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Biostimulants and Microorganisms Boost the Nutritional Composition of Buckwheat (*Fagopyrum esculentum* Moench) Sprouts

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Abstract: This study investigated the influence of biological control agents and plant growth promoters on the chemical composition of the cultivars Panda and Kora buckwheat sprouts. Before sowing, seeds were soaked in solutions containing *Bacillus subtilis* bacteria, *Pythium oligandrum* oospores, *Ecklonia maxima* algae extract, and/or nitrophenols. The sprouts of the Panda displayed higher levels of protein, fat, and dietary fiber fractions than the Kora. Measurable effects of biological control agents (BCAs) and plant growth promoters (PGPs) on the chemical composition of sprouts were also confirmed. Soaking the seeds in a solution containing *P. oligandrum* oospores resulted in a decrease in the level of crude ash in sprouts, while the addition of nitrophenols increased the level of both crude ash and protein. We also found statistically significant effects of interactions between the cultivar and BCA and/or PGP for each of the examined components.

Keywords: buckwheat sprouts; *Bacillus subtilis*; *Pythium oligandrum*; *Ecklonia maxima*; chemical composition

1. Introduction

Wheat, rice, and maize cover 40% of all arable land and constitute more than 50% of human caloric intake [1]. Therefore, modern cereal breeding programs should be replaced by breeding programs of so-called development opportunity crops, nutritious crops which are currently under-valued and under-utilized [2]. Such a shift from long-standing dietary habits will be undeniably difficult but necessary, requiring active involvement from the consumers [3]. Experiments with home gardens in India have shown that even a small arable area can provide essential deficient elements in the form of naturally nutrient-dense raw foods (i.e., vegetables) [1]. Western diets could be positively modified by micro-scale vegetable production, namely sprouts or microgreens of, e.g., alfalfa, azuki bean, black gram, chickpea, lentil, mung bean, soybean, barley, maize, oat, rice, wheat, amaranth, buckwheat, quinoa, almond, hazelnut, linseed, sesame, sunflower, broccoli, cabbage, carrot, celery, clover, fennel, kale, leek, lettuce, mustard, parsley, radish, arugula, spinach, onion, turnip, and watercress [4]. Sprouts are classified as a biogenic food, meaning that they are a living food source. The popularity of sprouts, microgreens, or "Baby leaf" is connected to their sensory attractiveness—vivid colors, delicate textures, and unique flavors—but can mainly be attributed to their biological value. Di Gioia and Santamaria [5] estimated that consumption of even a few grams of microgreens (1540 g) could entirely satisfy the recommended daily intake of vitamins C, E, and K. One popular source of sprouts is buckwheat (*Fagopyrum esculentum* Moench) [6,7]. If grown in the presence of light, they can be consumed raw, whereas those produced without light can be consumed following heat-treatment. In addition to essential nutrients, they contain beneficial ingredients such as phenolic compounds, vitamins, dietary fiber, and numerous minerals. Compared to buckwheat seeds, due to transformation of seed components during germination, the nutritional value of the sprouts is considerably higher [7]. Additionally, sprouts can be biofortified to increase levels of essential ingredients (i.e., phytonutrients) and thus, to additionally improve their nutritional value [8]. It can be performed by treating seeds with microorganisms that have proven beneficial to plants (biological control agents (BCAs)).

There are currently 48 biological control agents registered for use in Europe, including fungi, oomycetes, and bacterial microorganisms [9]. Numerous studies demonstrated their highly positive effect on plants [10–12]. Seed inoculation with the endophytic bacterium *Herbaspirillum* ST-B2 enhanced growth of sprouts and microgreens, promoted root elongation, and increased mass production of sprouts [13]. *Pythium oligandrum* has a documented impact on many plant processes, including a desirable effect on pathogen control and induction of resistance in the host plant [14]. Rice seeds treated with *Bacillus subtilis* and rutoside exhibited increased root and shoot lengths of seedlings. This, especially, is significant information in the context of this study because buckwheat seeds contain rutin which promotes the growth of fungi [15]. Additionally, algae extracts are gaining acceptance as plant growth promoters (PGPs) as they enhance plant tolerance to a wide range of abiotic stressors and promote growth [16–20]. Similar effects on plants have been observed with nitrophenols [21–23]. Synergistic action of biostimulators was proven and the second generation of this product is expected [24].

The hypothesis that BCAs and PGPs could modify quality of sprouts was considered. The aim of this study was to test the influence of BCAs and PGPs on the essential nutrients and dietary fiber components in buckwheat sprouts.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Common buckwheat (*Fagopyrum esculentum* Moench) seeds of the cultivars Panda and Kora were purchased from the Małopolska Plant Breeding Ltd., branch in Palikije, Poland. The experiment was performed at room temperature (ca. 21 °C) with the presence of natural light intensity occurring in July 2017 for a 16 h photoperiod for all time of the sprouting. The average light intensity measured every day at noon was 450 $\mu M/m^2/s$. Buckwheat seeds were soaked for 30 min in solutions of plant growth promoters (PGPs): Kelpak SL (Kelp Products International (Pty) Ltd., Simon's Town, South Africa,) and Asashi SL (Arysta LifeScience Ltd., Praha, Czech Republic), and/or biological control agents (BCAs): Polyversum WP (Biopreparaty Ltd., Úherce, Czech Republic) and Serenade ASO (Bayer AG, Leverkusen, Germany), according to the manufacturer's recommendations.

