. One-way analysis of variance (ANOVA) was used to determine statistical significance and compare mean values. The study groups were randomly sampled and values were assessed for significant differences (P≤0.05).

RESULTS AND DISCUSSION

Planting density is one of the key factors in soybean cultivation. The optimal density of soybean crops depends on the plant genotype, climatic conditions, tendency to lodging and other factors. Schmitz et al. [27] recorded the effectiveness of narrow row spacing and indicated that the optimal seeding rate per ha was 450,000-500,000 seeds. Kumagai [28] also confirmed that high planting densities with narrow row spacing resulted in high soybean yields. Puruker and Steinke [29] noted a 9 % increase in soybean grain yield with dense seeding of 1.8 times, but in further densing of seeding (2.6 and 3.4 times) no significant difference was observed.

Growing medium and different planting densities both in hydroponics and in soil had a significant impact on soybean yield (Table 1). Since the soybean seeds yield at S70 and S90 planting density was sharply low (6.2 and 4.5 g/plant, respectively), we did not consider it expedient to continue further research. In hydroponics, the maximum yield was obtained with the low planting density H30. The seed yield per plant was 1.8-4.5 times higher than that of the other hydroponic variances, and 1.9-5.4 times higher than that of the soil. The same results were obtained by Carciochi et al. [30]: per plant yield increased due to a decrease in plant density.

Although almost the same yield was obtained per square meter with a sowing density of H30, H50 and H70, we prefer a density of H30, taking into account less number of sown seeds (1.7-2.3 times), as well as low costs for harvesting.

Table 1. Effect of growing medium and planting density on the soybean seeds yield

Variants	Seed yield	
	g/plant	g/m^2
H30	38.7^a	1161
H50	21.6^b	1080
H70	16.7^b	1169
H90	8.6^c	774
S30	19.9^b	597
S50	7.2^d	360

Different letters indicate significant differences, P<0.05

Planting density and growing medium had a certain effect on the oil content in soybean seeds (Fig. 1). The oil content of all variants ranged from 19.6 to 24.4%, and the maximum content was observed in the plants of the S30 variant, which exceeded the rest of the variants by 1.2 times. This is consistent with the conclusions of Anwar et al. [3] who found that oil content from several varieties of soybean seeds ranged from 15.85 to 19.49%. In the other research Azam et al. [31] showed that in 333 soybean cultivars, the oil contents ranged from 14.6 - 22.8%.

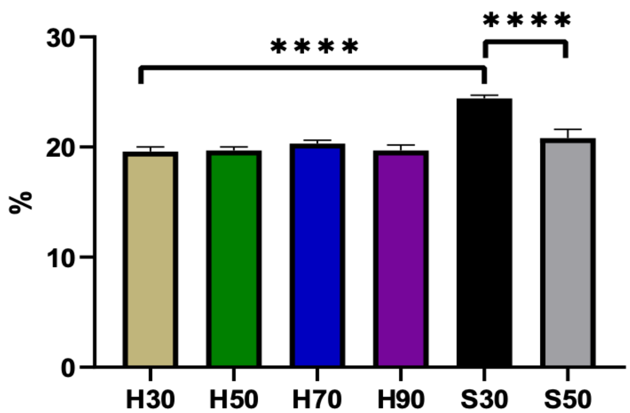


Figure 1. Oil content in soybean seeds of hydroponic and soil variants with different planting density P< 0.0001

Even though maximum oil content was recorded in S30 plants, maximum oil yield was observed in the H30 variant due to the high yield of the soybean seeds. It exceeded the other hydroponic variants by 1.8-4.5 times and soil by 1.6-5.1 times (Table 2).

Table 2. The oil yield of soybean depending on growing medium and planting density.

Variants	Oil yield	
	g/plant	g/m^2
H30	7.59^a	227.6
H50	4.26^b	212.8
H70	3.39^c	237.3
H90	1.69^d	152.5
S30	4.86^b	145.7
S50	1.50^d	74.9

Significant differences are indicated by different letters, P<0.05

Table 3 shows the FA composition of soybean seeds depending on the growing medium and planting density. Several studies are showing that changes in FA composition is dependent on both cultivar and environmental factors [9-10, 32]. When studying FA composition of oil of all variants, 11 FA were found. All listed FA were detected in all variants.

Table 3. Influence of growing medium and planting density on FA compositions of soybean seeds, %

