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EFFECT OF ELICITATION WITH SODIUM SILICATE AND IRON CHELATE ON THE COMPOSITION AND QUALITY OF FATTY ACIDS IN BUCKWHEAT SPROUTS*

Henryk Dębski, Marcin Horbowicz**Institute of Biological Sciences****Siedlce University of Natural Sciences and Humanities, Siedlce, Poland**

ABSTRACT

In laboratory experiments, the impact of elicitation with a mixture of sodium silicate and iron chelate or sodium silicate alone on fatty acid composition in sprouts of common buckwheat (*Fagopyrum esculentum* Moench) has been examined. Buckwheat seeds and then sprouts were soaked for 0.5 h each day over six days in an aqueous solution of the mentioned elicitors. The sprouts were grown in the light regime of 16/8 h, day/night. After seven days of germination (sprouting), the composition of fatty acids in freeze-dried and powdered sprout tissues was analyzed by gas chromatography. The results showed significant changes in the composition of fatty acids in sprouts in comparison to buckwheat seeds. The results indicate that the germination of buckwheat seeds leads to an increase in myristic (C14:0), palmitic (C16:0), stearic (C18:0) and behenic (C20:0) acid in sprouts, although the use of the elicitors, especially sodium silicate, inhibited this tendency. The sprouts of common buckwheat contained almost twice as much α -linolenic acid (C18:3) as the seeds did, and slightly less linoleic acid (C18:2). Moreover, the use of elicitors favored an increased accumulation of α -C18:3. As a result, germination drastically reduced the C18:2/ α -C18:3 ratio by about 4 times, which may have a positive health effect. The buckwheat sprouts contained traces of eicosatrienoic (C20:3), eicosatetraenoic (C20:4), docosaenoic (C22:1) and docosadienoic (C22:2) acids, while their content in the seeds was between 0.6 and 2.4%. The buckwheat sprouts had a higher atherogenic (AI) and thrombogenic index (TI) and a lower health index (HI) than seeds did, but the use of elicitors inhibited this tendency.

Keywords: common buckwheat, sprouts, elicitation, iron chelate, sodium silicate, fatty acids.

Marcin Horbowicz, Professor, Institute of Biological Sciences, Siedlce University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland, e-mail: mhorbowicz@uph.edu.pl

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INTRODUCTION

Plant sprouts are an important source of protein, minerals, dietary fiber, and vitamins in human diet (BENINCASA et al. 2019). During the germination of common buckwheat (*Fagopyrum esculentum* Moench), there is rapid accumulation of phenolic compounds in sprouts (KOYAMA et al. 2013, WICZKOWSKI et al. 2014). The content of crude protein, sugar, flavonoids and condensed tannins also increases, while the level of fat, phytic acid and activity of trypsin inhibitors decreases (ZHANG et al. 2015).

In buckwheat seeds, unsaturated fatty acids prevailed over saturated ones (BONAFACCIA et al. 2003). In buckwheat seeds of Turkish origin, oleic (C18:1), linoleic (C18:2) and palmitic acid (C16:0) are the major fatty acids, making up 33.1, 31.93 and 13.15% of total amount, respectively (GULPINAR et al. 2012). The same fatty acids were quantitatively prevalent in buckwheat seeds of American cultivars and average percentages of these three fatty acids in the total lipids were 36.3, 37.0 and 14.0 %, respectively (MAZZA 1988). Also, palmitic acid was the major saturated fatty acid, and oleic and linoleic acids were the major unsaturated fatty acids in buckwheat seeds of Korean and Chinese cultivars (CHO et al. 2016) and in Polish cultivars (DZIADEK et al. 2016). It is interesting that 60-80% fatty acids during the onset of buckwheat seeds' maturation were saturated ones, mostly represented by palmitic acid, but at the end of maturation, 65-80% acids were unsaturated, mostly linoleic and oleic acids (HORBOWICZ, OBENDORF 1992). This transition was associated with the rapid embryo growth and a 10-fold increase in storage lipid accumulation.