Bioregulator Kelpak SL is a commercial product (seaweed *Ecklonia maxima* extract) containing polysaccharides such as laminarin, alginates, and carrageenans; micro- and macronutrients; sterols; and N-containing compounds like betaines and hormones [25]. A 1:100 treatment solution of Kelpak SL was used. Biostimulant Asahi SL is a commercial product containing a mixture of three nitrophenolic compounds naturally occurring in plants (sodium p-nitrophenolate, sodium o-nitrophenolate, sodium 5-nitroguaiacolate). A 1:500 treatment solution of Asahi SL was used. Polyversum WP is a commercial product containing 10^6 *Pythium oligandrum* oospores per 1 g. The treatment solution used contained 2 g of Polyversum WP in 500 mL of water. Serenade ASO is a commercial product containing *Bacillus* *subtilis* QST 713 at a concentration of 13.96 g/L (1.34%) (minimum number of bacterial cells 1.042×10^{12} CFU/L). A 1:40 treatment solution of Serenade ASO was used.

The treatment solutions were: (1) *Ecklonia maxima* extract (Kelpak SL) only; (2) nitrophenols (Asahi SL) only; (3) *Pythium oligandrum* oospores (Polyversum WP) only; (4) *Bacillus subtilis* QST 713 (Serenade ASO) only; (5) *Ecklonia maxima* extract (Kelpak SL) plus *Pythium oligandrum* oospores (Polyversum WP); (6) nitrophenols (Asahi SL) plus *Pythium oligandrum* oospores (Polyversum WP); (7) *Ecklonia maxima* extract (Kelpak SL) plus *Bacillus subtilis* QST 713 (Serenade ASO); (8) nitrophenols (Asahi SL) plus *Bacillus subtilis* QST 713 (Serenade ASO); (9) nitrophenols (Asahi SL) plus *Ecklonia maxima* extract (Kelpak SL) and (10) control (without biostimulants or BCAs).

2.2. Sampling Preparation and Chemical Analysis

Following soaking, seeds were washed with distilled water and placed in a plastic sprouting plate on 15 July 2017, watered once daily. Sprouts were harvested 14 days after sowing (on 29 July 2017). The collected buckwheat sprouts were immediately dried at 40 °C. Dried sprouts were then ground into powder using a laboratory mill (KNIFETEC 1095, Foss Tecator, Höganäs, Sweden) and samples of about 200 g of dry matter was used to do chemical analysis.

To determine dry matter, samples were dried at 105 °C to constant weight. Basic chemical composition (crude protein, crude fat, crude fiber, crude ash) was determined according to the Association of Official Analytical Chemists [26]. Crude fat was determined using the Soxhlet extraction method with diethyl ether as solvent; crude ash by incineration in a muffle furnace at 580 °C for 8 h; crude protein (CP) ($N \times 6.25$) by Kjeldahl method using a Büchi B-324 distillation unit (Büchi Labortechnik AG, Flawil, Switzerland). Crude fiber (CF) was determined as the residue after sequential treatment with 1.25% H_2SO_4 and with 1.25% NaOH using an ANKOM^{220} Fiber Analyser (ANKOM Technology, New York, NY, USA). Total carbohydrates (TC) were estimated according to the following calculation:

$$\text{TC (\%)} = 100 - (\text{moisture \%} + \text{crude protein \%} + \text{crude fat \%} + \text{crude ash \%} + \text{crude fiber \%}) \quad (1)$$

Dietary fiber (DF) components were determined using the detergent method according to Van Soest et al. [27]. Determination of neutral detergent fiber (NDF) was conducted on an ash-free basis and included sodium dodecyl sulphate. Determination of acid detergent fiber (ADF) included hexadecyl-trimethyl-ammonium bromide, while acid detergent lignin (ADL) was determined by hydrolysis of the ADF sample in 72% H_2SO_4. The content of cell wall structural carbohydrates hemicellulose and cellulose was calculated as the following differences:

$$\begin{array}{c} \text{cellulose (CEL)} = \text{ADF} - \text{ADL} \\ \text{hemicellulose (HCEL)} = \text{NDF} - \text{ADF} \end{array} \quad (2)$$

2.3. Statistical Analyses

Two factorial analyses of variance (ANOVA) were done. Comparisons should be made separately for factors (1. cultivars and 2. treatment solution composition). The significance of differences between means was assessed using the Newman–Keuls test at $p = 0.05$. Additionally, principal component analysis (PCA) was done and presented as a biplot. Both analyses (ANOVA, PCA) were carried out using Statistica 13.1 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

The concentration of all basic nutrients in the sprouts of the two buckwheat cultivars (Panda, Kora) were significantly altered by soaking the seeds in the biostimulant solutions. Comparing the chemical composition of sprouts of the two tested cultivars without biostimulant treatment, a significantly higher level of dry matter, crude ash, and total carbohydrates was found in sprouts of the Kora cultivar, while sprouts of the Panda cultivar contained significantly more protein, fat, crude fiber, and all fractions of dietary fiber (Tables 1–3).

Table 1. Effect of cultivar and treatment on the dry matter, crude ash, protein, fat, and total carbohydrates (TC) of buckwheat sprouts.

