Bulgarian Journal of Agricultural Science, 32 (No 5) 2026, 1085–1093

DOI: https://doi.org/10.61308/WAKU8204

Physicochemical and fatty acid composition of spring canola seeds after treatment with biostimulants

Silviya Ivanova[1*], Iliyana Petrova[1], Milena Pavlova[1], Svetlana Stoyanova[2] and Ralitsa Mincheva[2]

[1] *Agricultural Academy, Institute of Cryobiology and Food Technology – Sofia, Bulgaria*
[2] *Agricultural Academy, Institute of Agriculture and Seed Science "Obraztsov chiflik" – Ruse, Bulgaria*
[*]*Corresponding author:* sylvia_iv@abv.bg

Abstract

Ivanova, S., Petrova, I., Pavlova, M., Stoyanova, S. & Mincheva, R. (2026). Physicochemical and fatty acid composition of spring canola seeds after treatment with biostimulants. *Bulg. J. Agric. Sci., 32*(5), 1085–1093

This study evaluates the effects of foliar application of newly developed natural biostimulants on the physicochemical composition and fatty acid profile of seeds of spring oilseed rape *(Brassica napus oleifera annua*), hybrid "Lakritz", grown under organic cultivation.

During 2 consecutive harvest years, in precise field trials conducted accordingly randomized block design at the Institute of Agriculture and Seed Science "Obraztsov Chiflik" – Ruse, the influence of 3 biostimulants with different characteristics applied in 2 doses was tested. Biostimulants were developed at the Institute of Cryobiology and Food Technologies – Sofia, based on vermicompost extract (HA), chitosan (Cs) and a humic complex containing a naturally identical growth regulator of auxin-type (HAR).

It has been established that the application of biostimulants in critical phenological phases leads to an increase in the content of crude proteins and crude fibers in the seeds. Treatment with Cs reduces the content of crude fats compared to the control group, while the influence of the humic extract and the humic complex was less pronounced in the first year. Crude fats increase in the second year of treatment when using the preparations HA 600, HAR 300 and HAR 600. The studied biostimulants improved the fatty acid composition of rapeseed seeds by reducing the total content of undesirable saturated fatty acids (Cs 300, Cs 600, HAR 300, and HAR 600) and erucic acid (Cs 600 and HAR 600), while increasing desirable polyunsaturated fatty acids, thereby enhancing the health properties of rapeseed oil.

Keywords: biostimulants; foliar treatment; canola seed; chemical composition; fatty acid profile
Abbreviations: Cs – chitosan; HA – humic extract; HAR – humic complex enriched with growth regulator; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; SFA – saturated fatty acids

Introduction

The composition of rapeseed is determined by genetic, environmental and agro-technical factors (Sikorska et al., 2021). Rapeseed production in Europe is primarily for oil, followed by animal feed, bio-oil and biodiesel (Chew, 2020). In recent years, students have been working on the impact of biostimulants in foliar treatments on the amount of fat (Ahmad et al., 2012; Chwil, 2016; Jankowski et al., 2016) and crude protein (Ahmad et al., 2007; Malhi et al., 2006) in the seeds. The influence of different types of biostimulants on yield, nutritional potential and abiotic stress in rapeseed and other conventional crops has also been studied (Neshev et al., 2021; Zhang et al., 2024; Kocira, 2019; Laurent et al., 2018; Bulgari et al., 2019; Szczepanek and Siwik-Ziomek, 2019; Nowak et al., 2023; Osvalde et al., 2024). Gugała et

al (2018), found crude protein content 349.1–367.7 g kg^{-1} d.m, crude fat from 440 to 490 g kg^{-1} d.m and crude fibber from 85.8 to 90.1 g kg^{-1} d.m. after foliar treatment of winter rapeseed with biostimulants.

Cold-pressed rapeseed oils have a distinct aroma and taste with a hint of nuts (Chew, 2020, Petrova et al., 2023; Nowak et al., 2023). Rapeseed oil was recognized as a healthy oil due to its attractive fatty acid profile, high in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and is low of SFA. This has led to interest in investigating various factors affecting the fatty acid profile.

The present study is to determine the change in the physicochemical and fatty acid composition of the seeds and to evaluate the quality indicators of the fat fraction in spring oilseed rape (*Brassica napus oleifera annua*) treated with biostimulants.

Material and Methods

In the experimental field of the Institute of Agriculture and Seed Science "Obraztsov chiflik" – Ruse, Bulgaria, during the period 2022–2023, to test the effect of foliar treatment with biostimulants, field trials have been conducted with spring oilseed rape, hybrid "Lakritz". Rapeseed was grown under organic conditions – without the application of pesticides, fertilizers and soil improvers prohibited for organic farming.

The experiment was arranged according to the Shanin block method, using randomly assessed block design, a harvest plot of 10 m^2 size, three plot replications for each treatment (Shanin, 1977). During the vegetation period, a two-time foliar treatment with biostimulants was carried out in the rosette phase (BBCH 18) and the beginning of flowering (BBCH 60).

Phytostimulants with different characteristics were applied. Three preparations were developed based on a two-stage extraction of vermicompost, hydrolysis of chitosan and a naturally identical auxin-type growth regulator. The composition was as follows:

- Cs, chitosan, applied at a concentration of 0.05% w/v;
- HA, 10% vermicompost extract with alkaline extractant, applied at a concentration of 20% v/v;
- HAR, combined treatment with humic extract and growth regulator in a 1:5 ratio.

The stimulants were applied in 2 doses – 300 ml da^{-1} and 600 ml da^{-1}, and thus the experiment included 6 treatment options and an untreated control.

The subject of analysis was the seeds harvested in the technological maturity phase (BBCH 98).

For each treatment option and the control, for analysis, 2 parallel pooled samples were collected from each of the three experimental plots (n = 6 per each variant).

