○ **Chemical and Biological Technologies in Agriculture**

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The effect of biodynamic preparations on growth and fruit quality of giant pumpkin (*Cucurbita maxima* D.)

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Abstract

Background: Food quality of agricultural crops depends on environmental conditions, production system and cultivation method. A plant-based nutrition with food rich in vitamins, minerals and secondary plant compounds with antioxidative properties promotes human health. This investigation was inspired by an increasing global issue on how to improve product quality while using alternative preparations. The main aim of a 3-year study was to investigate the influence of fermented manure and silica products on yield and nutritive components in peel, fruit and seeds of three pumpkin cultivars. In four replicates as block design, the effects of individually as well as combined application of biodynamic horn manure and horn silica preparation were compared to a control variant.

Results: Horn manure application significantly increased total and marketable yield. Marketable yield, contents of macroelements, total carotenoids, single carotenoids (lutein + zeaxanthin, lycopene, ß-carotene) and antioxidants (catechins, total phenols, leuco-anthocyanins) were significantly increased by horn silica use. The combination of both biodynamic preparations had a significantly increasing effect on total and marketable yield, net photosynthetic productivity, dry matter content and total and single carotenoid contents (lutein + zeaxanthin, lycopene, ß-carotene).

Conclusions: The pumpkin trial results indicated a general growth-promoting effect by horn manure, a quality-enhancing effect by horn silica and a compensatory effect through both preparations on a high qualitative level. In accordance with other investigations, these effects did not occur in the same way in all plant species. Therefore, the effects of the biodynamic preparations should be tested in further trials on a plant species-specific basis.

Keywords: Winter squash, Preparation, Horn manure, Horn silica, Pumpkin fruit, Secondary plant compounds, Antioxidants, Carotenoids

Background

Quality and yield of agricultural products such as fruits and vegetables are influenced among others by crop variety, environmental impacts and cultivation methods of the agricultural production system. An important part of product quality refers to their ingredients and health-conducive properties, e.g. amounts of minerals, vitamins, antioxidants and other phytochemicals [1]. A plant-based nutrition with vegetables rich in antioxidants is essential for human health and may protect from many diseases [2, 3]. Distinct differences in food quality caused by the crop production system were identified in a meta-analysis based on 343 peer-reviewed publications [4]. Organically versus conventionally produced crops contained significantly higher concentrations of antioxidants, vitamins and minerals. Negative food quality characteristics such as pesticide residues and toxic metal content (e.g. cadmium) were significantly higher in conventional produced crops [4].

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Plant development can be influenced by water, humus and nitrogen, which have a growth- and yield-promoting effect. Plant morphological differentiation processes and ripening are enhanced by light and warmth of solar radiation. Agricultural cultivation measures influence the relation between light intensity and nitrogen supply and can foster or inhibit growth and differentiation processes. This results in significant differences in the ingredient compositions of crops. For example, full light intensity, low N-supply and farmyard manure used (vs. shading, high N-supply and mineral fertiliser) enhanced value-added ingredients of rocket (e.g. ascorbic acid and glucosinolate contents) and wheat (true protein content) [5]. Higher light intensity tends to increase polyphenolic compound contents in plants [6], e.g. flavonoid contents in fruits [7] and total phenolic contents of leafy vegetables [8].

Furthermore, yield and crop quality can be affected by the application of biodynamic preparations. Biodynamic preparations (e.g. horn manure and horn silica) are an essential part of the biodynamic cultivation method. Their primary purpose is not to add nutrients, but to stimulate the processes of nutrient and energy metabolism and improve soil and crop quality [9]. As progenitor and eldest type of organic farming, biodynamic agriculture exists since more than 90 years [10]. In a review, [11] concluded that biodynamic preparations had a positive effect on soil quality (e.g. nutrients, enzymatic composition) and crop yield. Research has indicated that biodynamic preparations exhibited higher quality and better sensory properties and accumulated higher concentrations of antioxidant compounds [11].

Results of many experiments indicated that the preparations have different effects depending on the growth conditions of plants and whether they were used individually or in combination. Horn manure stimulated vegetative plant growth and increased leaf yield and beet yield from sugar beets. The additional application of horn silica further increased beet yield and decreased leaf yield [12]. Horn silica enhanced the quality of carrots in postharvest storage tests [13]. In studies of potatoes, horn manure led to lower and horn silica to higher levels of secondary plant compounds in two out of three cultivars [14]. The effect of horn silica was partly consistent with the effect of intense exposure to light on crops [5, 15, 16]. The combination of horn manure and horn silica often had a balancing effect on yield [17].