Germination process enhanced the nutritional value of fatty acids in buckwheat sprouts owing to the increase in the amount of α -linolenic acid (C18:3) (ZHANG et al. 2015). During the germination of Tartary buckwheat, the content of linoleic acid (C18:2) decreased, but palmitic (C16:0), stearic (C18:0) and eicosanoic acids (C20:0) became more abundant (YIMING et al. 2015). However, according to KIM et al. (2005), the content of C18:0 and oleic acid decreased whereas that of C18:2 and C18:3 increased after 7-day sprouting of common buckwheat.

Apart from the content and composition of fatty acids, indicators of their overall quality are important. Such indicators are the thrombogenicity index (IT), atherogenicity index (AI) and health index (HI). The IT is an indicator of the tendency for thrombotic formation in blood vessels, and the IA indicates the relationship between the sum of saturated fatty acids and that of unsaturated ones (ULBRICHT, SOUTHGATE 1991, SINYAVSKIY et al. 2016). It describes the ratio of prothrombogenic (saturated fatty acids) and anti-thrombogenic (mono- and polyunsaturated fatty acids).

The health index (HI) shows the ratio of the sum of polyunsaturated and monounsaturated fatty acids to saturated fatty acids, and the HI higher than 7 is found in vegetable oils (IOSYPENKO et al. 2019). Animal fats, which are

characterized by a low content of unsaturated fatty acids, have a health index of less than 2.

Elicitors promote plant defence reaction and synthesis of phytoalexin (BAENAS et al. 2014). The beneficial role of silicon (Si) in stimulating the growth and development of plants, and effectively mitigate various abiotic stresses, has been generally recognized (SIVANESAN, JEONG 2014, LUYCKX et al. 2017). LIANG et al. (2007) have shown that the addition of Si to the tissue culture medium enhances secondary metabolites production, and tolerance to both biotic and abiotic stresses. It has been demonstrated that the exogenous silicon treatment enhance the production of secondary metabolites in cucumber (DRAGISIC MAKSIMOVIC et al. 2007).

The sprouts of buckwheat are a rich source of flavonoids and phenolic acids, and scientific reports on the influence of various factors on the composition of phenolic compounds in buckwheat sprouts are numerous (KIM et al. 2004, WICZKOWSKI et al. 2014). Only a few published papers concern the content of fatty acids in sprouts of common buckwheat (KIM et al. 2005, ZHANG et al. 2015). There are no data regarding the influence of Si and iron chelate (Fe-EDTA) on the fatty acid composition of buckwheat sprouts in the available literature. Therefore, in this study, we evaluate the influence of these elicitors on the composition of fatty acids in buckwheat sprouts.

MATERIAL AND METHODS

Initially, four samples of surface-sterilized seeds of common buckwheat (100 -150 each, cv. Hruszowska) were soaked at 24°C in distilled water for 4 hours. Then, the wet seeds were placed on a layer of sterilized and moist cotton gauze stretched over an open 330 mL jar. The sprouts of buckwheat were then grown for 7 days at 20±1/16±2°C (day/night, 16/8 h) in a light flux 100 - 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (400 W high pressure sodium lamps, Osram, Germany). During the first six days, seeds and sprouts were soaked in distilled water (control) or elicitor solutions. The soaking lasting 15 min was carried out twice each day, at 9 am and 5 pm. After each treatment, the seeds were placed back on the gauze layer. On the seventh day, the sprouts were collected, cut into 2-3 mm pieces and freeze-dried in a laboratory freeze dryer (Alpha 1-2 ld plus, Martin Christ, Germany) for 48 h, and used for analyses of fatty acids.

The elicitors used in the study were solutions contained sodium silicate (Na_2SiO_3 , POCH, Poland), or a formulation containing sodium silicate and Fe-EDTA chelate (Optysil Eko, Interemag, Poland). The seed and sprout control samples were soaked in water, while three other sets of buckwheat seeds and sprouts were soaked in 4 mM of Na_2SiO_3 , or a mixture of 0.25 mM Fe-EDTA and 2 mM of Na_2SiO_3 , or of 0.5 mM Fe-EDTA and 4 mM of Na_2SiO_3 , respectively.