Treatment	Ingredient (%)														
	Dry Matter			Crude Ash			Crude Protein			Crude Fat			TC		
	Panda	Kora	Mean	Panda	Kora	Mean	Panda	Kora	Mean	Panda	Kora	Mean	Panda	Kora	Mean
Seaweed *E. maxima* extract	$91.27_{d,e}$	91.69_{f}	91.48^{e}	$5.37_{b,c,d}$	$5.74_{d,e,f}$	$5.55^{b,c}$	22.5_{f}	$22.65_{f,g}$	22.57^{b}	$3.82_{a,b,c}$	3.62_{a}	$3.72^{a,b,c}$	$38.93_{c,d,e}$	$38.35_{c,d,e}$	38.64^{a}
Nitrophenols	90.33_{a}	91.60_{f}	$90.96^{b,c}$	$5.45_{c,d,e}$	$5.47_{c,d,e}$	$5.46^{b,c}$	23.06_{g}	$21.99_{d,e}$	$22.52^{a,b}$	$3.79_{a,b,c}$	$3.71_{a,b}$	$3.75^{a,b,c}$	37.59_{b}	$38.8_{c,d,e}$	38.19^{a}
P. oligandrum	90.48_{a}	$91.50_{e,f}$	$90.99^{b,c}$	$5.47_{c,d,e}$	4.8_{a}	5.13^{a}	23.36_{g}	$21.09_{a,b}$	22.22^{a}	4.06_{c}	3.57_{a}	$3.82^{a,b,c}$	36.74_{a}	40.99_{i}	38.86^{a}
Bacillus subtilis	$90.60_{a,b}$	$91.11_{c,d}$	$90.85^{a,b}$	$5.26_{b,c}$	$5.54_{c,d,e}$	5.40^{b}	$22.25_{e,f}$	$22.57_{f,g}$	$22.41^{a,b}$	$3.94_{b,c}$	3.55_{a}	$3.75^{a,b,c}$	41.01_{i}	$39.66_{g,h}$	40.33^{b}
Seaweed *E. maxima* extract + *P. oligandrum*	$91.06_{c,d}$	$91.05_{c,d}$	$91.05^{b,c}$	$5.48_{c,d,e}$	$5.52_{c,d,e}$	$5.50^{b,c}$	23.27_{g}	21.27_{b}	$22.27^{a,b}$	4.05_{c}	$3.67_{a,b}$	3.86^{c}	$38.75_{c,d,e}$	$38.88_{c,d,e}$	38.81^{a}
Nitrophenols + *P. oligandrum*	$91.13_{c,d}$	$91.17_{c,d}$	$91.15^{c,d}$	$5.82_{e,f}$	$5.56_{c,d,e}$	5.68^{c}	22.98_{g}	23.4_{g}	23.19^{c}	$3.93_{b,c}$	3.55_{a}	$3.74^{a,b,c}$	38.16_{c}	$38.57_{c,d,e}$	38.36^{a}
Seaweed *E. maxima* extract + *B. subtilis*	91.68_{f}	$90.99_{c,d}$	$91.33^{d,e}$	$5.10_{a,b}$	5.89_{f}	$5.50^{b,c}$	22.5_{f}	22.5_{f}	$22.50^{a,b}$	$3.72_{a,b}$	3.51_{a}	3.61^{a}	40.14_{h}	40.67_{i}	40.40^{b}
Nitrophenols + *B. subtilis*	90.46_{a}	$91.05_{c,d}$	90.76^{a}	$5.51_{c,d,e}$	$5.07_{a,b}$	$5.29^{a,b}$	$22.67_{f,g}$	20.81_{a}	21.74^{a}	4.04_{c}	3.62_{a}	$3.83^{b,c}$	$39.14_{d,e,f}$	41.74_{j}	40.44^{b}
Seaweed *E. maxima* extract + nitrophenols	91.61_{f}	$90.93_{c,d}$	91.27^{d}	4.87_{a}	5.95_{f}	5.41^{b}	$21.64_{c,d}$	$22.33_{e,f}$	21.99^{a}	4.05_{c}	3.56_{a}	$3.80^{a,bc}$	40.01_{h}	$38.58_{c,d,e}$	39.29^{a}
Control	$91.26_{d,e}$	$90.8_{b,c}$	$91.03^{b,c}$	$5.24_{b,c}$	$5.55_{c,d,e}$	5.39^{b}	$21.65_{c,d}$	$22.18_{e,f}$	21.92^{a}	$3.72_{a,b}$	3.55_{a}	$3.63^{a,b}$	$39.15_{d,e,f,g}$	$39.58_{f,g,h}$	39.36^{a}
Mean	90.98^{a}	91.19^{b}	-	5.35^{a}	5.51^{b}	-	22.59^{b}	22.08^{a}	-	3.91^{b}	3.59^{a}	-	39.0^{a}	39.6^{b}	-

Means denoted by different letters differ statistically at $p = 0.05$ according to the Newman–Keuls test. Letters in the upper index denote the means of main effects (cultivar and treatment separately). Letters in the bottom index denote the means for cultivar under the treatment.

Table 2. Effect of cultivar and treatment on the crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) of buckwheat sprouts.

Treatment	Ingredient (%)								
	Crude Fiber			NDF			ADF		
	Panda	Kora	Mean	Panda	Kora	Mean	Panda	Kora	Mean
Seaweed *Ecklonia maxima* extract	20.88_{h}	20.16_{f}	$20.52^{e,f}$	40.44_{i}	35.71_{d}	38.07^{e}	31.16_{g}	$27.91_{b,c,e}$	29.54^{c}
Nitrophenols	20.27_{f}	$19.62_{d,e}$	19.95^{c}	$38.51_{f,g}$	35.82_{d}	37.16^{d}	$30.65_{f,g}$	$28.2_{c,d,e}$	29.42^{c}
Pythium oligandrum	$19.67_{d,e}$	20.93_{h}	$20.30^{d,e}$	35.90_{d}	$37.56_{e,f}$	36.73^{d}	$27.83_{b,c,e}$	$28.66_{d,e,f}$	28.24^{b}
Bacillus subtilis	20.21_{f}	17.11_{a}	18.65^{a}	38.25_{f}	32.61_{b}	35.43^{c}	$28.47_{d,e}$	25.00_{a}	26.74^{a}
Seaweed *Ecklonia maxima* extract + *Pythium oligandrum*	$19.38_{c,d}$	21.51_{i}	$20.45^{e,f}$	$37.73_{e,f}$	36.89_{e}	37.31^{d}	$29.24_{d,e,f,g}$	$28.69_{d,e,f}$	$28.97^{b,c}$
Nitrophenols + *Pythium oligandrum*	20.26_{f}	$19.91_{e,f}$	$20.08^{c,d}$	$40.00_{h,i}$	36.91_{e}	38.45^{e}	$30.53_{f,g}$	$29.73_{d,e,f,g}$	30.13^{c}
Seaweed *Ecklonia maxima* extract + *Bacillus subtilis*	19.01_{c}	18.29_{b}	18.6^{a}	34.50_{c}	31.00_{a}	32.75^{a}	26.04_{a}	25.38_{a}	25.71^{a}
Nitrophenols + *Bacillus subtilis*	$19.29_{c,d}$	18.99_{c}	19.14^{b}	35.94_{d}	33.24_{b}	34.59^{b}	$26.38_{a,b}$	$26.69_{a,b,c}$	26.54^{a}
Seaweed *Ecklonia maxima* extract + nitrophenols	20.14_{f}	20.49_{f}	$20.31^{d,e}$	$39.19_{g,h}$	37.15_{e}	38.17^{e}	$29.35_{d,e,f,g}$	$30.13_{e,f,g}$	29.74^{c}
Control	21.02_{h}	20.37_{f}	20.69^{f}	$39.64_{h,i}$	$37.9_{e,f}$	38.77^{e}	$30.15_{e,f,g}$	$29.24_{d,e,f,g}$	29.69^{c}
Mean	20.0^{b}	19.7^{a}	-	38.01^{b}	35.48^{a}	-	28.98^{b}	27.96^{a}	-