FA	Planting Density					
	H30	H50	H70	H90	S30	S50
TSFA	14.77±0.21	14.61±0.14	14.51±0.06	14.29±0.05	14.83±0.19	14.81±0.13
(C14:0) Myristic acid	0.07±0.005	0.06±0.005	0.06±0.005	0.06±0.005	0.06±0.005	0.06±0.005
(C16:0) Palmitic acid	10.79±0.13	10.58±0.10	10.34±0.08	10.33±0.04	10.38±0.11	10.62±0.12
(C18:0) Stearic acid	3.24±0.09	3.29±0.04	3.41±0.02	3.22±0.01	3.62±0.08	3.43±0.01
(C20:0) Eicosanoic acid	0.27±0.01	0.27±0.01	0.28±0.01	0.27±0.01	0.31±0.01	0.28±0.01
(C22:0) Behenic acid	0.28±0.02	0.28±0.01	0.29±0.01	0.28±0.01	0.31±0.01	0.29±0.01
(C24:0) Lignoceric acid	0.12±0.01	0.13±0.01	0.13±0.01	0.13±0.01	0.15±0.01	0.13±0.01
TUFA	85.24±0.21	85.39±0.14	85.49±0.10	85.72±0.05	85.20±0.21	85.21±0.18
MUFA	28.85±0.46	31.73±0.41	34.65±0.17	34.01±0.22	32.96±0.13	32.03±0.33
(C16:1) Palmitoleic acid	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.05±0.01	0.06±0.01
(C18:1) Oleic acid	28.53±0.45	31.42±0.40	34.32±0.19	33.69±0.23	32.6±0.14	31.72±0.32
(C20:1) Eicosenoic acid	0.26±0.01	0.25±0.01	0.27±0.01	0.26±0.01	0.31±0.01	0.25±0.01
PUFA	56.39±0.46	53.66±0.31	50.84±0.11	51.71±0.18	52.24±0.25	53.18±0.23
(C18:3) Linolenic acid	5.11±0.10	4.4±0.08	4.49±0.04	4.13±0.02	4.21±0.09	4.41±0.07
(C18:2) Linoleic acid	51.28±0.47	49.26±0.26	46.35±0.13	47.58±0.17	48.03±0.32	48.77±0.16
Main FA (PA, SA, OA, LA, LNA)	98.95	98.95	98.91	98.95	98.84	98.95
ω6:ω3	10.0:1	11.2:1	10.3:1	11.5:1	11.4:1	11.1:1

Growing medium had no significant effect on total saturated fatty acids content (TSFA); in all variants it ranged from 14.29% to 14.83%. Thus, myristic acid levels ranged between 0.06-0.07%, PA between 10.33-10.79%, SA between 3.22-3.62%, eicosanoic acid between 0.27-0.31%, behenic acid between 0.28-0.31% and lignoceric acid within the range of 0.12-0.15%.

Meanwhile, in hydroponics, along with an increase in planting density, a decrease in the content of TSFA was observed, which was significant in the case of the densest planting.

The content of PA and SA are in line with the reports of Anwar et al. [3], where their content in the examined soybean oils ranged from 10.01% to 13.5% and from 3.02% to 4.9%, respectively.

Abdelghani et al. [10] assessed the FA composition of 1025 Chinese soybeans and reported that PA and SA levels ranged from 10.2% to 15.2% and from 2.6% to

7.5%, respectively, which is also consistent with our findings. In a study conducted on 40 soybean genotypes from India, Rathod et al. [33] reported that PA and SA levels ranged from 8.45% to 13.23% and 2.28% to 9.52%, respectively.

The opposite pattern was observed for the content of total unsaturated fatty acids (TUFA): under hydroponic conditions, a gradual increase in TUFA content was noted along with an increase in planting density, which was significant in the case of the densest planting (H90).

The content of TUFA ranged from 85.20% to 85.72%, which is in agreement with the literature data [3, 34].

The content of monounsaturated fatty acids (MUFA) in the oil of "Menua" cultivar ranged from 31.73% to 34.65%, except for the H30 variety, where the lowest content was recorded (28.85%), which was 1.1-1.2 times lower than in others. Palmitoleic acid ranged from 0.05-0.06% regardless of growing medium and planting density.

One of the major ω-9 MUFA is OA. High intake of ω-9 MUFA in the diet has been associated with reduced anger and irritability, pain in patients with inflammatory arthritis [35]. Oils rich in MUFA are considered healthier and have a longer shelf life due to their increased resistance to oxidation [36].

OA content in the "Menua" cultivar seed oil ranged from 28.53% to 34.32% depending on planting density and growing medium. These data are higher than the amount obtained by Anwar et al. (22.6–24.0%) [3], but they are - by the results of Abdelghani et al. (13.3-36.1%) [10] and Choi et al. (14.97-38.74 %) [4].

The content of polyunsaturated fatty acids (PUFA) in the oil of seeds the soybean "Menua" cultivar underwent significant changes depending on the planting density and growing environment. In all variants it ranged from 50.84% to 56.39 %., which is in agreement with data from previous studies [31, 37].

The content of LNA in hydroponics ranged from 4.13% to 5.11%. The maximum content of 5.11% was observed at the lowest planting density (H30), and the minimum content of 4.13% at the 3 times denser planting density (H90). The content of LNA in the H30 variant exceeded other hydroponic variants by 1.1-1.2 times and soil variants by 1.2 times.

The content of LA in all variants ranged from 46.35% to 51.28%, and again the maximum content of 51.28% was observed at H30 variant.

As a conclusion we can maintain that H30 demonstrated better nutritional value due to the higher amount of EFA such as LNA and LA.

Anwar et al. [3] noted that LA and LNA content in soybean oil ranged between 49.03–53.00% and 6.50–8.00%, Abdelghani et al. [10] reported that it ranged between 40.4–63.9% and 3.9–12.8%, and Choi et al. [4] reported that it ranged between 43.22–60.26% and 5.37–12.33%, respectively.