Fatty acids were extracted with potassium hydroxide-methanol solution, which simultaneously combined extraction and saponification steps (HORBOWICZ, OBENDORF 1992). Briefly, samples of freeze dried and pulverized buckwheat tissues were homogenized in a mortar with methanol. After grinding, 0.1 M potassium hydroxide in methanol was added, and the mixture was heated for 2 h at 80°C (heating block, Lab-Line Instruments, USA) to facilitate extraction and saponification. After the mixture cooled, saturated solution of sodium chloride was added, and the mixture was extracted twice with hexane. The hexane and methanol-water layers were separated by centrifugation at 1000 g for 5 min, and the hexane fractions were discarded. The methanol-water layer was acidified with concentrated hydrochloric acid and extracted twice, each time with hexane. Hexane fractions were pooled, and hexane was evaporated in a stream of nitrogen. The fatty acids were subjected to methylation with a mixture of methanol, toluene, and sulphuric acid (80:20:2, v/v/v). For analyses, potassium hydroxide, toluene, sulphuric acid, hydrochloric acid and sodium chloride were purchased from POCH (Poland), while methanol and hexane from Merck (Germany) and Sigma-Aldrich (USA), respectively.

For analysis of fatty acids methyl esters (FAME), a Shimadzu GC 2010 Pro gas chromatograph (Japan) with a flame ionization detector (FID) was used. FAME were analyzed on a Fused Silica Carbowax Column, 30 m × 0.25 mm × 0.25 µm (Quadrex, USA). Separation and detection were performed under the following conditions: column oven temperature was raised from 80 to 230°C at a heating rate of 4°C min⁻¹ and then held isothermally at 230°C; injector temp. was 220°C, detector (FID) 250°C and injection volume 1 µL (split mode). The carrier gas was helium (He) at a total flow rate of 12 mL min⁻¹. The detector gases were hydrogen at 40 mL min⁻¹, air at 400 mL min⁻¹, and nitrogen (auxiliary gas) at 30 mL min⁻¹. Identification of fatty acids was carried out using a reference standard mixture of methyl esters (Supelco 37 Component FAME Mix, USA). The content of each of fatty acid was expressed as % of total fatty acids content.

The atherogenicity index (AI) and thrombogenicity indices (TI) were calculated from the equations proposed by ULBRICHT and SOUTHGATE (1991).

$$AI = [C12:0 + 4 \times (C14:0) + C16:0] / (MUFAs + PUFAs);$$

$$TI = [C14:0 + C16:0 + C18:0] / [0.5 \times MUFAs + 0.5 (FAs n-6) + 3 \times (FAs n-3) + (FAs n-3/FAs n-6)];$$

HI = [MUFAs + PUFAs]/SFAs was calculated according to BONAFACCIA et al. (2003) and KRUMINA-ZEMTURE, BEITANE (2017).

In these equations, MUFAs means monounsaturated fatty acids, PUFAs means polyunsaturated fatty acids, SFAs saturated fatty acids and FAs fatty acid.

Analyses of sprout and seed tissue were performed in three replicates. One-way analysis of variance (ANOVA) and the Tukey's post hoc test were used to check the significance of differences between seed and sprout tissues.

RESULTS AND DISCUSSION

Analysis of buckwheat seeds indicated the presence of saturated fatty acids (SFAs): caproic, palmitic, stearic, eicosanoic, docosanoic and tetracosanoic ones (C6:0; C16:0, C18:0; C20:0; C22:0 and C24:0, respectively) – Table 1.

Table 1

Composition of saturated fatty acids (% of total) in seeds and sprouts of common buckwheat

Fatty acid	Seeds	Sprouts, control	Sprouts + 0.25 mM Fe-EDTA + 2 mM Na ₂ SiO ₃	Sprouts +0.50 mM Fe-EDTA + 4 mM Na ₂ SiO ₃	Sprouts + 4 mM Na ₂ SiO ₃
C6:0	3.2± 0.3 ^{a*}	2.0±0.1 ^b	2.1±0.1 ^b	3.3±0.1 ^a	2.9±0.1 ^a
C14:0	<LOD	0.3±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.6±0.1 ^a
C16:0	10.2±0.2 ^c	15.7±0.3 ^a	11.9±0.3 ^b	12.3±0.1 ^b	11.6±0.1 ^b
C18:0	0.9±0.1 ^b	1.3±0.1 ^a	1.5±0.1 ^a	1.3±0.1 ^a	1.3±0.1 ^a
C20:0	5.4±0.5 ^{cd}	8.1±0.1 ^b	9.7±0.1 ^a	5.6±0.1 ^c	4.5±0.1 ^d
C22:0	0.9±0.1 ^b	2.1±0.1 ^a	2.0±0.2 ^a	1.5±0.1 ^a	0.8±0.1 ^b
C24:0	0.8±0.1 ^{bc}	0.3±0.1 ^c	0.8±0.1 ^{bc}	1.2±0.1 ^{ab}	0.6±0.1 ^c

* Results of means marked with the same letter were not significantly different at $P<0.05$ (Tukey's post hoc test). Comparisons were made within each fatty acid separately. LOD (limit of determination) = 0.1%.