Means denoted by different letters differ statistically at $p = 0.05$ according to the Newman–Keuls test. Letters in the upper index denote the means of main effects (cultivar and treatment separately). Letters in the bottom index denote the means for cultivar under the treatment.

Table 3. Effect of cultivar and treatment on the acid detergent lignin (ADL), hemicellulose (HCEL), and cellulose (CEL) of buckwheat sprouts.

Treatment	Ingredient (%)								
	ADL			HCEL			CEL		
	Panda	Kora	Mean	Panda	Kora	Mean	Panda	Kora	Mean
Seaweed *Ecklonia maxima* extract	12.10_{h}	$10.43_{e,f}$	11.27^{e}	$9.28_{d,e}$	$7.80_{b,c,d,e}$	8.54^{b}	$19.33_{c,d}$	$17.48_{a,b,c,d}$	$18.41^{c,d}$
Nitrophenols	11.44_{g}	$10.03_{c,d,e}$	10.74^{d}	$7.86_{b,c,d,e}$	$7.62_{b,c,d,e}$	$7.74^{a,b}$	$19.21_{c,d}$	$18.17_{a,b,c,d}$	$18.69^{c,d}$
Pythium oligandrum	$9.53_{a,b,c}$	10.74_{f}	10.13^{c}	$8.08_{b,c,d,e}$	$8.91_{c,d,e}$	8.49^{b}	$18.3_{a,b,c,d}$	$17.92_{a,b,c,d}$	$18.11^{b,c}$
Bacillus subtilis	$9.48_{a,b,c}$	8.86_{a}	9.17^{a}	9.79_{e}	$7.60_{b,c,d,e}$	8.69^{b}	$18.99_{c,d}$	16.15_{a}	$17.57^{b,c}$
Seaweed *Ecklonia maxima* extract + *Pythium oligandrum*	$9.68_{b,c,d}$	10.91_{f}	10.29^{c}	$8.49_{b,c,d,e}$	$8.20_{b,c,d,e}$	8.34^{b}	19.57_{d}	$17.79_{a,b,c,d}$	$18.68^{c,d}$
Nitrophenols + *Pythium oligandrum*	10.75_{f}	$10.27_{d,e,f}$	10.51^{c}	9.48_{e}	$7.18_{a,b,c,d}$	8.33^{b}	19.78_{d}	19.46_{d}	19.62^{d}
Seaweed *Ecklonia maxima* extract + *Bacillus subtilis*	$9.13_{a,b}$	$9.06_{a,b}$	9.09^{a}	$8.46_{b,c,d,e}$	5.63_{a}	7.04^{a}	$16.92_{a,b,c}$	16.32_{a}	16.62^{a}
Nitrophenols + *Bacillus subtilis*	$9.94_{c,d,e}$	$9.26_{a,b}$	9.60^{b}	9.56_{e}	$6.55_{a,b}$	$8.05^{a,b}$	$16.45_{a,b}$	$17.43_{a,b,c,d}$	$16.94^{a,b}$
Seaweed *Ecklonia maxima* extract + nitrophenols	11.49_{g}	$11.82_{g,h}$	11.65^{f}	9.84_{e}	$7.02_{a,b,c}$	8.43^{b}	$17.86_{a,b,c,d}$	$18.31_{a,b,c,d}$	$18.08^{b,c}$
Control	12.13_{h}	$10.4_{e,f}$	11.26^{e}	9.49_{e}	$8.67_{b,c,d,e}$	9.08^{b}	$18.03_{a,b,c,d}$	$18.84_{b,c,d}$	$18.43^{c,d}$
Mean	10.56^{b}	10.18^{a}	-	9.03^{b}	7.52^{a}	-	18.44^{b}	17.78^{a}	-

Means denoted by different letters differ statistically at $p = 0.05$ according to the Newman–Keuls test. Letters in the upper index denote the means of main effects (cultivar and treatment separately). Letters in the bottom index denote the means for cultivar under the treatment.

The crude ash concentration indicated the abundance of micro- and macroelements in the sprouts—in the tested cultivars, it was 5.35% and 5.51%, with the sprouts of the Kora cultivar showing significantly higher levels (Table 1). In an earlier study, Liu et al. [28] found as much as 9.4% ash in the sprouts of *Fagopyrum esculentum* and 6.4% in the sprouts of *Fagopyrum tataricum*. Kim et al. [29] notes that eight days after sowing, the amount of crude ash in sprouts increased from 2.8% (seeds) to 3.2%. The growing conditions were similar to our study control condition but ash content was significantly lover.

