The Influence of Biostimulants Used in Sustainable Agriculture for Antifungal Protection on the Chemical Composition of Winter Wheat Grain

Ewa Szpunar-Krok 1,*, Joanna Depciuch 2, Barbara Drygaś 3, Marta Jańczak-Pieniążek 1, Katarzyna Mazurek 4 and Renata Pawlak 5

1 Department of Crop Production, University of Rzeszow, Zelwerowicza 4 St., 35-601 Rzeszow, Poland
2 Institute of Nuclear Physics, Polish Academy of Sciences, 31-342 Krakow, Poland
3 Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, College of Natural Science, University of Rzeszow, Ćwiklińskiej 2D St., 35-601 Rzeszow, Poland
4 PTWP SA Warsaw Branch, Jana Pawła II 29 St., 00-867 Warsaw, Poland
5 Biostyma Sp. z o.o., Sikorskiego 38 St., 62-300 Września, Poland
* Correspondence: eszpunar@ur.edu.pl

Abstract: Field studies were conducted from 2016 to 2019 (south-eastern Poland; 49°58′40.6″ N 22°33′11.3″ E) with the aim to identify the chemical composition of winter wheat grain upon foliar application of biostimulants, of which PlanTonic BIO (containing nettle and willow extracts) showed antifungal activity. The main chemical compositions and their spatial distribution in wheat grain were characterized by Raman spectroscopy technique. It was established that applied biostimulants and hydro-thermal conditions changed the chemical composition of the grain during all the studied years. A similar chemical composition of the grain was achieved in plants treated with synthetic preparations, including both intensive and extensive variants. The second group, in terms of an increase in fatty acid content, consists of grains of plants treated with biostimulants PlanTonic BIO, PlanTonic BIO + Natural Crop and PlanTonic BIO + Biofol Plex. The future of using biostimulants in crop production, including those containing salicylic acid and nettle extracts, appears to be a promising alternative to synthetic crop protection products.

Keywords: plant protection; biostimulants; Triticum aestivum L.; grain quality; Raman spectroscopy

1. Introduction

Wheat is among the most significant crops grown worldwide, with a cultivated area of 219 million hectares [1,2]. The plant is important because of its high yield, chemical composition and technological properties of the grain [3,4]. Wheat grain is rich in carbohydrates and has a higher protein content than other grains, and contains large amounts of minerals (Zn, Fe) and vitamins, making it a valuable food source [5,6]. Wheat flour has unique viscoelastic properties that allow it to be processed as dough for bread, pasta and other food products due to the presence of the gluten fraction. Gluten is a mixture of proteins formed after dough hydration and is responsible for its structure [7,8]. The main goal of growing wheat is not only to obtain a high grain yield but also to improve its quality. Grain yield and quality depend on the interaction between environmental, agronomic and biological factors [9,10]. Weather conditions and, in particular, the distribution of precipitation and temperatures prevailing during the growing season largely contribute to the formation of grain yield and quality [11,12]. The growth and development of plants grown under stressful conditions, such as drought, waterlogging, excessively high or low temperatures, and deficiency or excess mineral compounds in the substrate that negatively affect their metabolism, leading to weakened growth and, consequently, reduced yields [13]. Most grains show strong sensitivity to abiotic stress early in pollen development [13,14]. This can result in irregularities at the stage of grain formation and unfavourable changes...
in the chemical composition of the grain. These parameters are particularly adversely affected by fungal diseases and pests, the presence of which is a major constraint on wheat production [15].

With the growing interest in reducing the use of chemicals in plant cultivation, such as fertilizers and pesticides, a recent priority has been to find environmentally friendly ways to promote plant growth and development and increase crop yields. The increase in consumer demand for foods that do not contain potentially toxic residues is also not insignificant [16]. Therefore, the use of natural plant growth enhancers [17], referred to as biostimulants, which can be made from a variety of products (seaweeds, protein hydrolysates, humic substances, and microorganisms [18–20]), and which improve crops without causing unwanted side effects, is becoming increasingly important [21,22]. Biostimulants can perform numerous agronomic functions. Their use in crop cultivation contributes to increased crop yields while reducing dependence on chemical fertilizers. They influence basic processes and defence mechanisms in plants, enabling them to maintain homeostasis to ensure long-term adaptation, medium-term acclimatization and short-term response to changing environmental conditions [22]. This makes them safer for the environment and contributes to sustainable crop production [23]. They differ from fertilizers in that they do not directly provide nutrients but promote in plants an improvement in the efficiency of nutrient uptake and assimilation [24], grain yield quality traits [25], and increase tolerance to biotic stresses (pest and pathogen activity) and abiotic stresses (drought, frost, salinity, heavy metal environmental pollution, etc.) [18,21,26–28]. Metabolites in biostimulants protect plants from environmental stresses, mainly by activating plant secondary metabolite pathways and mobilizing signalling molecules to activate defence responses [29]. The role of biostimulants in crop cultivation is growing, as indicated by the value of the global market for these products [30].

Biostimulants showing antifungal activity include salicylic acid (SA) and plant extracts, including aqueous extracts of nettle (Urtica dioica L.). SA is a phenolic compound synthesized by plants. In the conducted experiment, SA and extract of nettle were included in PlanTonic BIO.