Fats were determined according to BDS EN ISO 1211: 2002, ISO 9622.

Crude protein BDS EN ISO 5983-2:2009

Fibers by automatic extraction system for determining fibre content Dosi-Fiber- Selecta, Spain.

Extraction of fat was achieved by Bligh and Dyer (1959) method. The FAME were determined on Shimadzu-2010 gas chromatograph (Kioto, Japan) by FID, CP 7420 capillary column (100 m × 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA), carrier gas- hydrogen and make-up gas – nitrogen on the four-step furnace mode was programmed – the column's initial temperature was 80°C / min, maintained for 15 minutes, then increased by 12°C / min to 170°C and maintained for 20 minutes, followed by a further increase of 4°C / min to 186°C for 19 minutes and up to 220°C with 4ºC / min until the process is complete.

The lipid preventive score (LPS), atherogenic (AI) and thrombogenic index (TI) (Ulbricht and Southgate, 1991), the ratio between hyper- and hypocholesterolemic (h/H) fatty acids, trans fatty acids (TFA) and the amount of saturated fatty acids were calculated.

LPS = FAT + 2 × SFA – MUFA-0.5PUFA,

AI = 12:0 + 4 × 14:0 + 16:0/[ΣMUFAs + PUFA n-6 + PUFAn-3]

TI = (14:0 + 16:0 + 18:0)/[0.5 × ΣMUFAs + 0.5 × PUFAn-6 + 3 × PUFAn-3 + PUFAn-3/PUFA n-6]

h/H = (C18:1n-9 + C18:1n-7 + C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3)/(C14:0 + C16:0).

The results were processed in Excel using Student's t-test and are presented in the tables of the current study.

Results and Discussion

The functional properties of rapeseed were determined by the amount of fat, protein, carbohydrates, crude fiber, ash, minerals (e.g. calcium, magnesium, potassium, phosphorus and sulphur), vitamins (e.g. vitamin E, vitamin K and vitamin B complex) and moisture content, and vary depending on the variety, growing conditions, harvesting methods and storage conditions. The level and ratio of the individual components determine their nutritional value and quality for different industrial applications (Anwar et al., 2015; Barthet and Daun, 2011; Guirrou et al., 2023).

Oil, the most important component, varies from 40 to 50% depending on the variety and weight of rapeseed (Petrie et al., 2020; Mohtashami et al., 2020). Fig. 1 presents the data on crude fat.

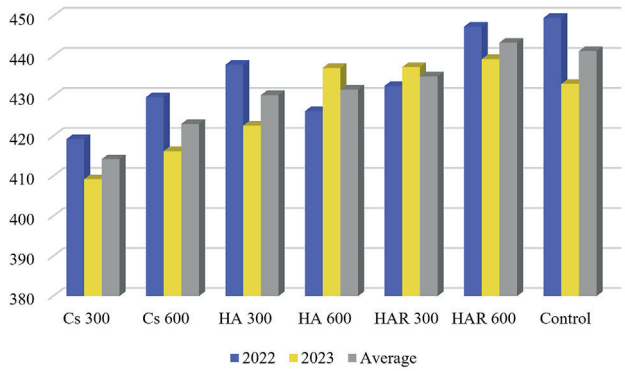


Fig. 1. Effect of foliar treatment with biostimulants on crude fats of canola seed, g kg^{-1} d.m.
*Cs – chitosan; HA – vermicompost extract;
HAR – humic complex with growth regulator
Source: Authors' own elaboration

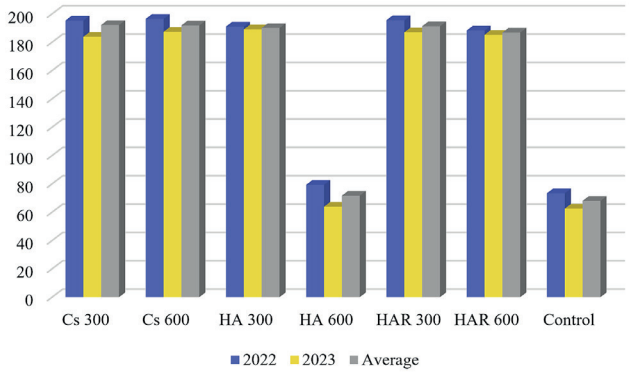


Fig. 2. Effect of foliar treatment with biostimulants on crude proteins of canola seed, g kg^{-1} d.m.
*Cs – chitosan; HA – vermicompost extract;
HAR – humic complex with growth regulator
Source: Authors' own elaboration

The application of the biostimulants Cs 300 (***P < 0.001), Cs 600 (**P < 0.01), HA 600 (**P < 0.01) and HAR 600 (**P < 0.01) during the two years of rapeseed treatment significantly reduced the concentration of crude fat (Fig.1). On the first year of treatment, a significant decrease in the amount of crude fat was found in all variants compared to the control group. Highly significant differences were found in Cs 300, Cs 600, HA 300 and HAR 300 (***P < 0.001) and low significance of the results in HA 600 and HAR 600 (*P < 0.05). The treatment of rapeseed with the biostimulant Cs 600 had a significantly higher fat content compared to Cs 300 (**P < 0.01) on the second year. The use of the lower dose of HA 300 resulted in significantly higher crude oil content in rapeseed compared to the higher concentration of HA 600 (**P < 0.01). The application of HAR 600 to rapeseed resulted in the synthesis of more oil in rapeseed compared to HAR 300 (***P < 0.001; Fig. 1).

Protein content was another important quality characteristic of rapeseed and determines the quality of rapeseed meal for animal feed. The protein in rapeseed ranges from 17 to 26% and was moderately heritable (Li et al., 2012).