Buckwheat sprouts are considered to be a good source of protein, the level of which differed significantly between the cultivars. Sprouts of the Panda cultivar contained 22.59% crude protein, compared to 22.08% in the Kora sprouts (Table 1). Paredes-Lopez and Mora-Escobedo [30] reported the level of protein in amaranth sprouts (*Amaranthus hypochondriacus*), a pseudocereal like buckwheat, at 20.8%. Moreover, the authors observe that during germination, the crude protein levels increased after three days by more than 40% compared to the level measured on the first day of incubation (21.9% and 15.5%, respectively).

Buckwheat sprouts are also a good source of raw fat, which is well absorbed by the human body [6]. Sprouts of the Panda cultivar contained significantly more crude fat than sprouts of the Kora cultivar (3.91% and 3.59%, respectively) (Table 1). The obtained results are consistent with an earlier study by Gimenez-Bastida and Zieliński [31] who report a 3.8% fat level in buckwheat sprouts. Liu et al. [27] determined the fat level in sprouts of *Fagopyrum esculentum* at 5.28%, and in sprouts of *Fagopyrum tataricum* at 4.05%.

Total carbohydrates (TC), consisting of simple sugars, starch, dextrins, and organic acids, comprised the dominant share of the dry matter. The tested sprouts contained on average 39.3% TC, with the Panda cultivar containing significantly less (39.0%) than Kora (39.6%) (Table 1).

Crude fiber includes the sum of fibrous substances (cellulose, lignin, hemicellulose, etc.) that are resistant to enzymes of the digestive tract. In the studied sprouts, we measured 19.7% (Kora) and 20% (Panda) crude fiber. Similar to protein and fat content, sprouts of the Panda cultivar were richer in this material (Table 2). The obtained values were twice as high as those given by Liu et al. [28], which may be due to the fact that the sprouts used in our analysis, like sprouts served to consumers, included some amount of seed coat and cotyledons, a part of the seed which is particularly rich in this nutrient [32].

Nowadays, increasing attention is being paid to nutrient components which are difficult for the human gastrointestinal tract to digest, called dietary fiber. The varied components of dietary fiber have different physical and chemical properties, characterized by their physiological effects on the human body. Dietary fiber contains both raw insoluble fiber fractions and soluble non-starch polysaccharides [33]. As a result of the use of different definitions and assay methods in research on dietary fiber, considerable problems are encountered when attempting to determine its actual level in food. For this reason, it is crucial to define not only the total level of dietary fiber in the diet, but also its fractional composition, since individual fractions are characterized by diverse action in the human body. Therefore, in our study we determined the share of NDF, ADF, ADL, HCEL, and CEL fractions. The largest fraction was neutral detergent fiber (NDF), which includes the sum of hemicellulose, cellulose, and lignin, followed by the acid detergent fiber fraction (ADF), which consists of lignin and cellulose. Buckwheat sprouts, like buckwheat seeds, are characterized by low hemicellulose levels (HCEL), which is usually the major component of dietary fiber in cereal bran (60% hemicellulose) [34]. Sprouts of the Panda cultivar were characterized by significantly higher levels of all fiber fractions (Tables 2 and 3).

Statistical analysis also confirmed an effect of PGPs and/or BCAs on the dry matter level of buckwheat sprouts. The lowest dry matter level was observed after soaking the seeds in a solution containing *Bacillus subtilis* bacteria alone or in combination with nitrophenols. The highest level of dry matter was measured in sprouts obtained after soaking in a solution containing *Ecklonia maxima* algae extract alone or in combination with *Bacillus subtilis* bacteria. Sprouts collected after germination in water (control) contained 91.03% dry matter. In general, a higher level of dry matter was observed in sprouts germinated in solutions containing both PGPs and BCAs or algae extract alone (Table 1).

The PGP and/or BCA solutions caused measurable cultivar-specific alterations in the levels of dry matter. Soaking the seeds in solutions containing *Ecklonia maxima* algae extract and *Bacillus subtilis* bacteria, as well as algae extract and nitrophenols, caused an effect of cultivar similar to that observed in controls, i.e., the sprouts of Panda cultivar contained more dry matter than the sprouts of Kora cultivar. The use of *Pythium oligandrum* solution together with one of the PGPs resulted in a similar dry matter level in buckwheat sprouts of both cultivars. On the other hand, all other solutions (containing PGPs and BCAs individually and those containing nitrophenols and *Bacillus subtilis* bacteria together) caused the dry matter level in sprouts of Panda cultivar to be lower than in the Kora cultivar (Table 1).

The composition of the solution in which the seeds were soaked also modified the level of crude ash in the buckwheat sprouts. The lowest level of crude ash was observed after soaking the seeds in the solution containing *Pythium oligandrum* oospores alone, but the highest level resulted when nitrophenols were added (Table 1).

Like dry matter, the crude ash level in sprouts of the tested buckwheat cultivars was significantly altered after soaking. Buckwheat sprouts of the Panda cultivar had a lower crude ash level than sprouts of the Kora cultivar after soaking the seeds in five solutions: (1) control; (2) extract of *Ecklonia maxima* algae + nitrophenols; (3) algae extract + *Bacillus subtilis*; (4) extract of algae and (5) *Bacillus subtilis* bacteria. However, when the solution contained oospores of *Pythium oligandrum*, nitrophenols and *Pythium oligandrum*, or nitrophenols and *Bacillus subtilis*, sprouts of the Panda cultivar contained more crude ash than the Kora sprouts. The use of extract from *Ecklonia maxima* + *Pythium oligandrum* algae or of nitrophenols resulted in a similar level of crude ash in sprouts of both tested buckwheat cultivars (Table 1).