The most abundant SFAs were C16:0 (10.2%), and C20:0 (5.4%). According to an earlier Polish study, the contribution of C16:0 in the total fatty acids in seeds of four buckwheat cultivars reached 14 - 15.5% (DZIADEK et al. 2016). Also data published by MAZZA (1988) and CHO et al. (2016) indicate that the content of C16:0 in the total fatty acid of buckwheat seeds reached 13.4 - 14.6% and 15 - 16.1%, respectively. In our analyses, the C20:0 content was 5.4%, which was much higher than the mentioned Polish, Korean and American reports, in which the share of this acid was between 0.5 and 1.8%.

Among the monounsaturated fatty acids (MUFAs), oleic (C18:1), eicosenoic (C20:1, erucic (C22:1) and nervonic (C24:1) ones were found in measurable quantities in buckwheat seeds (Table 2). Among MUFAs, the major acid in seeds was C18:1, whose share in the total pool of fatty acids was 25.5%. An earlier report indicates that the percentage of C18:1 in the seeds of Polish buckwheat cultivars was 39 - 41% (DZIADEK et al. 2016). Similar contents of this acid were found in seeds of buckwheat of Chinese and Korean origin (CHO et al. 2016). On the other hand, a study of North American origin cultivars showed that the C18:1 content was in the range of 33.9 - 38.2% of all the fatty acids composition in buckwheat seeds (MAZZA 1988).

Numerous polyunsaturated acids (PUFAs) were found in buckwheat seeds: linoleic (C18:2), α -linolenic (C18:3), eicosadienoic (C20:2), eicosatrienoic (C20:3), eicosatetraenoic (C20:4) and docosadienoic acid (C22:2) – Table 2.

Table 2

Composition of unsaturated fatty acids (% of total) and C18:2/C18:3 ratio in seeds and sprouts of common buckwheat

Fatty acid	Seeds	Sprouts, control	Sprouts + 0.25 mM Fe-EDTA + 2 mM Na ₂ SiO ₃	Sprouts + 0.50 mM Fe-EDTA + 4 mM Na ₂ SiO ₃	Sprouts + 4 mM Na ₂ SiO ₃
C16:1 ω-7	<LOD	0.3±0.1 ^a	0.5±0.1 ^a	0.5±0.1 ^a	0.3±0.1 ^a
C18:1 ω-9	25.5±0.5 ^a	21.7±0.5 ^b	22.0±0.5 ^b	21.3±0.1 ^b	23.1±0.2 ^b
C18:2 ω-6	38.9±2.0 ^b	40.1±1.0 ^b	39.5±0.5 ^b	44.3±0.5 ^a	45.4±0.5 ^a
C18:3 ω-3	2.7±0.1 ^d	5.3±0.1 ^c	6.1±0.1 ^b	6.8±0.1 ^a	7.3±0.1 ^a
C20:1 ω-9	1.8±0.1 ^a	1.3±0.1 ^a	1.7±0.1 ^a	1.5±0.1 ^a	1.8±0.1 ^a
C20:2 ω-6	2.1±0.1 ^a	0.1±0.1 ^b	0.2±0.1 ^b	0.2±0.1 ^b	0.3±0.1 ^b
C20:3 ω-3	2.4±0.1 ^a	<LOD	<LOD	<LOD	<LOD
C20:4 ω-6	2.4±0.1 ^a	<LOD	<LOD	<LOD	<LOD
C22:1 ω-9	0.6±0.1 ^a	<LOD	<LOD	<LOD	<LOD
C22:2 ω-6	0.7±0.1 ^a	<LOD	<LOD	<LOD	<LOD
C24:1 ω-9	1.4±0.1 ^a	1.6±0.1 ^a	0.6±0.1 ^b	0.3±0.1 ^b	0.3±0.1 ^b
C18:2/C18:3 ratio	14.4±0.9 ^a	4.1±0.3 ^b	3.6±0.2 ^b	3.1±0.3 ^b	3.2±0.1 ^b

* Results marked with the same letter were not significantly different at $P < 0.05$ (Tukey's post hoc test). Comparisons were made within each fatty acid separately. LOD (limit of determination) = 0.1%.