The application of the biostimulants Cs 300 (***P < 0.001), Cs 600 (**P < 0.01), HA 600 (**P < 0.01) and HAR 300 (**P < 0.01) and the control group (**P < 0.01) during the two years of rapeseed treatment led to a statistically significant decrease in crude protein content. Crude protein synthesis increased after treatment with the used types of biostimulants (***P < 0.001) compared to the control group, with the exception of the application of HA 600, in which the changes compared to the control group of rapeseed were low reliable (Fig. 2). On the first year of treatment, a significant decrease in the amount of crude protein was found in rapeseed treated with HA 600 compared to HA 300 and in HAR 300 compared to HAR 600 (***P < 0.001), while the application of Cs 300 and Cs 600 did not cause significant changes. On the second year of application the biostimulants, the synthesis of crude protein was maintained as on the first, but in lower concentrations and under the influence of the biostimulants, similar dependencies were observed (Fig. 2).

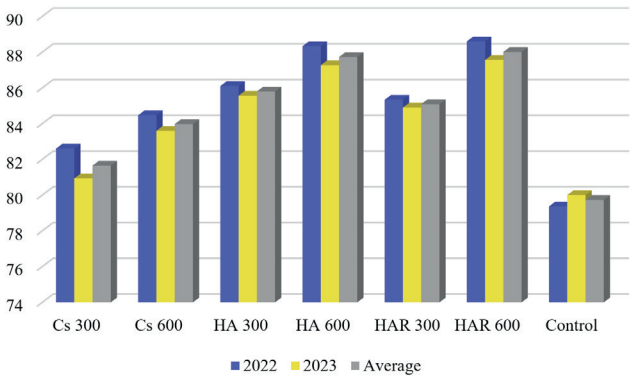


Fig. 3. Effect of foliar treatment with biostimulants on crude fibbers of canola seed, g kg^{-1} d.m.
*Cs – chitosan; HA – vermicompost extract;
HAR – humic complex with growth regulator
Source: Authors' own elaboration

The use of biostimulants in the treatment of rapeseed in the two studied years leads to an increase the crude fibre content compared to the control group and on the first year compared to the second year (Fig. 3). The changes in the

amount of crude fibre between the two years were not statistically significant. Low reliability of the increase in fibre content was found in the treatment with Cs 600 compared to Cs 300 (*P < 0.05) on the first year. The application of the biostimulants Cs 600 (*P < 0.05), HA 300 (*P < 0.05), HA 600 (**P < 0.01) and HAR 600 (**P < 0.01) significantly increases the crude fibre content in the first year compared to the control group, while with Cs 300 and HAR 300, the fibre content increases insignificantly. The used by Cs 600, HA 300 and HA 600 (*P < 0.05) led to an increase in crude fibre content in rapeseed with low reliability compared to the control group on the second year and with HAR 600 (**P < 0.01), while with Cs 300 and HAR 300 the differences were not statistically significant (Fig. 3).

Carré et al. (2016) found crude fibre content ranging from 27 to 44% dry matter in unbleached seeds and from 3–12% in peeled seeds. Sikorska et al. (2021) found an increased crude fibre content after treatment with biostimulants in rapeseed from 81 to 84 g kg^{-1} d.m, which in turn was close to the results obtained in our studies.

Rapeseed oil was recognized as one of the healthiest vegetable oils due to high content of MUFA (50–70%) – mostly oleic acid (C18:1) (~60%) and PUFA, mainly a-linolenic acid (7–10%) and linoleic acid (17–21%), as well as a low saturated fatty acids amount (5–7%). The ratio of omega-6 / omega-3 in rapeseed oil is about 2:1, which was considered optimal for consumption. They were characterized by oxidative stability, specific taste, good nutritional value and has anti-inflammatory, antioxidant and anticancer properties (Ghazani and Marangoni, 2013; Johnson et al, 2007; Sey et al., 2020; Chew, 2020; Goyal et al., 2021). The fatty acid composition was an important quality characteristic of rapeseed, as affects the nutritional and industrial oil properties. Rapeseed oil contains mainly unsaturated fatty acids, such as oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), which have a beneficial effect on human health, rapeseed oil was a high oleic acid and low linolenic acid, which determined and improved the stability and functional-