Similarly, the level of raw protein in buckwheat sprouts was also modified by the composition of the soaking solution. Buckwheat sprouts grown from seeds soaked in a solution containing nitrophenols and *Pythium oligandrum* oospores were characterized by a significantly higher level of crude protein (Table 1).

Statistical analysis of crude protein level in buckwheat sprouts also confirmed an effect of interaction between the studied factors (Table 1). A similar reaction of cultivars to that seen in controls (higher levels in Kora sprouts) was observed for four solutions: (1) *Ecklonia maxima* extract + nitrophenols; (2) nitrophenols + *Bacillus subtilis*; (3) *Bacillus subtilis*; (4) *Ecklonia maxima* extract. Conversely, a higher level of protein in the sprouts of the Panda cultivar was observed after treatment with: 1. nitrophenols, 2. oospores of *Pythium oligandrum*, 3. algae extract of *Ecklonia maxima* + oospores of *Pythium oligandrum*, and 4. nitrophenols + bacteria of *Bacillus subtilis*. In a study by Yang et al. [35], spraying sprouts with jasmonic acid methyl ester did not cause a change in protein level in seven-day old buckwheat sprouts, but the levels found (17.09%–17.42%) were significantly lower than those observed in our study. On the other hand, they observed an increase in phenolic compounds and flavonoids. Yiming et al. [36] also confirmed an increase in flavonoid and chlorophyll levels with the growth of buckwheat sprouts, as well as modifications in the amino acid composition of proteins that occur at that time. Elumali and Rengasamy [37], who observed a growth of *Vigna radiata* sprouts after application of *Sargassum* algae fertilizer in combination with *Bacillus* sp., showed that microgreens/"Baby leaf" derived from this combination were characterized by a significantly higher protein level (3.160 mg/g) compared to control (1.0 mg/g). They were also characterized by a higher level of chlorophyll, which would have had a positive effect on their appearance. Selvam and Sivakumar [38] sprayed *Arachis hypogea* seedlings with *Hypnea musciformis* algae extract from the 15th day and found that the concentration of algae extract in the solution affected the level of chlorophyll, carbohydrates, protein, and fat in plants. The highest level of these nutrients was observed when the spray liquid contained 2% of the extract. On the other hand, a 1.5% concentration of *Sargassum wightii* extract in the solution sprayed onto 20-day *Cyamopsis tetragonoloba* plants caused changes in the chemical composition of leaves, increasing the level of chlorophyll and protein and reducing sugars and ascorbic acid [39]. Di Fan et al. [40] confirmed an increase in the protein level in vitro in spinach after the application of commercial extract of *Ascophyllum nodosum*.

In our study, soaking the seeds in solutions containing PGPs and/or BCAs also resulted in modification of the crude fat level in buckwheat sprouts. The highest fat level was found in sprouts obtained from seeds soaked in solution containing *Ecklonia maxima* + *Pythium oligandrum* algae extract, and the lowest fat levels in those soaked in a solution containing *Ecklonia maxima* extract + *Bacillus subtilis* (Table 1).

The reaction of the cultivars to PGPs and/or BCAs varied in terms of fat content. In the Kora cultivar, the fat level in its sprouts was altered slightly after soaking compared to control. The Panda cultivar, on the other hand, after treatment with the majority of PGP and BCA solutions, accumulated significantly more crude fat than was observed in controls (Table 1).

Soaking seeds in PGP and/or BCA solutions resulted in varied effects on TC levels in buckwheat sprouts. Only sprouts obtained from seeds treated with *Bacillus subtilis* bacteria, alone or in combination with *Ecklonia maxima* algae extract or nitrophenols, were characterized by a higher level of carbohydrates (Table 1). In an earlier study, Kim et al. [6] found that during the growth of buckwheat sprouts, their monosaccharide level increases, making it highly nutritional vegetable. The sprouts of the tested buckwheat cultivars grown from seeds soaked in water (control) did not differ significantly in total carbohydrate content. After the application of *Pythium oligandrum*, a significant increase in carbohydrate level in sprouts of the Kora cultivar and a decrease in the level in sprouts of the Panda cultivar were observed. After the application of *Bacillus subtilis*, an increase in the carbohydrate level in sprouts of the Panda cultivar was observed, with no change in comparison to the control of carbohydrate level in sprouts of the Kora cultivar. Application of the solution containing *Ecklonia maxima* algae extract and *Bacillus subtilis* bacteria increased the level of this nutrient in sprouts of both cultivars, whereas the solution containing nitrophenols and *Bacillus subtilis* bacteria increased the total carbohydrate level only in sprouts of the Kora cultivar (Table 1).

Statistical analysis of the crude fiber level in buckwheat sprouts confirmed an effect of PGPs and/or BCAs solutions, with most solutions causing a slight decrease in this nutrient (Table 2). The highest crude fiber level was found in buckwheat sprouts grown from seeds soaked in water (control). A slightly lower crude fiber level was observed in sprouts grown from seeds soaked in a solution containing *Ecklonia maxima* algae extract. The lowest level of crude fiber was found in sprouts grown from seeds soaked in a solution containing *Bacillus subtilis* bacteria, consistent with the fact that these bacteria are known for the ability to degrade lignin [41], one of the components of raw fiber. Crude fiber levels in the tested cultivars following soaking were significantly different than in the control (Table 2). The presence of *Bacillus subtilis* bacteria reduced the crude fiber level of sprouts of the Kora cultivar much more strongly than those of Panda. However, a solution containing *Pythium oligandrum* oospores alone or in combination with *Ecklonia maxima* algae extract resulted in an increase in the crude fiber level in the sprouts of the Kora cultivar. Soaking buckwheat sprouts in the solutions of PGPs and/or BCAs significantly modified the proportion of NDF (Table 2). The lowest level of this fraction of dietary fiber (32.75%–35.43%) was observed in sprouts grown from seeds soaked in solutions containing *Bacillus subtilis* bacteria. The highest level of NDF (38.77%) was found in the control condition.