Knowledge of the chemical composition of cereal grains, including wheat protected with biostimulants showing antifungal activity, analyzed using Raman spectroscopy, is still insufficient so far, so the present study was undertaken. Raman spectroscopy is a technique used in biological, biomedical, food and agricultural research that allows a simultaneous analysis of various chemical compounds and an evaluation of molecular changes occurring in the objects under study [31,32]. It involves measuring the radiation of so-called Raman scattering (inelastic scattering of photons); it is a method for studying the rotational and oscillatory spectra of molecules. With Raman scattering, it is possible to obtain information on the structure of a molecule, which allows the chemical and structural characterization and identification of complex biological material. The main advantages of this analytical technique include the accuracy of the measurements, the large amount of information obtained (at a relatively low cost), the ability to examine the sample without complex preparation and processing, and non-destructiveness [33,34].

Previous field studies with biostimulants are based mainly on the effect of these products on crop yields [35]; however, there is a lack of data on their effect on the chemical composition. The purpose of this research was to determine the effect of foliar application of biostimulants, including PlanTonic BIO, which contains salicylic acid and aqueous nettle extracts with antifungal activity, on the chemical composition of winter wheat grain using Raman spectroscopic technique. A comparison of the chemical composition of plant grains treated with biostimulants and plant grains treated with synthetic fungicides was also made.
2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment with winter wheat (*Triticum aestivum* L. subsp. *aestivum*) of the cultivar Hondia (breeder DANKO Plant Breeding, Choryń, Poland; a company belonging to the National Agricultural Support Center) was conducted in the growing seasons from 2016/2017 to 2018/2019, in the village of Pelnatycz (south-eastern Poland; 49°58′40.6″ N 22°33′11.3″ E).

A factor of the experiment was the various options of plant protection against diseases caused by fungi, including protection with synthetic preparations and biostimulants. The experimental sites are summarized in Table 1, and the chemical composition of the biostimulants is given in Table 2. The biostimulant showing antifungal activity was PlanTonic BIO. Due to the phytotoxicity of SA observed on plants [36,37], plant growth stimulants were additionally used to alleviate possible plant stress after its application in selected variants (5 and 6): BioFol Plex and Natural Crop SL.

Table 1. Conventional plant protection products and biostimulants used in the cultivation of winter wheat—doses and dates of application.

| Plant Protection | Treatment Time (BBCH Scale) | Trade Name       | Active Substance (g dm⁻³) | Terms and Doses | Preparation (dm³ ha⁻¹) | Active Substance (gha⁻¹) |
|------------------|-----------------------------|------------------|---------------------------|-----------------|------------------------|--------------------------|
| (1) Control      | -                           | -                | -                         | -               | -                      | -                        |
| (2) Intensive    | 31–33                       | Duett Star 334 SE (BASF) | Fenpropimorph (250)       | 1.0             | 250                    | 84.0                     |
|                  |                              |                   | Epoxiconazole (84)        |                 |                        |                          |
|                  | 45–47                       | Acanto 250 SC (DuPont) | Azoxystrobin (250)        | 0.6             | 150                    |                          |
|                  |                              | Bumper 250 SC (ADAMA) | Propiconazole (250)       | 0.7             | 175                    |                          |
|                  | 65–69                       | Mystic 250 EC (Nufarm) | Tebuconazole (250)        | 0.9             | 225                    |                          |
| (3) Extensive    | 31–33                       | Duett Star 334 SE (BASF) | Fenpropimorph (250)       | 1.0             | 250                    | 84.0                     |
|                  |                              |                   | Epoxiconazole (84)        |                 |                        |                          |
|                  | 45–47                       | Acanto 250 SC (DuPont) | Azoxystrobin (250)        | 0.6             | 150                    |                          |
|                  |                              | Bumper 250 SC (ADAMA) | Propiconazole (250)       | 0.7             | 175                    |                          |
| (4) PlanTonic BIO| 31–33                       | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  | 45–47                       |                   |                           | 4.0             |                        |                          |
|                  | 65–69                       |                   |                           | 4.0             |                        |                          |
| (5) PlanTonic BIO + Natural Crop | 31–33     | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  |                              | Natural Crop SL   |                           | 1.5             |                        |                          |
|                  | 45–47                       | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  |                              | Natural Crop SL   |                           | 1.5             |                        |                          |
|                  | 65–69                       | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  |                              | Natural Crop SL   |                           | 1.5             |                        |                          |
| (6) PlanTonic BIO + BioFol Plex | 31–33   | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  |                              | BioFol Plex       |                           | 2.0             |                        |                          |
|                  | 45–47                       | PlanTonic BIO     |                           | 4.0             |                        |                          |
|                  |                              | BioFol Plex       |                           | 2.0             |                        |                          |
|                  | 65–69                       | PlanTonic BIO     |                           | 4.0             |                        |                          |
Table 2. Chemical composition of biostimulants used in the experiment.

| Biostimulators                                | Biostimulator Characteristics                                                                 | 
|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| BioFol Plex (Biostyma, Września, Poland)      | Complexed with humic acids                                                                                                   |
| Natural Crop SL (Natural Crop, Italy)         | Concentrate of peptides and L-amino acids obtained by enzymatic hydrolysis of collagen                                        |
| PlanTonic Bio (OGET Innovations GmbH, Allerheiligen bei Wildon, Austria) | Nettle (*Urtica dioica* L.) extract, willow (*Salix* sp.) extract, sunflower (*Helianthus annuus* L.) oil                     |

The experiment was implemented as a field experiment. Each variant of the experiment included a wheat canopy of 200 m², which was divided into 4 smaller experimental units—plots (repetitions for a given variant), each of 50 m².