Numerous studies have shown that low ω6:ω3 ratios are generally healthful [38]. In the study by Shostak et al. [37] the ω6:ω3 ratio ranged from 7.5 to 8.2. Butler et al. [39] recorded a dramatically high ratio for high LNA and low LNA varieties: 13.7-16 and 27.9-28.3, respectively. In the study by Anwar et al. [3], this indicator ranged between 6.1-8.1, in the study by Abdelghany et al. [10] it ranged between 3.16-16.4 for 1025 soybean accessions and in the study by Choi et al. [4] it ranged between 3.5-11.2.

In our study ω6:ω3 ratio varied between 10:1 and 11.5:1, depending on the growing medium and planting density.

The H30 variant was the best option as a functional food since the ω6:ω3 ratio was the lowest in this variant and it is in the range of WHO recommendation (5-10)[17].

The variants did not differ in the total content of the 5 main FA (98.84-98.95%), and the total content of the remaining 6 FA did not exceed 1.16% in any variant (Table 3).

In Figure 2 the quantity of 5 main FA is presented per square meter. As can be seen from Figure 2, hydroponics provided the optimal conditions for the accumulation of main FA per square meter. At the same planting densities, growing medium significantly affected the yield of fatty acids obtained per square meter. Thus, in the case of 30 plant/m^2 the quantity LA, LNA, OA, SA and PA obtained from plants per square meter of hydroponics exceeded the soil by 1.7, 1.9, 1.4, 1.4 and 1.6 times, respectively. The difference in the case of 50 plants/m^2 was more noticeable: the amount of LA, LNA, OA, SA and PA obtained per square meter in the soil was inferior to the hydroponic one by 2.9, 2.8, 2.8, 2.7 and 2.8 times, respectively.

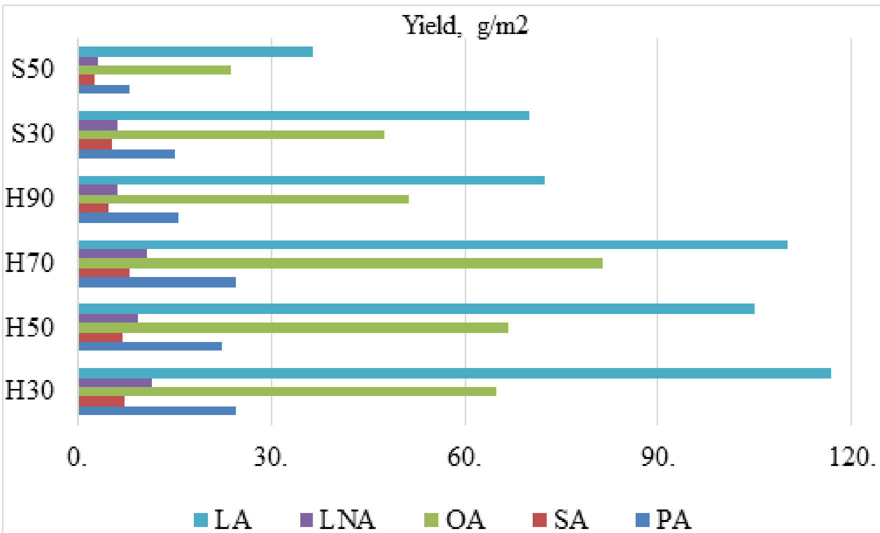


Figure 2. Yield of PA, SA, OA, LNA and LA per square meter

In the densest planting in hydroponics the minimum yield of LA (72.4 g), LNA (6.3), OA (51.3 g), SA (4.9 g) and PA (15.7 g) was obtained per square meter, which is inferior to other hydroponic variants by 1.4-1.6, 1.5-1.8, 1.3-1.6, 1.4-1.7 and 1.4-1.6 times, respectively. In the case of H30 it is possible to obtain a maximum 116.7 g LA and 11.6 g LNA.

CONCLUSION

In the FA composition of the soybean "Menua" cultivar seeds oil, regardless of the growing conditions and planting density, the main FA formed the following descending series: LA>OA>PA>LNA>SA. The same pattern was recorded in other studies [4, 10].

The obtained results (yield, oil content and FA composition) confirm the feasibility of using H30 as a plant-based functional food directed to improve overall health because of the high levels of LNA (5.11%) and the lowest ω6:ω3 ratio (10:1) [17, 40-41].

H70 meets the confectionery industry's needs for high melting point and oxidative stability because it contains high levels of SA (3.41%) and high levels of OA (34.32%), as well as low levels of PUFA (50.84%) [33, 42].

Based on our research, we recommend using H30 as a functional food for daily consumption and H70 for industrial and confectionery purposes.

Authors' Contribution: All authors contributed to this research and wrote this paper.

Abbreviations: GMO: genetically modified organism, FA: fatty acids, EFA: essential fatty acid, TSFA: total saturated fatty acids TUFA: total unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, PA: palmitic acid, SA: stearic acid, OA: oleic acid, LNA: linolenic acid, LA: linoleic acid.

Competing Interest: None.

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