The studied buckwheat cultivars, when soaked in PGP and/or BCA solutions, showed a different reaction than controls. A significant decrease in the level of NDF in sprouts of the Kora cultivar occurred after soaking the seeds in a solution containing *Bacillus subtilis* bacteria. The level of this fraction in the Panda cultivar sprouts did not decrease as significantly in comparison to the control. The use of a solution containing only *Pythium oligandrum* resulted in a higher NDF level in the Kora cultivar compared to the Panda cultivar (Table 2).

The level of the ADF fraction in buckwheat sprouts was also modified by the composition of the treatment solution. The lowest level of this fraction (25.71%–26.74%) was observed for *Bacillus subtilis*-treated sprouts. Sprouts grown from seeds soaked in solutions containing *Pythium oligandrum* oospores displayed a higher level of ADF. The highest level of ADF was found in sprouts from seeds soaked in *Pythium oligandrum* + nitrophenols (30.13%) (Table 2).

The sprouts of the tested cultivars in the majority of treatment conditions did not differ significantly in their level of ADF. The exceptions were those whose seeds were soaked in solutions containing only *Ecklonia maxima* algae extract, only nitrophenols, or only *Bacillus subtilis* bacteria, which resulted in a significantly lower level of ADF in sprouts of the Kora cultivar (Table 2).

The levels of ADL in the sprouts grown from seeds soaked in solutions containing PGPs and/or BCAs varied significantly (Table 3). The lowest level of this fiber fraction was observed in BCA-treated sprouts, especially those treated with *Bacillus subtilis*, wherein the ADL fraction level ranged from 9.09% to 9.60%. Lignin degradation by bacteria has already been mentioned above [42]. On the other hand, a lack of BCAs in the solution caused a significant increase in the level of this fiber fraction (from 10.74% to 11.65%) in sprouts. The negative effect of algae extract on the lignin level in the biomass of two grass species (orchard grasses and Braun's festulolium) was reported previously by Ciepiela et al. [43]. In the mentioned study foliar application of *Ecklonia maxima* algae extract resulted in a decrease in the level of this fiber fraction, from 43.6 g/kg to 39.7 g/kg dry matter (d.m.). This negative change in the level of lignin and cellulose in young sprouts not only affects their nutritional value, but also affects the maintenance of upright position in young plants, which is especially important in the production of microgreens and "Baby leaf".

Statistical analysis of the ADL fraction in buckwheat sprouts also confirmed an effect of interaction between the factors studied. The use of solutions containing only PGPs resulted in an ADL level similar to control in both cultivars. However, the presence of *Pythium oligandrum* oospores in the solution changed the relations between the levels of ADL fractions, reducing ADL in Panda sprouts and increasing it in Kora (Table 3).

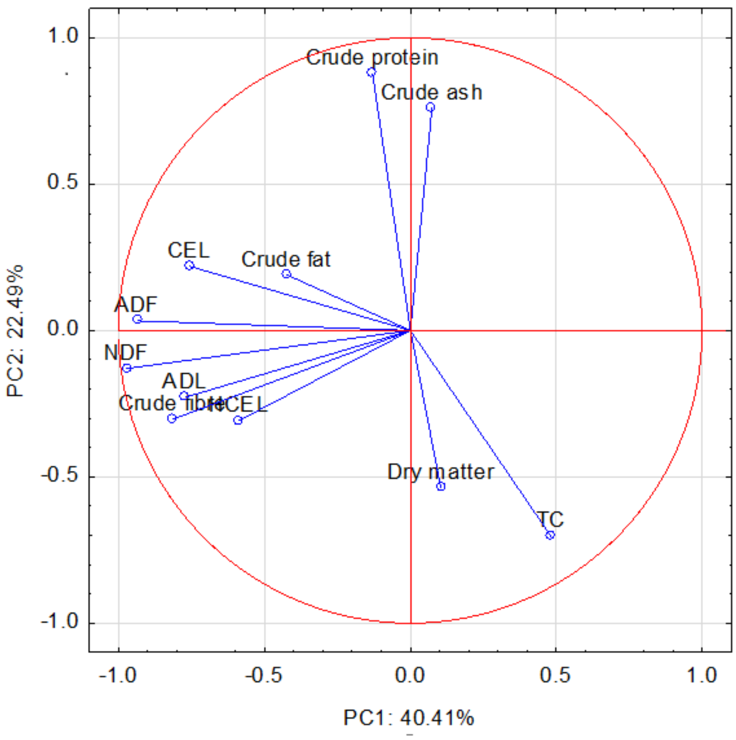
Likewise, the level of hemicellulose in buckwheat sprouts was altered by the composition of solutions (Table 3). The lowest level of hemicellulose was observed after soaking seeds in the solution containing *Ecklonia maxima* algae extract and *Bacillus subtilis* bacteria. The highest level of this fraction of dietary fiber was found in control buckwheat sprouts. Similar to our results, a study by Ciepiela et al. [43] showed the negative impact of an algae extract on the hemicellulose level in the biomass of two grass species. The average hemicellulose level in the biomass of both grass species in the control condition was 198 g/kg d.m. and after foliar application of an algae extract, it decreased to 181 g/kg d.m., with the difference being statistically significant. The concentration of this fiber fraction in grass was not comparable to that found in buckwheat sprouts, but the effect of algae extract on the level of this component was applicable.

The studied buckwheat cultivars, when soaked in PGP and/or BCA solutions, showed a different reaction than controls. Sprouts of the Panda cultivar generally contained significantly more hemicellulose than sprouts of the Kora cultivar, with this difference magnified by most soaking solutions. The exception was the solution containing only *Pythium oligandrum* oospores, which resulted in lower levels in the sprouts of the Panda cultivar compared to the Kora cultivar (Table 3).