Fertilisers were soil- and foliar-applied. The doses and dates of fertilizer application are presented in Table 3. The following amounts of nutrients were provided with the fertilizers:

- **Soil application** (kg ha⁻¹ year⁻¹)—189 N, 70 P₂O₅, 105 K₂O, 135 SO₃;
- **Foliar application** (g ha⁻¹ year⁻¹)—59 N, 260 P₂O₅, 338.5 K₂O, 669.5 MgO, 1450.4 SO₃, 23.65 Fe, 2.95 B, 19.55 Cu, 88.2 Mn, 9.25 Zn, 0.79 Mo.

Table 3. Doses and dates of application of fertilisers.

| Type of Fertiliser | Trade Name | Chemical Composition | Fertiliser Dose (kg ha⁻¹) | Dose (ha⁻¹ Year⁻¹) | Application Term |
|--------------------|------------|----------------------|---------------------------|-------------------|------------------|
| Soil-applied       | Polifoska 6| 6% N-NH₄⁺, 20% P₂O₅, 30% K₂O, 2% SO₃ | 350                       | 21 kg N, 70 kg P₂O₅, 105 kg K₂O, 24.3 kg SO₃ | before sowing |
|                    | Saletrosan® 26 | 26% N (including 19% N-NH₄⁺, 7% N-NO₃⁻, 32.5% SO₃) | 300                       | 88.4 kg N, 110.5 kg SO₃ | BBCH 25-27 |
|                    | Zaksan® | 32% N (including 16% N-NH₄⁺, 16% N-NO₃⁻) | 250                       | 80 kg N | BBCH 33-35 |
| Foliar fertilisers | GranuFol Fosfor | 10.0% N, 41% P₂O₅, 12% K₂O, 2.3% MgO, 2.5% SO₃, 0.03% B, 0.03% Cu, 0.16% Fe, 0.07% Mn, 0.002% Mo, 0.07% Zn | 1.0 | 10 g N, 410 g P₂O₅, 120 g K₂O, 23 g MgO, 25 g SO₃, 0.3 g B, 0.3 g Cu, 1.6 g Fe, 0.7 g Mn, 0.02 g Mo, 0.7 g Zn | BBCH 31-32 |
|                    | GranuFol CuMan | 43.3% SO₃, 5% Cu, 25% Mn | 0.3 | 129.9 g SO₃, 15 g Cu, 75 g Mn | |
|                    | Wuxal mikro | 5% N, 10% K₂O, 3% MgO, 5.2% S, 0.3% B, 0.5% Cu, 1% Fe, 1.5% Mn, 0.01% Mo, 1% Zn | 0.5 | 39 g N, 78.5 g K₂O, 23.5 g MgO, 40.5 g S, 2.35 g B, 3.95 g Cu, 7.85 g Fe, 11.8 g Mn, 0.75 g Mo, 7.85 g Zn | BBCH 31-32 |
|                    | Granufol Mag | 20% MgO, 41% SO₃, 0.42% Fe | 1.5 | 600 g MgO, 1230 g SO₃, 12.6 g Fe | BBCH 31-32 BBCH 54-55 |
|                    | Granufol Potas | 10.0% N, 12% P₂O₅, 41% K₂O, 2.3% MgO, 2.5% SO₃, 0.03% B, 0.03% Cu, 0.16% Fe, 0.07% Mn, 0.002% Mo, 0.07% Zn | 1.0 | 10 g N, 120 g P₂O₅, 410 g K₂O, 23 g MgO, 25 g SO₃, 0.3 g B, 0.3 g Cu, 1.6 g Fe, 0.7 g Mn, 0.02 g Mo, 0.7 g Zn | BBCH 54-55 |

The forecrop for winter wheat was winter rapeseed. Wheat was sown in the last weeks of September 2016, 2017 and 2018 (the optimal date for this region of Poland), at a density of 350 grains m⁻². The seed was treated with a seed treatment (Scenic 080 FS: fluoxastrobin 37.5 g dm⁻³, prothioconazole 37.5 g dm⁻³, and tebuconazole 5.0 g dm⁻³). The crop was harvested in the second or third week of August at full maturity (BBCH 97, plant dead and collapsing).

2.2. Soil Conditions

According to the FAO/WRB classification [38], the soil type was Haplic Cambisol (Eutric) formed from loess. At the start of the experiment, the abundance of available
forms of P (5.84–6.32 mg 100 g⁻¹) and K (13.1–18.7 mg 100 g⁻¹) were average, and Mg (14.5–17.1 mg 100 g⁻¹) was very high.

2.3. Weather Conditions

Weather conditions were given according to the records of the Experimental Station for Variety Testing in Skołoszów (49°53’ N, 22°44’ E, altitude 230 m), Poland. The course of weather conditions in the research years was variable. Annual precipitation was higher in 2016 and 2019 (by 21.3 and 8.9%, respectively) and lower in 2017 (by 6.3%) compared to the average for 1980–2015, while in 2018, annual precipitation was close to the multi-year average. In all years of the study, the average annual air temperature was higher than average for the years 1980–2015.

Thermal and rainfall conditions during the growing season of the plants were evaluated based on Sielianinov’s hydro-thermal coefficient K (Figure 1). According to this criterion, the vegetation of winter wheat experienced water scarcity conditions from May 2017 until harvest (relatively dry—May, dry—June and July, extremely dry—August). Deficiencies in spring precipitation also occurred in 2018 (very dry—April and dry—May), while hydro-thermal conditions were favourable for wheat in the subsequent period. For this reason, the course of hydro-thermal conditions in 2018 was considered the most favourable for wheat during the entire research period. In contrast, in 2019, thermal and rainfall conditions in April were optimal, and May was extremely humid, after which moisture conditions deteriorated later in the growing season (extremely dry—June, rather dry—July).