Table 1. Fatty acids (g/100 g fat) in rapeseed treated with biostimulants, n = 6

2022														
	Cs 300		Cs 600		HA 300		HA 600		HAR 300		HAR 600		Control	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
C-14:0	0.33	0.00	0.16	0.00	0.96	0.00	0.33	0.00	0.14	0.00	0.12	0.00	0.25	0.08
C-15:0	0.07	0.00	0.04	0.00	0.14	0.00	0.06	0.00	0.03	0.00	0.03	0.00	0.04	0.01
C-16:0	5.29	0.06	4.74	0.04	7.20	0.00	5.01	0.01	4.64	0.00	4.58	0.11	5.25	0.32
C-16:1n7	0.32	0.00	0.31	0.00	0.55	0.00	0.19	0.00	0.32	0.00	0.45	0.01	0.24	0.08
C-17iso	0.02	0.00	0.02	0.00	0.07	0.00	0.00	0.00	0.02	0.00	0.11	0.00	0.01	0.00
C-17aiso	0.09	0.00	0.07	0.00	0.12	0.00	0.06	0.00	0.08	0.00	0.06	0.00	0.05	0.02
C-17:0	0.09	0.00	0.04	0.00	0.14	0.00	0.10	0.00	0.09	0.00	0.09	0.00	0.08	0.03
C-17:1n7	0.16	0.00	0.11	0.00	0.16	0.00	0.16	0.00	0.08	0.00	0.15	0.00	0.13	0.04
C-18iso	0.10	0.00	0.08	0.00	0.09	0.00	0.06	0.00	0.00	0.00	0.07	0.00	0.06	0.02
C-18.0	1.51	0.02	1.26	0.01	2.38	0.00	1.92	0.01	1.29	0.00	1.50	0.03	1.81	0.09
C-18:1c9	63.64	0.43	66.65	1.11	60.24	0.03	65.63	0.19	65.47	0.02	65.98	0.22	65.40	1.03
C-18:2c9,12	19.43	0.22	18.12	0.17	18.54	0.01	17.33	0.05	19.00	0.01	18.55	0.74	17.97	0.93
gC-18:3n6	0.16	0.00	0.66	0.01	0.31	0.00	0.18	0.00	0.17	0.00	0.16	0.00	0.17	0.06
aC-18:3n3	7.49	0.09	7.43	0.07	7.15	0.00	6.75	0.02	7.11	0.00	6.87	0.16	6.49	0.09
C-22:1n9	0.89	0.01	0.05	0.00	0.91	0.00	0.70	0.41	1.03	0.00	1.12	0.03	1.04	0.34
C-20:5n3	0.04	0.00	0.04	0.00	0.06	0.00	0.29	0.00	0.06	0.00	0.10	0.00	0.14	0.05
SFA	7.41	0.07	6.32	0.06	11.17	0.00	7.57	0.02	4.96	0.00	6.39	0.14	7.52	0.27
MUFA	65.09	0.42	67.42	1.10	61.97	0.03	66.97	0.55	67.14	0.02	67.87	0.18	67.00	1.56
PUFA	27.13	0.31	26.35	0.25	26.11	0.01	24.59	0.07	26.40	0.01	25.71	0.57	24.79	0.73
Σn-3	7.53	0.09	7.57	0.07	7.20	0.00	7.07	0.02	7.23	0.00	6.99	0.16	6.66	0.14
Σn-6	19.60	0.23	18.78	0.18	18.91	0.01	17.51	0.05	19.17	0.01	18.71	0.74	18.14	0.87
Σn-6/Σn-3	2.60	0.17	2.48	0.25	2.62	0.23	2.48	0.12	2.65	0.21	2.68	0.17	2.73	0.19
Σ C-18:1cis-FA	63.64	0.43	66.65	1.11	60.24	0.03	65.63	0.19	65.47	0.02	65.98	0.22	65.40	1.03
BFA	0.25	0.00	0.22	0.00	0.56	0.00	0.18	0.00	1.42	0.00	0.26	0.01	0.17	0.06

Source: Authors' own elaboration

ity (Orsavova et al., 2015; McVetty and Duncan 2015; Spasibionek et al., 2020).

The experimental biostimulants reduced the content of saturated fatty acids compared with the control group of rapeseed seeds, with the exception of the treatment with HA 300 and HA 600 (HA 300/ HA 600 2022, ***P < 0.001), in which their concentration increased compared with the control group of seeds on the first year (Tables 1 and 3). The application of Cs 300, Cs 600, HAR 300 and HAR 600 reduced the amount of saturated fatty acids compared to the control group seeds. The treatment of rapeseed with Cs 300, HA 300 and HA 600 reduced the concentration of monounsaturated fatty acids compared to the control group on the first year, while no significant changes were found for the other stimulants. Polyunsaturated fatty acids increased compared to the control group rapeseed at the expense of a decreased in saturated fatty acids or monounsaturated compared to the control group with the exception of rapeseed treated with HA 600, in which they decreased slightly compared to the baseline group. The used biostimulants for rapeseed treatment led to a decreased in the accumulation of oleic acid in Cs 300 and HA 300. Linoleic acid in the studied variants increased compared to the control group with the exception of rapeseed treated with HA 600. Gamma linolenic acid had the highest value when using Cs 300 – 0.66 g/100 g fat. Alpha linolenic acid increased as a result of the treatment rapeseed with three types of stimulants at two different concentrations compared to the control. The fatty acid that determines the quality of rapeseed oil was erucic acid (C-22:1n9), which causes a serious toxic effect. The concentration in the control rapeseed was 1.04 g/100 g fat on the first year. Treatment of rapeseed with Cs 600 red uced the amount of erucic acid to 0.05 g/100 g fat and of HAR 600 to 0.70 g/100 g fat (table 1 and 3). Erucic acid was a natural plant toxin found in rapeseed and mustard oil (Hulan et al., 1976; Kramer et al., 1982; Wildt and Speijers, 1984; Russo et al., 2021). Epidemiological studies have shown that in regions where mustard oil was still used in a traditional way (raw, unrefined), there was a higher number of cardiovascular diseases (Rastogi et al., 2004). It adversely affects the heart tissue (FSANZ, 2003),