However, no research evidence was found on the effects of biodynamic preparations on the improvement of giant pumpkin (*Cucurbita maxima* D.) fruit quality and on the increasing of biologically active compounds content. *Cucurbita maxima* is an economically important species cultivated worldwide for human consumption. According to the Food and Agriculture Organization of the United Nations, the world production of pumpkins in 2019 was estimated over 22.9 million tons harvested from 1.5 million hectares [18]. Pumpkins contain large amounts of fibre, free sugars, minerals and vitamins such as B1 and C, as well as secondary plant compounds, including carotenoids and polyphenols, which provide various human health promoting functions [19, 20].

According to the sustainable and holistic approach of organic farming [21], a farm should be considered as an organism. An increasing number of studies have proven that organic farming led to higher soil quality and more biological activity in soil compared to conventional farming [22–26]. A basic statement of organic agriculture derived from this idea, implied that a healthy soil leads to better, more healthy plant growth and a healthy diet [27].

This research aimed to explore and assess the effects of biodynamic preparations (HM: horn manure and HS: horn silica) on the properties of soil, yield and quality of pumpkin fruits. Effects of the biodynamic preparations on soil enzyme activity, soil nutrients and pumpkin yield resulting from this 3-year experiment were presented in Juknevičienė et al. [28]. The application of horn manure significantly increased soil enzymatic activity (urease and saccharase) and soil nutrient content (phosphorus and potassium). The pH value of the soil decreased over the growing period, thus influenced phosphorus uptake in pumpkin plants [28]. Effects of horn manure on soil enzymatic activity were also determined in research of potatoes by Vaitkevičienė et al. [14].

In this paper, the effects of horn manure and horn silica on quality and nutritive components of pumpkin fruits are represented. The following research hypotheses were examined: applications of horn manure and horn silica increase the yield of pumpkin fruit. The contents of secondary plant compounds are reduced by horn manure application and increased by horn silica. If the preparations are applied in combination, an increase in secondary plant substances is expected. The impact of biodynamic preparations on the development of various pumpkin cultivars of *Cucurbita maxima* D. was examined in this trial. Therefore, the quality of morphological parts of pumpkin fruit—peel, flesh and seeds—were analysed to determine the effect of biodynamic preparations on contents of biologically active compounds. Finally, together with the results on soil properties of Juknevičienė et al. [28], it should be verified whether the basic statement of organic agriculture—a healthy soil leads to better, more healthy plant growth and a healthy diet—can be positively supported by the application of the biodynamic preparations horn manure and horn silica.

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Materials and methods

Experimental design and management

The investigation was carried out on an organic farm in Kaunas district, Lithuania from 2012 to 2014 (see [28]). The three trial years were carried out each year on a new field of the organically managed farm. The respective trial area with pumpkin was fertilised in the trial year with 30 t ha-1 of plant compost (pH$_{KCl}$ 6.97, available P$_2$O$_5$ 1932 mg kg^{-1}, and mineral nitrogen 52.73 mg kg^{-1}, the compost was 2 years old). In a two-factorial field trial, different cultivars of winter squash and the use of biodynamic preparations were examined in four field replications as block design. Therefore, the three giant pumpkin (*Cucurbita maxima* Duchesne) cultivars 'Justynka F$_1$', 'Karowita' and 'Amazonka' developed by breeders at the Warsaw University of Life Sciences in Poland were cultivated. The effect of biodynamic preparations was examined by four variants. Horn manure (HM: fermented manure) and horn silica (HS: ground silica SiO$_2$) were each tested individually and in combination. In the control variant, no treatment was applied.

The soil of the experimental site was a *Calc(ar)i-Endo-hypogleyic Luvisol* [29] with light loamy soil texture (limnoglace clay loam on boulder clay, deeply gleyic luvisol). The soil was characterised by soil organic matter 2.4%, available P$_2$O$_5$ 139–173 mg kg^{-1} and available K$_2$O 173–209 mg kg^{-1} contents, 0.29% nitrogen and a pH value almost neutral (pH ~ 7).

Pumpkinseeds were sown in plastic pots in May (2–3 seeds were put into one hole of 2–4 cm depth) and greenhouse-grown till they were plant out at the end of May. Each field trial plot was 12 m^2 in total with a harvested plot area of 6 m^2 and marginal strip of 0.5 m width. Six plants were cultivated in each core plot. The pumpkins were harvested at ripeness within the first 10 days of September. All three pumpkin cultivars have a bushy growth habit. The growing season takes 120–150 days. Pumpkin fruits of these three varieties are dark orange, round to elliptical and weigh 1–1.5 kg (Amazonka) or 3–4 kg (Justynka, Karowita), respectively. Justynka is the most productive variety (50–60 t ha^{-1}), followed by Karowita. Smaller fruits of Amazonka often result in lower yield compared to Karowita [30]).