![Figure 1. Sielianinov’s hydro-thermic coefficients K described for Poland by Skowera et al. [39]: K ≤ 0.4 extremely dry (ed), 0.4 < K ≤ 0.7 very dry (vd), 0.7 < K ≤ 1.0 dry (d), 1.0 < K ≤ 1.3 relatively dry (rd), 1.3 < K ≤ 1.6 optimal (o), 1.6 < K ≤ 2.0 relatively humid (rh), 2.0 < K ≤ 2.5 humid (h), 2.5 < K ≤ 3.0 very humid (vh), K > 3.0 extremely humid (eh).](image-url)

2.4. Analysis of the Chemical Composition of the Grain

Unground wheat grain was used to assess chemical composition using Raman spectroscopy. The Raman spectra were taken with a Nicolet NXR 9650 FT-Raman spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an Nd laser: YAG (1064 nm) and germanium detector. Measurements were carried out in the range from 150 to 3700 cm⁻¹ at a laser power of 1 W. An out-of-focus laser beam with a diameter of about 100 μm and a spectral resolution of 8 cm⁻¹ was used. Each spectrum was collected using 128 scans. The Raman spectra were analyzed using OPUS 7.0.129 software.
2.5. Statistical Analysis

To determine the regularity between the variables (to verify that the changes in chemical composition occurring between the study groups, as measured by Raman spectroscopy, are distinguishable), principal component analysis (PCA) was performed. Obtained Raman spectra give a large amount of data. Therefore, it is necessary to show the most important data. Consequently, PCA was chosen because it is a method for analyzing large datasets with a high number of features per observation. PCA algorithm can increase the interpretability of data, while preserving the maximum amount of information and enabling the visualization of multidimensional data. To summarize, PCA is a statistical technique for reducing the dimensionality of a dataset. In the presented study, we used so-called “fingerprint” region, i.e., between 800 cm\(^{-1}\) and 1800 cm\(^{-1}\), for PCA analysis. Consequently, we used 259 data from each obtained spectrum. Furthermore, to show which groups and samples were similar to each other, hierarchical cluster analysis (HCA) was performed using the same data as for PCA analysis. HCA analysis was conducted using paired group (UPGMA) algorithm with Euclidean similarity index. Both analyses were carried out using Past 3.0 software.

3. Results and Discussion

The present study investigated the possibility of using the Raman spectroscopy technique to evaluate the effect of the foliar application of crop protection products on winter wheat grain quality. Among the measures used, in addition to standard fungicide protection, biostimulants that are increasingly common in modern agriculture were used. In particular, their role is to maximize yields and improve quality, especially under unfavourable environmental conditions for plant growth and development [16,21].

Conventional methods for identifying the chemical composition of cereal grains are time-consuming and labour-intensive, making it difficult to analyze large quantities of samples quickly and economically [40]. The Raman spectroscopy technique provides valuable information on grain chemical composition (including the presence of starch, lipids, protein, cellulose and lignin), as indicated by a study by Yang, et al. [41] performed on corn grain. A measurement using the Raman spectroscopy technique was also performed to identify ash and protein in flour obtained from wheat grain in a study by Czaja, et al. [42].

In the conducted experiment, the Raman spectra of wheat grains showed areas characteristic of vibrations of functional groups that build nucleic acids, sugars, proteins and fats. The characteristic Raman intensities of the measured grains are (Figure 2a):

- 810–975 cm\(^{-1}\) (stretching vibration of C-C group);
- 920–960 cm\(^{-1}\) (vibration of C-C group of amylose);
- 1190 cm\(^{-1}\) (vibration of C-C group of sugars);
- 1260 cm\(^{-1}\) (vibration of III-row amide—protein);
- 1382–1338 cm\(^{-1}\) vibration of the secondary structure of proteins (α-helix);
- 1455 cm\(^{-1}\) (vibration of C-H group of proteins and sugars);
- 1550 cm\(^{-1}\) (vibration of protein groups included in amyllopectins);
- 1637 cm\(^{-1}\) (vibration of protein groups included in amyllopectins);
- 1740 cm\(^{-1}\) (vibration of C-O group of proteins and fats);
- 2800–3000 cm\(^{-1}\) (stretching vibration of C-H groups included in carbon chains of fatty acid residues) [42,43].
Conventional methods for identifying the chemical composition of cereal grains are time-consuming and labour-intensive, making it difficult to analyze large quantities of samples quickly and economically [40]. The Raman spectroscopy technique provides valuable information on grain chemical composition (including the presence of starch, lipids, protein, cellulose and lignin), as indicated by a study by Yang, et al. [41] performed on corn grain. A measurement using the Raman spectroscopy technique was also performed to identify ash and protein in flour obtained from wheat grain in a study by Czaja, et al. [42].

In the conducted experiment, the Raman spectra of wheat grains showed areas characteristic of vibrations of functional groups that build nucleic acids, sugars, proteins and fats. The characteristic Raman intensities of the measured grains are (Figure 2a):

- 810–975 cm$^{-1}$ (stretching vibration of C-C group);
- 920–960 cm$^{-1}$ (vibration of C-C group of amylose);
- 1190 cm$^{-1}$ (vibration of C-C group of sugars);
- 1260 cm$^{-1}$ (vibration of amide—protein);
- 1382–1338 cm$^{-1}$ vibration of the secondary structure of proteins ($\alpha$-helix);
- 1455 cm$^{-1}$ (vibration of C-H group of proteins and sugars);
- 1550 cm$^{-1}$ (vibration of protein groups included in amylopectins);
- 1637 cm$^{-1}$ (vibration of protein groups included in amylopectins);
- 1740 cm$^{-1}$ (vibration of C-O group of proteins and fats);
- 2800–3000 cm$^{-1}$ (stretching vibration of C-H groups included in carbon chains of fatty acid residues) [42,43].