Linoleic acid (C18:2) appeared to be the most dominant among PUFAs acids and all the fatty acids of buckwheat seeds, reaching 38.9% of their total content. This result was similar to previously published American, Korean and Polish data (MAZZA 1988, CHO et al. 2016, DZIADEK et al. 2016). However, our research showed that the content of C20:2 and C20:3 was higher than that in the seeds of Korean and Chinese buckwheat cultivars (CHO et al. 2016). Besides, our study confirmed for the first time the presence of eicosatetraenoic acid (C20:4) and docosadienoic acid (C22:2) in buckwheat seeds.

In the summary to this part of the discussion, the following can be considered: differences in the composition of fatty acids in our research with previous reports are probably due to genetic differences between the buckwheat cultivars and/or cultivation conditions.

The set of SFAs in buckwheat sprouts was similar to that in seeds. Moreover, the sprouts contained C14:0 acid, which was found in the seeds in trace amounts (Table 1). Similarly to seeds, the most abundant SFAs in sprouts were C16:0 and C20:0. However, in untreated sprouts (control) and those treated with elicitors, most of the saturated fatty acids were found higher concentration than in buckwheat seeds. This was the case of C16:0, C18:0 and C20:0 amounts, while the content of C6:0 and C22:0 acids

decreased during the sprouting process (Table 1). In the case of control sprouts, the C16:0 acid content was more than 50% higher than in seeds. The use of the elicitors reduced differences between the seeds and sprouts for C16:0, C20:0, C22:0 and C24:0 acids, but not for C18:0. These findings resemble the results obtained YIMING et al. (2015), who showed that the content of these SFAs increased during germination of Tartary buckwheat (*Fagopyrum tataricum* Gertn.). However, in contrast to these results, KIM et al. (2005) showed that a 7-day germination process significantly reduced C16:0, C18:0 and C20:0 acids in buckwheat sprouts.

During the germination process, fatty acids are first released from triacylglycerides and then they can be converted to sugars through β -oxidation and glyoxylation cycles. The conversion is dependent on the pre-germination treatments, and levels of some individual FAs either progressively increased or decreased over germination time (GRAHAM 2008, BENINCASA et al. 2019). This is probably the main reason for the differences in the fatty acid composition of buckwheat sprouts.

Among the MUFAs, the major acid in buckwheat sprouts was C18:1, whose share in the total pool of acids exceeded 21% (Table 2). Furthermore, small contents of C16:1 were found in buckwheat sprouts, whereas in the seeds its content was below the limit of determination (LOD), i.e. <0.1%. In sprouts level C22:1 acid were below the LOD. The use of elicitors during germination slightly decreased content of C18:1 acid, independently of their concentration. Furthermore, these elicitors has significantly reduced the C24:1 acid content. The process of buckwheat germination in a study conducted by KIM et al. (2005) caused a 50% decrease in the level of C18:1 acid and a slight loss of C20:1 acid. Also, a decline of C18:1 content was found during the germination of Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] (YIMING et al., 2015)

Among the PUFAs in buckwheat sprouts, there were high levels of C18:2 but much less of α -C18:3 (Table 2). In the control sprouts, as well as in the ones treated with the elicitors, the content of C20:3, C20:4, and C22:2 was below LOD (Table 2). Control buckwheat sprouts contained twice as much α -C18:3 acid as seeds. The use of the elicitors led to a further growth in the content of this acid in the sprouts, especially through the application of high concentrations of iron chelate and sodium silicate. According to KIM et al. (2005), after 7-days of the germination of buckwheat seeds, the content of α -C18:3 and C18:2 acids increased by 1.3 and 5.4 times, respectively. However, the conditions of the germination process are unknown and these seem to be important. For example, a short germination process of buckwheat (24 - 48 h) did not cause significant changes in the fatty acid composition of the sprouts compared to the seeds (KRUMINA-ZEMTURE, BEITANE 2017).