Table 2. Fatty acids (g/100 g fat) in rapeseed treated with biostimulants, n = 6

2023														
	Cs 300		Cs 600		HA 300		HA 600		HAR 300		HAR 600		Control	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
C-14:0	0.16	0.00	0.13	0.20	0.62	0.00	0.40	0.01	0.19	0.15	0.32	0.00	0.17	0.00
C-15:0	0.03	0.00	3.51	0.00	0.08	0.01	0.06	0.00	0.03	0.02	0.04	0.01	0.04	0.00
C-16:0	4.65	0.32	1.89	0.18	6.06	0.01	5.46	0.11	3.41	0.13	5.13	0.01	4.71	0.01
C-16:1n7	0.21	0.05	0.12	0.02	0.25	0.00	0.32	0.01	0.18	0.01	0.25	0.00	0.28	0.00
C-17iso	0.02	0.00	0.04	0.00	0.03	0.00	0.04	0.00	0.04	0.03	0.00	0.00	0.03	0.00
C-17aiso	0.05	0.00	0.06	0.01	0.06	0.00	0.04	0.00	0.06	0.02	0.05	0.00	0.06	0.00
C-17:0	0.04	0.00	0.07	0.02	0.05	0.00	0.05	0.00	0.05	0.01	0.06	0.00	0.04	0.00
C-17:1n7	0.04	0.00	1.10	0.20	0.07	0.00	0.06	0.00	0.07	0.02	0.05	0.00	0.04	0.00
C-18iso	0.06	0.00	0.12	0.04	0.07	0.00	0.06	0.00	0.17	0.02	0.07	0.00	0.07	0.00
C-18.0	1.45	0.01	1.35	0.23	1.88	0.00	1.82	0.04	1.32	0.02	1.35	0.07	1.44	0.00
C-18:1c9	65.26	0.26	63.06	0.13	62.93	0.08	64.73	1.09	62.17	0.92	62.17	0.92	65.10	0.10
C-18:2c9.12	17.81	0.07	18.19	0.04	17.65	0.02	17.44	0.34	18.88	0.31	18.88	0.31	17.70	0.03
gC-18:3n6	0.04	0.00	0.04	0.02	0.03	0.00	0.04	0.00	0.13	0.05	0.12	0.03	0.12	0.00
aC-18:3n3	8.08	0.03	8.88	0.02	8.22	0.01	7.83	0.15	9.29	0.66	9.29	0.46	8.14	0.01
C-22:1n9	1.30	0.01	0.45	0.05	1.22	0.00	1.26	0.02	0.74	0.05	0.74	0.05	1.34	0.00
C-20:5n3	0.17	0.00	0.06	0.02	0.12	0.00	0.13	0.00	0.07	0.03	0.07	0.01	0.13	0.00
SFA	6.73	0.59	7.35	0.16	8.92	0.31	7.93	0.15	8.20	2.30	7.06	0.06	6.54	0.03
MUFA	67.09	0.27	64.94	0.22	64.69	0.08	66.56	1.05	63.34	0.81	63.37	0.79	67.03	0.10
PUFA	26.11	0.11	27.25	0.06	26.03	0.03	25.46	0.49	28.44	1.01	28.42	0.99	26.11	0.04
Σn-3	8.27	0.03	9.00	0.02	8.34	0.01	7.97	0.15	9.43	0.71	9.43	0.71	8.28	0.01
Σn-6	17.84	0.07	18.25	0.07	17.68	0.02	17.49	0.34	19.01	0.34	19.00	0.33	17.83	0.03
Σn-6/Σn-3	2.16	0.00	2.03	0.01	2.12	0.00	2.19	0.00	2.02	0.12	2.02	0.12	2.15	0.00
Σ C-18:1cis-FA	65.26	0.26	63.06	0.13	62.93	0.08	64.73	1.09	62.17	0.92	62.17	0.92	65.10	0.10
BFA	0.16	0.00	0.35	0.07	0.26	0.00	0.20	0.00	0.37	0.04	0.20	0.00	0.21	0.00

Source: Authors' own elaboration

Table 3. Significant changes in fatty acid composition in rapeseed treated with biostimulants during the two years, n = 6

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
C-14:0	***		***	**		***		***	***	*		***	
C-15:0	***		**	*				***	***	*		*	
C-16:0	***		***	*		*		***	***			**	
C-16:1n7	***		***	***		***			***	**		**	
C-17iso	***	***	***	***		***	*	***	***	***	***	***	
C-17aiso	***		***	***		**		***	***	**		***	
C-17:0	***		***	***	**	***		***	***	*			
C-17:1n7	***		***	***		***		***	***	***		**	
C-18iso	***		***		**	*		***	***	***		*	
C-18.0	*		***	*		*	*	***	***	**			
C-18:1c9	**	*	***		*	*		*	***		***		
C-18:2c9,12	**		***					**	***		**		
gC-18:3n6	***	***	***	***				***	***	*		**	
aC-18:3n3	**	***	***	**	*	*	***		***		***	*	
C-22:1n9	***		***					***		*			
C-20:5n3	***		***	***				***	***	***	**	*	
SFA		***	***	*		**	*	***	***	**		**	
MUFA	**	*	***		*	**		*	**	*	***		
PUFA	*	*	*			*		*	***		***		
Σn-3	**	***	***	**	*	*	**		**		***	*	
Σn-6	**	*	***					**	***		**		
Σn-6/Σn-3	***	***	***	***	**	**	*	***	***		**	***	
Σ C-18:1cis-FA	**	*	***		*	*		*	***		***		
BFA	***		***	**		**		***	***	***	*	***	

I – Cs 300 2022/2023; II – Cs 600 2022/2023; III – HA 300 2022/2023; IV – HA 600 2022/2023; V – HAR 300 2022/2023; VI – HAR 600 2022/2023; VII – Control 2022/2023; VIII – Cs 300/ Cs 600 2022; IX – HA 300/ HA 600 2022; X – HAR 300/HAR 600 2022; XI – Cs 300/ Cs 600 2023; XII – HA 300/ HA 600 2023; XIII – HAR 300/HAR 600 2023; *P < 0.05; **P < 0.01; ***P < 0.001

Source: Authors' own elaboration

was absorbed by the myocardial tissue and was not metabolized (Kramer et al., 1982). According to Regulation No. 1881/2006 of the EC, permissible concentrations of erucic acid in oils were 50 g/kg and in baby foods up to 10 g/kg.

Application of the studied biostimulant doses in the second year of rapeseed reduced the content of saturated fatty acids (Tables 2 and 3) due to an increase in polyunsaturated fatty acid concentration. The control group was characterized by a lower content of saturated fatty acids – 6.54 g/100 g fat (*P < 0.05), the concentration of monounsaturated fatty acids was preserved – 67.03 g/100 g fat and polyunsaturated fatty acids increase to 26.11 g/100 g fat. On the second year, the amount of saturated fatty acids was preserved when using HA 600 and their decrease was established in Cs 300 and HA 300, while in the other variants of treatment with biostimulants they increase significantly. Monounsaturated fatty acids increase significantly when treating rapeseed with Cs 300 and HA 300 on the second year and decrease significantly by Cs 600, HAR 300 and HAR 600, while in HA 600 they did not change significantly (Tables 2 and 3). Polyunsaturated fatty acids significantly decreased on the second year when rapeseed was treated with Cs 300, HA 300 and significantly increased with Cs 600 and HAR 600.