Figure 2. FT-Raman spectra with marked analyzed region (a) for wheat grown in 2017 (b), 2018 (c), and 2019 (d); crop protection variants: (1) control (black line); (2) intensive (red line); (3) extensive (green line); (4) PlanTonic BIO (blue line); (5) PlanTonic BIO + Natural Crop (azure line); (6) PlanTonic BIO + Biofol Plex (pink line).

Compared to the control (wheat grain from facilities without antifungal plant protection) (Figure 2b,d), for 2017 and 2019, grain from the crop with plant protection showed a higher content of C-C group stretching vibrations (810–975 cm$^{-1}$), C-C group vibrations from amylose (920–960 cm$^{-1}$), C-C group vibrations from sugars (1190 cm$^{-1}$), protein-specific amide III vibrations (1260 cm$^{-1}$), protein secondary structure ($\alpha$-helix) vibrations (1382, 1338 cm$^{-1}$), C-H group vibrations from proteins and sugars (1455 cm$^{-1}$), and a lower content of vibrations of protein groups included in amylopectins (1550 cm$^{-1}$, 1637 cm$^{-1}$), vibrations of C-O group of proteins and fats (1740 cm$^{-1}$), stretching vibrations of C-H groups included in carbon chains of fatty acid residues (2800–3000 cm$^{-1}$). A larger decrease in fat functional groups is observed for 2019. Summarizing the Raman spectroscopic measurements of 2017 and 2019, the application of selected plant protection to wheat resulted in an increase in amylose (included in starch) and other sugars and proteins, and a decrease in amylopectin (included in starch) and fat. With regard to the controls, the grains of winter wheat grown in 2018 (Figure 2c), variants 3, 4, 5 and 6, were characterized by lower contents of C-C group stretching vibrations (810–975 cm$^{-1}$), C-C group vibrations from amylose (920–960 cm$^{-1}$), C-C group vibrations from sugars (1190 cm$^{-1}$), vibrations of the third-row amide—protein (1260 cm$^{-1}$), vibrations of the secondary structure of proteins (\textit{\(\alpha\)}-helix) (1382, 1338 cm$^{-1}$), vibrations of the C-H group from proteins as well as sugars (1455 cm$^{-1}$); a higher content of vibrations of protein groups included in amylopectins.
(1550 cm\(^{-1}\), 1637 cm\(^{-1}\)), vibrations of the C-O group building proteins as well as fats (1740 cm\(^{-1}\)). Furthermore, variant 2 showed very similar contents of chemical compounds whose functional groups can be attributed to Raman shift values up to 1740 cm\(^{-1}\). In the case of the stretching vibrations of C-H groups included in the carbon chains of fatty acid residues (2800–3000 cm\(^{-1}\)), it can be seen that intensive protection causes a decrease in their content. In variants 4, 5 and 6, an increase in the content of a stretching C-H groups included in the carbon chains of fatty acid residues (2800–3000 cm\(^{-1}\)) was observed, while variant 3 did not change the amount of these functional groups in comparison with control one. Summarizing the Raman measurements for 2018, the use of synthetic preparations in variant 2 resulted in a decrease in fatty acids, while in variant 3, it led to an increase in amylopectins, proteins and fat and a decrease in sugars and some proteins.

On average, the analysis of the chemical composition in our study showed an increase in amylose, sugars and proteins and a decrease in fatty acids as a result of the application of crop protection products. A similar relationship was also obtained by Ciolek et al. \[44\] in a study that compared the impact of growing crops under organic and conventional systems. The grain from cultivation in an organic system, where chemical protection was not used, had a higher content of fatty acids, especially valuable unsaturated acids. The protein content of the wheat grain is an important determinant of its quality, determining the nutritional and rheological properties of dough, which is of great importance in the face of a changing climate. Variable weather conditions prevailing during the experiment may have significantly affected the formation of quality indicators in wheat grain \[45,46\]. Under the influence of rainfall deficiency and increased temperature, the protein content of the grain increases, as also shown by Hotea et al. \[47\]. In our study, such a relationship was observed in 2019, when there was a shortage of precipitation in the grain filling phase. The most favourable conditions for starch accumulation in wheat grain prevailed in 2019. Similar to Cociu and Alionte \[48\], it has been shown that greater starch accumulation is particularly favoured by warm and dry conditions during grain maturation.

The paper also presents the results of a principal component analysis (PCA), the preferred method for extracting features that is used to reduce the size of a set of features \[49\], and a hierarchical cluster analysis (HCA) is used to group them \[50\]. PCA analysis shows that each of the crop protection options significantly affects the chemical composition of winter wheat grain. This is a trend observed in each year of the study. In addition, in 2017, a similar effect on chemical composition was found in variants 2 and 3, with synthetic fungicides and biostimulant application in variants 4 and 5 (Figure 3a). In 2018, protection variants 2 and 3 and the biostimulant in variant 4 had similar effects on the chemical composition of wheat grain (Figure 3b). In 2019, similarity in chemical composition was observed for wheat grain treated with biostimulants in variants 4, 5 and 6, as well as in variant 3 using synthetic formulations (Figure 3c).