The biodynamic preparations horn manure and horn silica used in this study were obtained from a Demeter certified farm in Germany, specialised in the production of biodynamic preparations (CvW KG, Internationale Biodynamische Präparatezentrale, Künzelsau, Germany). Horn manure is made by collecting manure from several cows and putting it into cow horns, which are buried in the soil during winter and excavated in spring. Horn manure preparation is the 'humus mixture' that resulted from this fermentation. The horn manure preparation used in the experiment was neutral (pH$_{KCl}$ 6.96), contained a huge amount of phosphorus (1960 mg kg^{-1} total amount in dry matter), potassium (259 mg kg^{-1} total amount in dry matter) and nitrogen (2.10% total amount in dry matter).

Horn manure was sprayed to soil surface as 1% solution (200 l solution ha^{-1}, 200 g horn manure preparation for 1 ha) 2 weeks before planting pumpkin seedlings. Pumpkin leaves were sprayed twice with a 0.5% horn silica solution at the beginning of flowering (BBCH 605: 5th flower opened on main stem) and at the beginning of fruit formation (BBCH 702: 2nd fruit on main stem has reached typical size and fruit). Horn manure was sprayed in the afternoon and horn silica in the morning. Both solutions were stirred one hour before spraying.

Parameters and methods

Total yield (t ha^{-1}) of pumpkin fruits was calculated by weight of harvested fruits. Healthy, fully ripened fruits were classified as marketable quality.

Net photosynthetic productivity (Fpr) was calculated according to the formula:

$$Fpr = 2(M_2 - M_1) / (L_1 + L_2) T,$$

with (M_2-M_1): dry matter increases during a given time; L_1 and L_2: leaf area at the beginning and at the end of the period; T: time duration in days [31].

Leaf area was determined at stage of foliation (BBCH 110 and BBCH 115), flowering (BBCH 605 and BBCH 610) and fructification (BBCH 702 and BBCH 703). BBCH identification keys used in this research based on the methodology of Feller et al. [32] for pumpkin plants.

For chemical analyses, a composite sample of fruit was drawn up as follows: four fruits were randomly selected from each variant in four replicates. This resulted in 16 fruits, of which a random sample of 5 fruits was drawn to analyse. Chemical composition of the flesh was tested by a sample of at least 1000 g taken from this pooled sample of each variant of the pumpkin (according to LST ISO 2859-10:2007), by cutting pieces of flesh at random from several places. Fruit peel was analysed by taken ~ 1000 g from the pooled sample. Seeds were analysed by a pooled sample size of 100 g. Chemical analyses of the peel, pulp and seeds of pumpkin fruits were carried out in four replicates. Standard methods were used to analyse different parameters in fresh mass (FM) of fruit flesh and in dry matter (DM) of peel and flesh. A spectrophotometer was used to analyse total carotenoid content (mg·100 g^{-1} FM) (Spectro UV–VIS dualbeam UVS-2800, Labomed Inc., USA). The individual carotenoids lutein, zeaxanthin, lycopene and β-carotene (mg·100 g^{-1} FM) were determined by high-performance liquid chromatography (HPLC). Dry matter (% DM) was determined by drying

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samples at 105 °C to constant weight (LST ISO 751:2000). Contents of macroelements (K, P, Ca, Mg in % DM) were measured by atomic absorption spectrometry.

At the Institute of Chemistry and Biology of Immanuel Kant Baltic Federal University, the following parameters were determined according to the method of Gupta and Verma [33] using a spectrophotometer SF-2000 (ZAO OKB SPECTRUM, Russia): contents of leuco-anthocyanins (mg·100 g^{-1}), catechins (mg·100 g^{-1}) and total phenolics (mg·g^{-1}) in the dry matter of pumpkin peel and flesh. In addition, contents of anthocyanins (mg·100 g^{-1}), leuco-anthocyanins (mg·100 g^{-1}) and total carotenoids (mg·100 g^{-1}) were measured in the fresh mass of pumpkin seeds.