The quality indicators of rapeseed oil during the study period improved as a result of treatment with various biostimulants and depending on environmental factors. On the second year of rapeseed cultivation, were obtained lower values for the lipid preventive score, atherogenic and thrombogenic index in the control group and higher for the hypo-hypercholesterolemic index (Tables 4 and 5). The application of HAR 300 on the first year led to the best results for the lipid preventive score – 12.79, while the treatment with HAR 600 had the lowest values for the thrombogenic (0.09) and atherogenic (0.05) index and the highest hypo-hypercholesterolemic index – 19.49, while in Cs 600 the content of saturated fatty acids was the lowest compared to the control group – 2.72 g/100g product (Table 4). The lipid preventive score on the second year was lowest by Cs 300 – 13.64, the atherogenic index when using HAR 300 – 0.05, the thrombogenic index at Cs 600 – 0.05, the highest hypo-hypercholesterolemic index –

Table 4. Quality parameters of lipid fraction on the first year

2022														
	Cs 300		Cs 600		HA 300		HA 600		HAR 300		HAR 600		Control	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
LPS	15.15	0.20	13.77	0.21	20.72	0.21	15.29	0.10	12.79	0.40	14.34	0.06	16.02	0.08
AI	0.07	0.01	0.06	0.04	0.13	0.03	0.07	0.03	0.06	0.01	0.05	0.01	0.07	0.02
TI	0.11	0.04	0.09	0.01	0.16	0.01	0.11	0.02	0.12	0.02	0.09	0.01	0.11	0.02
h/H	16.16	0.53	18.98	0.26	10.58	0.58	16.92	0.92	19.24	0.21	19.49	0.34	16.43	0.43
SFA (g/ 100 g product)	3.10	0.21	2.72	0.16	4.89	0.35	3.23	0.27	3.03	0.17	2.86	0.11	3.38	0.26

Source: Authors' own elaboration

Table 5. Quality parameters of lipid fraction on the second year

2023														
	Cs 300		Cs 600		HA 300		HA 600		HAR 300		HAR 600		Control	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
LPS	13.64	0.06	15.04	0.09	16.95	0.04	15.99	0.07	17.01	0.08	16.10	0.14	14.26	0.06
AI	0.06	0.01	0.03	0.01	0.10	0.01	0.08	0.02	0.05	0.01	0.07	0.01	0.06	0.01
TI	0.09	0.03	0.05	0.02	0.13	0.03	0.11	0.01	0.11	0.03	0.10	0.03	0.09	0.02
h/H	19.02	0.02	44.64	0.08	13.33	0.33	15.43	0.97	25.21	0.69	16.61	0.21	18.71	0.69
SFA (g/ 100 g product)	2.75	0.09	3.06	0.12	3.77	0.25	3.47	0.15	3.59	0.23	3.11	0.22	2.83	0.19

Source: Authors' own elaboration

44.64 at Cs 600, and the lowest content of saturated fatty acids in product with Cs 300 – 2.75 g/100g product (Table 5).

Conclusions

The use of biostimulants on the first year of treatment leads to a decrease a decrease in fat content, while on the second an increase was found in treatment with HA 600, HAR 300 and HAR 600 compared to the control group. Crude protein synthesis increases after treatment with the used types of biostimulants (***$P < 0.001$) compared to the control group, with the exception of the application of HA 600, in which the changes compared to the control group of rapeseed were low reliable. On the second year of application biostimulants, crude protein synthesis was maintained as in the first, but in lower concentrations. The used biostimulants for treatment of rapeseed in the two studied years leads to an increase in the content of crude fibre compared to the control group and on the first year compared to the second year. The application of Cs 300, Cs 600, HAR 300 and HAR 600 reduces the amount of saturated fatty acids compared to the control group of seeds on the first year. Saturated fatty acids decreased on the second year in all variants. Lower values were obtained for the lipid preventive score, atherogenic and thrombogenic index and higher values for the hypo-hypercholesterolemic index on the second year of rapeseed cultivation by biostimulants treatment. Within the framework of the conducted research, the tested experimental biostimulants, developed on the basis of vermicompost and chitosan, have shown effectiveness in improving both the chemical-technological characteristics of the seeds and the biological value of the production, with the changes also being determined by the agro-ecological conditions of the harvest year. The obtained results reveal the potential of the developed products for application in ecological production practices.

Acknowledgements

The research was conducted with the financial support of the National Science Fund, Ministry of Education and Science, Bulgaria, within the implementation of the project "Use of biostimulants in biological crop cultivation-assessment of the contributions to bioeconomy", Contract SNF№ KP-06-N46/6, 27.11.2020.

Conflict of interest

No conflict of interest exists.