The results of the HCA analysis for each of the years analyzed indicate the formation of two groups of similar plants (in terms of chemical composition). In addition, each year, the separation of grain from plants without the application of preparations is observed, which means that its chemical composition significantly differs from that observed for grains in facilities with preparations for protection against fungal diseases. In 2017, wheat grains from variants 2 and 3, in which plants were protected with synthetic preparations, were found to be similar in chemical composition. In addition, plants treated with a Plantonic biostimulant in each configuration (variants 4, 5 and 6) show similar grain chemistry (Figure 4a). In 2018, one can see a similarity in the chemical composition of grain after the application of synthetic preparations in variants 2 and 3, and in variants 4, 5 and 6, in which biostimulants were used for plant protection (Figure 4b). In 2019, the first group of similarities is grains from variants 2 and 3, while the second group of similarities is grains after biostimulant application (variants 4, 5 and 6) (trend as in 2017) (Figure 4c).
The results of the HCA analysis for each of the years analyzed indicate the formation of two groups of similar plants in terms of their chemical composition. Wheat grains of plants treated with biostimulants (PlanTonic BIO, PlanTonic BIO + Natural Crop and PlanTonic BIO + Biofol Plex) showed similar grain chemistry compared to synthetic preparations and nettle extracts, appearing to be a promising alternative, despite their often weaker action against phytopathogenic organisms compared to synthetic crop protection products. This research could inspire further studies on the effect of biostimulants on the quality of seed yield in other crop species.

4. Conclusions
The study confirms the applicability of the Raman technique characterized by fast and non-destructive measurements to assess the quality of winter wheat grain. Using this measurement technique, the effect of applied crop protection products, including biostimulants, and hydro-thermal conditions during the years of the study on the chemical composition of wheat grain was demonstrated. It was indicated that hydro-thermal conditions and applied biostimulants changed the chemical composition of the grain during the studied years. The results of the HCA analysis for each of the years analyzed indicate the formation of two groups of similar plants in terms of their chemical composition. Wheat grains of plants treated with synthetic preparations in variants with intensive and extensive protection were found to be similar in chemical composition. The second group of similarities is grains from variants 2 and 3, in which plants were protected with synthetic preparations, as in variant 3 using synthetic formulations (Figure 3c). In 2017, a similar effect on chemical composition was found in variants 2 and 3, with synthetic fungicides and biostimulant application in variants 4 and 5 (Figure 3a). In 2018, one can see a similarity in the chemical composition of grain after biostimulant application (variants 4, 5 and 6) (trend as in 2017) (Figure 4c).

Figure 3. PCA analysis of FT-Raman spectra of wheat grains grown in 2017 (a), 2018 (b), 2019 (c); crop protection variants: (1) control (black dot); (2) intensive (red dot); (3) extensive (green dot); (4) PlanTonic BIO (blue dot); (5) PlanTonic BIO + Natural Crop (azure dot); (6) PlanTonic BIO + Biofol Plex (pink dot).

Figure 4. HCA analysis of FT-Raman spectra of wheat grains grown in 2017 (a), 2018 (b), 2019 (c); crop protection variants: control (1); intensive (2); extensive (3); Plantonic (4); Plantomic + Natural Crop (5); Plantonic + Biofol Plex (6).
Author Contributions: Conceptualization, E.S.-K.; methodology, E.S.-K. and J.D.; formal analysis, J.D.; investigation, E.S.-K., R.P., M.J.-P., B.D. and K.M. (field experiment) and J.D. (laboratory experiment); writing, E.S.-K., J.D., B.D. and M.J.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: During the research conducted from 2016 to 2019 (the results of which are included in the manuscript), Dr. Renata Pawlak was a PhD student at the University of Rzeszów and did not work for the Biostyma company. She started working for the Biostyma company in 2021. The authors declare no conflict of interest.