Germination of buckwheat seeds drastically changed the α -C18:3/C18:2 ratio (Table 2). In the seeds, it reached 14.4 while in the sprouts it was 3.2 - 4.1. Similarly, this ratio varied between 13.54 and 16.04 in buckwheat

flours (KRUMINA-ZEMTURE, BEITANE 2017). A proper ratio of α -C18:3/C18:2 is important for health and in the prevention of obesity (SIMOPOULOS 2016). In buckwheat seeds, the HI ratio in the current research was 3.67 (Table 3). This result was similar to data reported by BONAFACCIA et al. (2003) as well as by KRUMINA-ZEMTURE and BEITANE (2017), but higher in comparison with the data by ALVAREZ-JUBETE et al. (2009). In control sprouts and treated with

Table 3

Contents of total SFAs, UFAs, MUFAs and PUFAs, and their quality factors (AI, TI, HI) in seeds and sprouts of common buckwheat

Fatty acids/ /quality of FAs	Seeds	Sprouts, control	Sprouts + 0.25 mM Fe-EDTA + 2 mM Na ₂ SiO ₃	Sprouts + 0.50 mM Fe-EDTA + 4 mM Na ₂ SiO ₃	Sprouts + 4 mM Na ₂ SiO ₃
Total SFAs	21.4±1.3 ^{b*}	29.8±0.9 ^a	28.3±1.0 ^a	25.5±0.7 ^{ab}	22.3±0.7 ^b
Total UFAs	78.5±3.3 ^a	70.1±2.0 ^b	70.6±1.5 ^b	74.9±1.1 ^{ab}	78.7±0.7 ^a
MUFAs	29.3±0.8 ^a	24.6±0.8 ^b	24.8±0.8 ^b	23.6±0.4 ^b	25.7±0.5 ^b
PUFAs	49.2±2.5 ^{ab}	45.5±1.7 ^b	45.8±1.2 ^b	51.3±0.7 ^a	53.0±0.7 ^a
AI	0.13±0.01 ^b	0.22±0.01 ^a	0.17±0.01 ^{ab}	0.16±0.01 ^b	0.15±0.01 ^b
TI	0.40±0.01 ^c	0.62±0.02 ^a	0.56±0.02 ^a	0.47±0.01 ^b	0.39±0.01 ^c
HI	3.67±0.16 ^a	2.35±0.10 ^c	2.49±0.08 ^c	2.94±0.10 ^b	3.53±0.12 ^a

* Results marked with the same letter were not significantly different at $P<0.05$ (Tukey's post hoc test). Comparisons were made within each line separately. SFAs – saturated fatty acids, UFAs – unsaturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids, AI – atherogenic index, TI – thrombogenic index, HI – health index.

the elicitors, the HI was significantly lower, but in those treated with sodium silicate it was similar to the one detected in seeds. The AI and TI reached 0.13 and 0.40 in seeds and were lower than in the control sprouts, but the elicitors used caused the AI and TI levels of seeds and sprouts to be similar. In comparison, in seeds of Slovenian buckwheat cultivated for two seasons, the AI and TI reached values of 0.22 - 0.23 and 0.39 - 0.40, respectively (SINKOVIČ et al. 2020). AI and TI are nutritionally optimal when they range from 0.18 to 0.52 and 0.34 to 0.73, respectively. For nutritional purposes, lower AI and TI are considered better (ULBRICHT, SOUTHGATE 1991).

CONCLUSIONS

The current results indicate that the germination of buckwheat seeds leads to an increase in SFAs although the elicitors used inhibited these tendencies, especially after the application of sodium silicate. The sprouts

of buckwheat contained almost twice as much α -linolenic acid as the seeds did, and slightly less linoleic acid, and the elicitors applied favored increased accumulation of α -linolenic acid. As a result of the increase in the level of α -C18:3 acid in buckwheat sprouts, the C18:2/C18:3 ratio was reduced by about 4 times, which may have positive health effects. In sprouts, the content of long-chain unsaturated acids was below 0.1%, while in seeds their content was between 0.6 and 2.4%. The germination of buckwheat seeds raised the AI and TI and lowered the HI of fatty acids in the sprouts, but the use of the elicitors inhibited these tendencies.

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