References

Ahmad, G., Jan, A., Arif, M., Jan, M. T. & Khattak, R. A. (2007). Influence of nitrogen and sulfur fertilization on quality of canola (*Brassica napus* L.) under rainfed conditions. *J. Shejiang Univ. Sci. B., 8*(10), 731–737. https://doi.org/10.1631/jzus.2007.B0731

Ahmad, W., Zia, M. H., Malhi, S. S., Niaz, A. & Saifullah, U. (2012). Boron defciency in soils and crops: A review. *Crop Plant*, 1, 77–114. https://doi.org/10.5772/36702

Anwar, M. M., Ali, S. E. & Nasr, E. H. (2015). Improving the nutritional value of canola seed by gamma irradiation. *J. Radiat. Res. Appl. Sci.*, *8*(3), 328–333. https://doi.org/10.1016/j.jrras.2015.05.007

Barthet, V. J. & Daun, J. K. (2011). 5-Seed morphology, composition, and quality. In: *Canola*; Daun, J. K., Eskin, N. A. M., Hickling, D., Eds., AOCS Press: Hoboken, NJ, USA, 119–162. ISBN 978-0-9818936-5-5

Bulgari, R., Franzoni, G. & Ferrante, A. (2019). Biostimulants application in horticultural Crops under abiotic stress conditions. *Agronomy, 9*(6), 306. https://doi.org/10.3390/agronomy9060306

Bligh, E.G. & Dyer, W. J. (1959).A rapid method of total lipid extraction and purification. *J. Biochem. Physiol., 37*, 911–917.

Carré, P., Citeau, M., Robin, G. & Estorges, M. (2016). Hull content and chemical composition of whole seeds, hulls and germs in cultivars of rapeseed (*Brassica napus*). *OCL*, *23*(3), A302. https://doi.org/10.1051/ocl/2016013

Chew, S. C. (2020). Cold-pressed rapeseed (*Brassica napus*) oil: Chemistry and functionality. *Food Res. Int.*, *131*, 108997

Chew, S. C. (2020). Chapter 7–cold pressed rapeseed (*Brassica napus*) oil. M.F. Ramadan (Ed.), Cold pressed oils. *Academic Press*, 65–80. 10.1016/B978-0-12-818188-1.00007-4

Chwil, S. (2016). The effect of foliar feeding under different soil fertilization conditions on the yield structure and quality of winter oilseed rape (*Brassica napus L.*). *EJPAU*, *19*(3).

COMMISSION REGULATION (EU) amending Regulation (EC) № 1881/2006 as regards maximum levels for erucic acid in vegetable oils and fats and in foods containing vegetable oils and fats, Council of the European Union Brussels, 12 March 2014. (OR. En) 7650/14 DENLEG 65 AGRI 207.

FSANZ–Food Standards Australia New Zealand. (2003). Erucic acid in food: A toxicological review and risk assessment. *Technical report series, No. 21*, Page 4, paragraph 1. ISBN 0- 642-34526-0, ISSN 1448-3017.

Ghazani, S. M. & Marangoni, A. G. (2013). Minor components in canola oil and effects of refining on these constituents: A review. *J. Am. Oil Chem. Soc.*, *90*(7), 923–932. https://doi.org/10.1007/s11746-013-2254-8

Goyal, A., Tanwar, B., Sihag, M. K., Kumar, V., Sharma, V. & Soni, S. (2021). Rapeseed/Canola (*Brassica napus*) Seed. In *Oilseeds: Health Attributes and Food Applications*; Tanwar, B., Goyal, A., Eds.; *Springer*: Singapore, 47–71. ISBN 9789811541940.

Gugała, M., Sikorska, A., Zarzecka, K., Kapela, K. & Mystkowska, I. (2018). The effect of biostimulators on the content of crude oil and total protein in winter oilseed rape (*Brassica napus* L.) seeds. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *69*(2), 121–125. https://doi.org/10.1080/09064710.2018.1513063

Guirrou, I., El Harrak, A., El Antari, A., Hssaini, L., Hanine, H., El Fechtali, M. & Nabloussi, A. (2023). Bioactive compounds assessment in six Moroccan rapeseed (*Brassica napus* L.) varieties grown in two contrasting environments. *Agronomy*, *13*(2), 460. https://doi.org/10.3390/agronomy13020460. https://doi.org/10.1016/j.foodres.2020.108997

Hulan, H. W., Kramer, J. K., Mahadevan, S. & Sauer, F. D. (1976). Relationship between erucic acid and myocardial changes in male rats. *Lipids, 11*(1), 9–15. doi:10.1007/BF02532578

Jankowski, K. J., Sokólski, M., Dubis, B., Krzebietke, S., Żarczyński, P., Hulanicki, P. & Hulanicki, P. S. (2016). Yield and quality of winter oilseed rape (*Brassica napus* L.) seeds in response to foliar application of boron. *Agricultural and Food Science*, *25*(3), 164–176. https://doi.org/10.23986/afsci.57413

Johnson, G. H., Keast, D. R. & Kris-Etherton, P. M. (2007). Dietary modeling shows that the substitution of canola oil for fats commonly used in the united states would increase compliance with dietary recommendations for fatty acids. *J. Am. Diet. Assoc.*, *107*, 1726–1734. https://doi.org/10.1016/j.jada.2007.07.015

Kocira, S. (2019). Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chilean journal of agricultural research, 79*(1), 17–25. http://dx.doi.org/10.4067/S0718-58392019000100017

Kramer, J. K., Farnworth, E. R., Thompson, B. K., Corner, A. H. & Trenholm, H. L. (1982). Reduction of myocardial necrosis in male albino rats by manipulation of dietary fatty acid levels. *Lipids*, *17*(5), 372–382. DOI: 10.1007/BF02535197

Laurent, E. A., Lamaze, T., Grieu, P., Durieu, C. & Ahmed, N. (2018). Marine and fungal biostimulants (DPI4913 and AF086 Extracts) improve grain yield, plant nitrogen absorption and allocation to ear in durum wheat plants. 15. ESA Congress, Aug 2018, Genève, Switzerland, 180. hal-02735684.