References

1. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 8 August 2022).
2. Jarecki, W.; Czernicka, M. Yield and quality of winter wheat (Triticum aestivum L.) depending on multi-component foliar fertilization. J. Elem. 2022, 27, 559–567. [CrossRef]
3. Zargar, M.; Polityko, P.; Pakina, E.; Bayat, M.; Vandyshhev, V.; Kavhiza, N.; Kiselev, E. Productivity, quality and economics of four spring wheat (Triticum aestivum L.) cultivars as affected by three cultivation technologies. Agron. Res. 2018, 16, 2254–2264. [CrossRef]
4. Brouns, F.; van Rooij, G.; Shewry, P.; Rustgi, S.; Jonkers, D. Adverse reactions to wheat or wheat components. Compr. Rev. Food Sci. Food Saf. 2019, 18, 1437–1452. [CrossRef] [PubMed]
5. Zörb, C.; Ludewig, U.; Hawkesford, M.J. Perspective on wheat yield and quality with reduced nitrogen supply. Trends Plant Sci. 2018, 23, 1029–1037. [CrossRef] [PubMed]
6. Horvat, D.; Dvokjović, K.; Novoselović, D.; Tucak, M.; Andrić, L.; Magdić, D.; Drezner, G. Response of wheat yield and protein-related quality on late-season urea application. Agronomy 2022, 12, 886. [CrossRef]
7. Shewry, P.R. Wheat. J. Exp. Bot. 2009, 60, 1537–1553. [CrossRef]
8. Jańczak-Pieniążek, M.; Buczek, J.; Bobrecka-Jamro, D.; Szpunar-Krok, E.; Tobiasz-Salach, R.; Jarecki, W. Morphophysiology, productivity and quality of soybean (Glycine max (L.) Merr.) cv. Merlin in response to row spacing and seeding systems. Agronomy 2021, 11, 403. [CrossRef]
9. Gasanova, I.; Yerashova, M.; Astakhova, Y.; Drumova, O. Influence of mineral fertilizers and other agrotechnical cultivation methods on yield and grain protein content of winter wheat. Am. J. Agric. For. 2021, 9, 89–94. [CrossRef]
10. Kulyk, M.I.; Rozhkov, A.O.; Kalinichenko, O.V.; Taranenko, A.O.; Onoprienko, O.V. Effect of winter wheat variety, hydrothermal coefficient (HTC) and thousand kernel weight (TKW) on protein content, grain and protein yield. Agron. Res. 2020, 18, 3. [CrossRef]
11. Ljubičić, N.; Popović, V.; Ćirić, V.; Kostić, M.; Ivošević, B.; Popović, D.; Pandžić, M.; El Musafah, S.; Janković, S. Multivariate interaction analysis of winter wheat grown in environment of limited soil conditions. Plants 2021, 10, 604. [CrossRef]
12. Golba, J.; Studnicki, M.; Gozdowski, D.; Madry, W.; Rozbicki, J. Influence of genotype, crop management, and environment on winter wheat grain yield determination based on components of yield. Crop Sci. 2018, 58, 660–669. [CrossRef]
13. Ashraf, M. Stress-Induced Changes in Wheat Grain Composition and Quality. Crit. Rev. Food Sci. Nutr. 2014, 54, 1576–1583. [CrossRef] [PubMed]
14. Dolférs, R.; Ji, X.; Richards, R. Abiotic stress and control of grain number in cereals. Plant Sci. 2011, 181, 331–341. [CrossRef] [PubMed]
15. Figueroa, M.; Hammond-Kosack, K.E.; Solomon, P.S. A review of wheat diseases—A field perspective. Mol. Plant Pathol. 2018, 19, 1523–1536. [CrossRef]
16. Roupheal, Y.; Colla, G. Toward a sustainable agriculture through plant biostimulants: From experimental data to practical applications. Agronomy 2020, 10, 1461. [CrossRef]
17. Mukherjee, D. Microbial Interventions in Soil and Plant Health for Improving Crop Efficiency. In Microbial Interventions in Agriculture and Environment; Singh, D., Prabha, R., Eds.; Springer: Singapore, 2009; pp. 17–47. [CrossRef]
18. Corsi, S.; Ruggeri, G.; Zamboni, A.; Bhakti, P.; Espen, L.; Ferrante, A.; Noseda, M.; Varanini, Z.; Scarafoni, A. A bibliometric analysis of the scientific literature on biostimulants. Crit. Rev. Food Sci. Nutr. 2011, 51, 331–341. [CrossRef] [PubMed]
19. Szczepanek, M.; Wszelaczycyńska, E.; Pobereży, J. Effect of seaweed biostimulant application in spring wheat. AgroLife Sci. J. 2018, 7, 131–136. Available online: https://www.cabdirect.org/cabdirect/abstract/20183195796 (accessed on 15 August 2022).
20. Lamparski, R.; Szczepanek, M. Effect of bioregulator Kelpak application in spring wheat on the occurrence of phytophagous insects. Prog. Plant Prot. 2013, 53, 1. (In Polish) [CrossRef]
21. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hort. 2015, 196, 3–14. [CrossRef]
22. Du Jardin, P.; Xu, L.; Geelen, D. Agricultural functions and action mechanisms of plant biostimulants (PBs): An Introduction. In *The Chemical Biology of Plant Biostimulants*; Geelen, D., Xu, L., Eds.; Wiley Online Library: Hoboken, NJ, USA, 2020; pp. 1–29. [CrossRef]

23. Xu, L.; Geelen, D. Developing biostimulants from agro-food and industrial by-products. *Front. Plant Sci.* **2018**, *9*, 1567. [CrossRef]

24. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in plant science: A global perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [CrossRef] [PubMed]

25. Rouphael, Y.; Colla, G. Editorial: Biostimulants in Agriculture. *Front. Plant Sci.* **2020**, *11*, 40. [CrossRef] [PubMed]

26. Caradonna, F.; Battaglia, V.; Righi, L.; Pascali, G.; La Torre, A. Plant biostimulant regulatory framework: Prospects in Europe and current situation at international level. *J. Plant Growth Regul.* **2019**, *38*, 438–448. [CrossRef]

27. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]

28. Ambrosini, S.; Sega, D.; Santì, C.; Zamboni, A.; Varanini, Z.; Pandolfini, T. Evaluation of the potential use of a collagen-based protein hydrolysate as a plant multi-stress protectant. *Front. Plant Sci.* **2021**, *12*, 63. [CrossRef]

29. Sharma, H.S.S.; Selby, C.; Carmichael, E.; McRoberts, C.; Rao, J.R.; Ambrosino, P.; Chiurazzi, M.; Pucci, M.; Martin, T. Physico-chemical analyses of plant biostimulant formulations and characterisation of commercial products by instrumental techniques. *Chem. Biol. Technol.* **2016**, *3*, 13. [CrossRef]

30. Marketsandmarkets. Global Biostimulants Market (2021–2026) by Active Ingredient, Application Method, Crop Type, Form, Geography and the Impact of COVID-19 with Ansoff Analysis. 2021, p. 138. Available online: https://www.marketresearch.com/Infogeneity-Marketing-Advisory-Services-v4010/Global-Biostimulants-Active-Ingredient-Application-14533191/ (accessed on 16 August 2022).