The level of cellulose in sprouts was also affected by the composition of the solution used for seeds soaking (Table 3). The lowest level of cellulose was observed after soaking the seeds in a solution containing *Ecklonia maxima* algae extract and *Bacillus subtilis* bacteria, and the highest for nitrophenols and *Pythium oligandrum* oospores. This was in line with the results of the aforementioned study by Ciepiela et al. [43], who report that spraying grasses with an algae extract also reduced the cellulose level in biomass from 303 g/kg d.m. to 284 g/kg d.m.

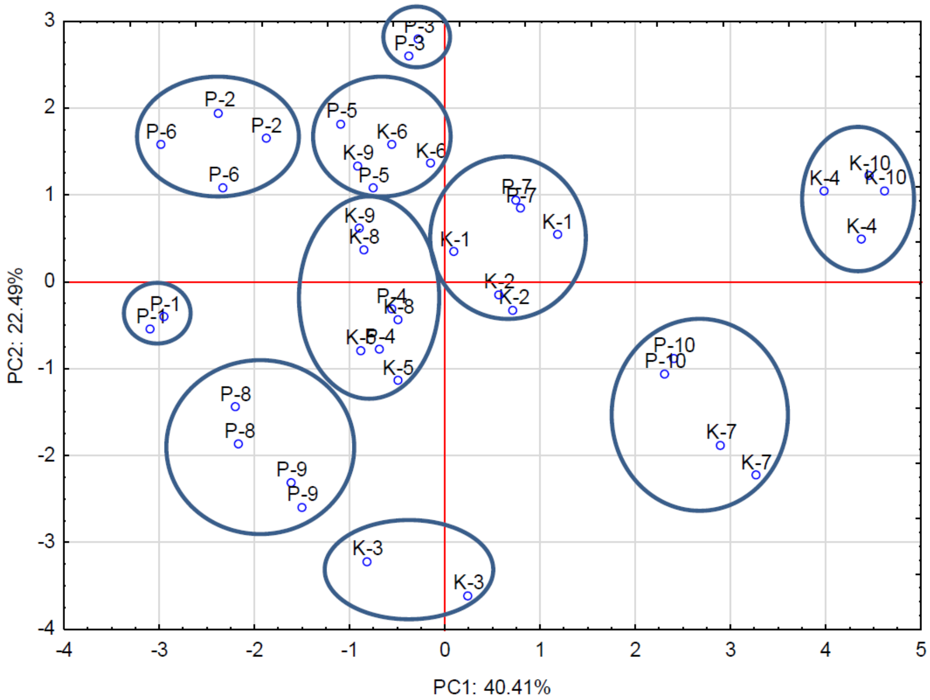
The interactions between the examined factors allowed us to state that cellulose levels in the tested buckwheat cultivars reacted differently to changes in the composition of the solutions used to soak seeds (Table 3). Nitrophenols combined with *Bacillus subtilis* bacteria or with *Ecklonia maxima* algae extract caused a reaction similar to that observed in controls—namely, higher levels in Kora sprouts. In the other treatment conditions, the reaction of cultivars was different, as a higher level of cellulose was observed in the sprouts of the Panda cultivar.

Biplot analysis was used to confirm the relationships between the buckwheat seed treatments and chemical composition of buckwheat sprouts, which were expressed in analyses of variance. The first two principal components (PCs) of the method explained 62.90% of the variance of the original variables (Figure 1). This analysis was a good approach to identify better chemical composition of buckwheat sprouts dependently on seeds treatment. Biplot analysis confirmed correlation between the studied traits (Figure 1A). Acute angles between the trait vectors means the positive correlation, obtuse angles means negative correlation and right angle means no correlation. The PCAs projection of the traits data showed a very different trend depending on both cultivar and treatment. When both axes were considered simultaneously, few groups were identified. The cultivar /treatment objects with lower PC 1 were superior in terms of fiber fractions. The objects with upper PC 1 and lower PC 2 were better in terms of dry matter and TC concentration (sprouts of Kora grew out from seed soaking with seaweed *Ecklonia maxima* extract and *Bacillus subtilis* or sprouts of Panda grew out in control condition) (Figure 1B). The designated groups of data consist of both cultivars in different way prepared by soaking. It confirmed the interactions of main factors which was in line with analyses of variance.



(**A**)

Figure 1. *Cont.*



(**B**)

Figure 1. Biplot based on first two principal component axes for chemical composition of buckwheat sprouts (**A**) and distribution of two cultivars on buckwheat sprouts based on the first two components obtained from principal component analysis (**B**). K denotes cultivar Kora, P denotes cultivar. Panda and number from 1 to 10 denote buckwheat seed treatments precisely described in Materials and Methods.

4. Conclusions

This study confirms a significant effect of biological control agents and plant growth promoters on the level of basic nutrients, crude fiber, and dietary fiber fractions in buckwheat sprouts. Sprouts t of the Kora cultivar contained more crude ash and total carbohydrates, while sprouts of the Panda cultivar contained significantly more protein, fat, crude fiber and all its fractions. The low proportion of hemicellulose in the total fiber of buckwheat sprouts distinguishes it from the dietary fiber derived from cereal seeds. Soaking the seeds in a solution containing *Ecklonia maxima* algae extract promoted the accumulation of dry matter in sprouts, while the use of *Bacillus subtilis* bacteria resulted in a decrease in the dry matter level. The use of *Pythium oligandrum* oospore solution reduced the ash level in sprouts, while the addition of nitrophenols increased the level of both ash and protein. The presence of only *Bacillus subtilis* bacteria in the solution enabled the production of sprouts with the lowest crude fiber content.

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