Li, N., Qi, G., Sun, X. S., Stamm, M. J. & Wang, D. (2012). Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite. *J. Am. Oil Chem. Soc.*, *89*(5), 897–908. https://doi.org/10.1007/s11746-011-1977-7

Malhi, S. S., Gan, Y. & Raney, J. P. (2006). Yield, seed quality, and sulfur uptake of Brassica oilseed crops in response to sulphur fertilization. *Agron. J.*, *99*(2), 570–577. https://doi.org/10.2134/agronj2006.0269

McVetty, P. B. E. & Duncan, R. W. (2015). Canola, rapeseed and mustard: for biofuels and bioproducts. In *Industrial crops: breeding for bioenergy and eioproducts*; Cruz, V.M.V., Dierig, D.A., Eds.; Handbook of Plant Breeding; Springer: New York, NY, USA, 133–156. ISBN 978-1-4939-1447-0.

Mohtashami, R., Movahhedi Dehnavi, M., Balouchi, H. & Faraji, H. (2020). Improving yield, oil content and water productivity of dryland canola by supplementary irrigation and selenium spraying. *Agric. Water Manag*, *232*, 106046. https://doi.org/10.1016/j.agwat.2020.106046

Neshev, N., Balabanova, D., Yanev, M., Mitkov, A., Tonev, T. & Vasilev, A. (2021). Study on biostimulant application at oilseed rape damaged by simulated herbicide drift. *Bulg. J. Agric. Sci., 27*(Suppl. 1), 155–160.

Nowak, R., Szczepanek, M., Błaszczyk, K., Kobus-Cisowska, J., Przybylska-Balcerek, A., Stuper-Szablewska, K., Poberężny, J., Hassanpouraghdam, M. B. & Rasouli, F. (2023). Impact of the farming system and amino-acid biostimulants on the content of carotenoids, fatty acids, and polyphenols in alter-

native and common barley genotypes. *Agronomy, 13*(7), 1852. https://doi.org/10.3390/agronomy13071852

Orsavova, J., Misurcova, L., Vavra Ambrozova, J., Vicha, R. & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *Int. J. Mol.Sci.*, *16*(6), 12871–12890. https://doi.org/10.3390/ijms160612871

Osvalde, A., Karlsons, A., Cekstere, G., Āboliņa, L. & Malecka, S. (2024). Foliar application of commercial humic substances for possible increase of nutrient status and yield of oilseed rape. *Environmental and Experimental Biology*, *22*(1), 1–9. https://doi.org/10.22364/eeb.22.01

Petrie, J. R., Zhou, X.-R., Leonforte, A., McAllister, J., Shrestha, P., Kennedy, Y., Belide, S., Buzza, G., Gororo, N. & Gao, W. (2020). Development of a *Brassica napus* (Canola) crop containing fish oil-like levels of DHA in the seed oil. *Front. Plant Sci.* 2020, *11*, 727. https://doi.org/10.3389/fpls.2020.00727

Petrova, I., Ivanova, S., Stoyanova, S., Mincheva, R. & Pavlova, M. (2023). Influence of Biostimulants and Humic Extracts Treatment on the Fatty Acid Profile of the Spring Oilseed Rape Variety. *J. Agric. Sci. Technol.*, *15*(1), 52–59.

Rastogi, T, Reddy, K. S., Vaz, M., Spiegelman, D., Prabhakaran, D., Willett, W. C., Stampfer, M. J. & Ascherio, A. (2004). Diet and risk of ischemic heart disease in India. *American Journal of Clinical Nutrition*, *79*(4), 582–592.

Russo, M., Yan, F., Stier, A., Klasen, L. & Honermeier, B. (2021). Erucic acid concentration of rapeseed (Brassica napus L.) oils on the German food retail market. *Food Science and Nutrition*, *9*(7), 3664–3672. DOI: 10.1002/fsn3.2327

Sey, A. A., Pham, T.H., Kavanagh, V., Kaur, S., Cheema, M., Galagedara, L. & Thomas, R. (2020). Canola Produced under Boreal Climatic Conditions in Newfoundland and Labrador Have a Unique Lipid Composition and Expeller Press Extraction Retained the Composition for Commercial Use. *J. Adv. Res.*, *24*, 423–434. https://doi.org/10.1016/j.jare.2020.05.002

Shanin, Y. (1977). Methodology of Polish Experience. In: Block Method. *Zemizdat*, Sofia, 65–73 (Bg).

Sikorska, A., Gugała, M. & Zarzecka, K. (2021). The response of different kinds of rapeseed cultivars to foliar application of nitrogen, sulphur and boron. *Sci Rep., 11*(1), 21102. https://doi.org/10.1038/s41598-021-00639-2

Spasibionek, S., Mikołajczyk, K., Ćwiek–Kupczyńska, H., Piętka, T., Krótka, K., Matuszczak, M., Nowakowska, J., Michalski, K. & Bartkowiak-Broda, I. (2020). Marker assisted selection of new high oleic and low linolenic winter oilseed rape (*Brassica napus* L.) inbred lines revealing good agricultural value. *PLoS ONE*, *15*, e0233959. https://doi.org/10.1371/journal.pone.0233959

Szczepanek, M. & Siwik-Ziomek, A. (2019). P and K accumulation by rapeseed as affected by biostimulant under different NPK and S fertilization doses. *Agronomy, 9*(9), 477. https://doi.org/10.3390/agronomy9090477

Ulbricht, T. L. & Southgate, D.A. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, *338*, 985–992.

Wildt, D. J. & Speijers, G. J. (1984). Influence of dietary rapeseed oil and erucic acid upon myocardial performance and hemodynamics in rats. *Toxicology and Applied Pharmacology*, *74*(1), 99–108. DOI: 10.1016/0041-008X(84)90275-8

Zhang, X., Yin, J., Ma, Y., Peng, Y., Fenton, O. & Wang, W. (2024). Unlocking the potential of biostimulants derived from organic waste and by-product sources: improving plant growth and tolerance to abiotic stresses in agriculture. *Environ. Technol. Innov., 34*(2024), Article 103571. 10.1016/j.eti.2024.103571

Received: **December, 22, 2024**; *Approved*: **February, 15, 2025**; *Published*: **October, 2026**