31. Piot, O.; Autran, J.-C.; Manfait, M. Spatial distribution of protein and phenolic constituents in wheat grain as probed by confocal Raman microspectroscopy. *J. Cereal Sci.* **2000**, *32*, 57–71. [CrossRef]

32. Stawoska, I.; Weselucha-Brzyczka, A.; Skoczowski, A.; Dziurka, M.; Waga, J. FT-Raman spectroscopy as a tool to study the secondary structures of wheat gliadin proteins. *Molecules* **2021**, *26*, 5388. [CrossRef]

33. Yaseen, T.; Sun, D.-W.; Cheng, J.-H. Raman imaging for food quality and safety evaluation: Fundamentals and applications. *Foods* **2017**, *6*, 127–189. [CrossRef]

34. Qin, J.; Chao, K.; Kim, M.S. Raman chemical imaging system for food safety and quality inspection. *Trans. ASABE* **2010**, *53*, 1873–1882. [CrossRef]

35. Pačuta, V.; Rašovský, M.; Michalska-Klimczak, B.; Wyszyński, Z. Grain yield and quality traits of durum wheat (*Triticum durum* Desf.) treated with seaweed- and humic acid-based biostimulants. *Agronomy* **2021**, *11*, 1270. [CrossRef]

36. Uzunova, A.N.; Popova, L. Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. *Photosynthetica* **2000**, *38*, 243–250. [CrossRef]

37. Durango, D.; Pulgarin, N.; Echeverri, F.; Escobar, G.; Quiñones, W. Effect of salicylic acid and structurally related compounds in the accumulation of phytoalexins in cotyledons of common bean (*Phaseolus vulgaris* L.) cultivars. *Molecules* **2013**, *18*, 10609–10628. [CrossRef] [PubMed]

38. World Reference Base for Soil Resources 2014. Update 2015. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. FAO. Available online: http://www.fao.org/fileadmin/templates/nr/images/resources/pdf_documents/wrb2007_red.pdf (accessed on 29 December 2021).

39. Skowera, B.; Jedrzsycz, E.; Kopcińska, J.; Ambroszczyk, A.M.; Kolton, A. The effects of hydrothermal conditions during vegetation period on fruit quality of processing tomatoes. *Pol. J. Environ. Stud.* **2017**, *23*, 195–202. Available online: http://www.ipjjes.com/The-Effects-of-Hydrothermal-Conditions-r-nduring-Vegetation-Period-on-Fruit-Quality,89183,0,2.html (accessed on 16 August 2022).

40. Huang, M.; Wang, Q.; Zhu, Q.; Qin, J.; Huang, G. Review of seed quality and safety tests using optical sensing technologies. *Seed Sci. Technol.* **2015**, *43*, 337–366. [CrossRef]

41. Yang, G.; Wang, Q.; Liu, C.; Wang, X.; Fan, S.; Huang, W. Rapid and visual detection of the main chemical compositions in maize seeds based on Raman hyperspectral imaging. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2018**, *200*, 186–194. [CrossRef]

42. Czaja, T.; Sobota, A.; Szostak, R. Quantification of ash and moisture in wheat flour by Raman Spectroscopy. *Foods* **2020**, *9*, 280. [CrossRef]

43. Wiercigroch, E.; Szafarianiec, E.; Czamara, K.; Pacia, M.Z.; Majzer, K.; Kochan, K.; Kacor, A.; Baranska, M.; Malek, K. Raman and infrared spectroscopy of carbohydrates: A review. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *185*, 317–335. [CrossRef]

44. Ciolek, A.; Makarska, E.; Wesolowski, M. Content of selected nutrients in wheat, barley and oat grain from organic and conventional farming. *J. Elem.* **2012**, *17*, 181–189. [CrossRef]

45. Asseng, S.; Martre, P.; Maiorano, A.; Rötter, R.P.; O’Leary, G.J.; Fitzgerald, G.J.; Girousse, C.; Mottoz, R.; Giunta, F.; Babar, M.A.; et al. Climate change impact and adaptation for wheat protein. *Glob. Change Biol.* **2019**, *25*, 155–173. [CrossRef]

46. Nuttall, J.G.; O’Leary, G.J.; Panozzo, J.E.; Walker, C.K.; Barlow, K.M.; Fitzgerald, G.J. Models of grain quality in wheat—A review. *Field Crops Res.* **2017**, *202*, 136–145. [CrossRef]

47. Hotea, I.; Dragomirescu, M.; Colbar, O.; Tirzii, E.; Herman, V.; Berbeceoa, A.; Radulov, I. The influence of climate conditions and meteorological factors on the nutritional value of wheat (*Triticum aestivum* L.) used for human nutrition, in Romania. IOP Conf. Ser. Earth Environ. Sci. **2021**, *906*, 012019. [CrossRef]
48. Cociu, A.I.; Alionte, E. Effect of different tillage systems on grain yield and its quality of winter wheat, maize and soybean under different weather conditions. *Rom. Agric. Res.* **2017**, *34*, 1222–4227. Available online: [http://www.incda-fundulea.ro/rar.htm](http://www.incda-fundulea.ro/rar.htm) (accessed on 15 August 2022).

49. Hwang, J.; Choi, N.; Park, A.; Park, J.-Q.; Chung, J.H.; Baek, S.; Cho, S.G.; Baek, S.-J.; Choo, J. Fast and sensitive recognition of various explosive compounds using Raman spectroscopy and principal component analysis. *J. Mol. Struct.* **2013**, *1039*, 130–136. [CrossRef]

50. Shanmukh, S.; Jones, L.; Zhao, Y.P.; Driskell, J.D.; Tripp, R.A.; Dluhy, R.A. Identification and classification of respiratory syncytial virus (RSV) strains by surface-enhanced Raman spectroscopy and multivariate statistical techniques. *Anal. Bioanal. Chem.* **2008**, *390*, 1551–1555. [CrossRef]