Statistical analysis

Research data were statistically evaluated by the analysis of variance (ANOVA) using the program Systat 10 (Systat 10, Statistics I, SPSS Inc., Chicago, IL, USA). Statistical significance of differences between means was assessed by Fisher's LSD test ($p < 0.05$). Correlation analysis was performed to determine the strength and nature of the relationship between the variables. For data evaluation, experimental years were assumed to be "random". No interactions were found between treatments of horn manure and experimental years.

Results

Total and marketable yield and the non-marketable yield calculated out of it are shown in Table 1. Justynka was the most productive variety, followed by Karowita and Amazonka. In the control group, the total yield of Justynka cv. was 35% higher than that of Karowita cv. and 95% higher than that of Amazonka cv. All three biodynamic preparation variants had a mostly significant effect on total and marketable yield of the three cultivars. Horn manure significantly increased total and marketable yield of all cultivars compared to their control group (total yield: Justynka: +26,63%, Karowita: +6,3%, Amazonka: +17,32%). Within the cultivars of Justynka and Amazonka, horn manure led to highest results compared to all other spray treatments. The application of horn silica resulted in a lower yield increase (total and marketable yield) compared to horn manure. Horn silica vs. horn manure led to a further increase of total and marketable yield of Karowita cv. The combination of both preparations led to a significant increase in all cultivars compared to the respective control groups. The application of both preparations resulted in the highest total and marketable yield of Karowita cv., while total and marketable yield of Justynka and Amazonka cvs. were significantly more effected by horn manure.

Table 1 Total yield, marketable yield and non-marketable yield of pumpkin fruits of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014

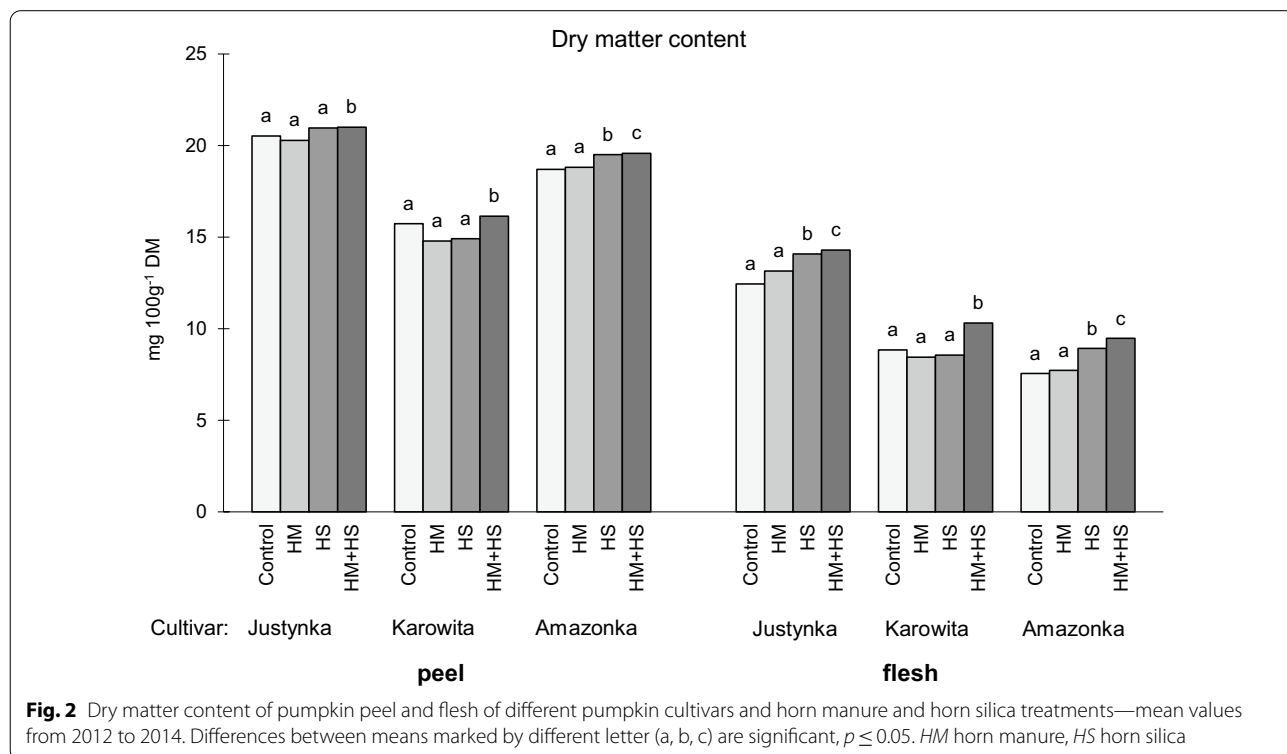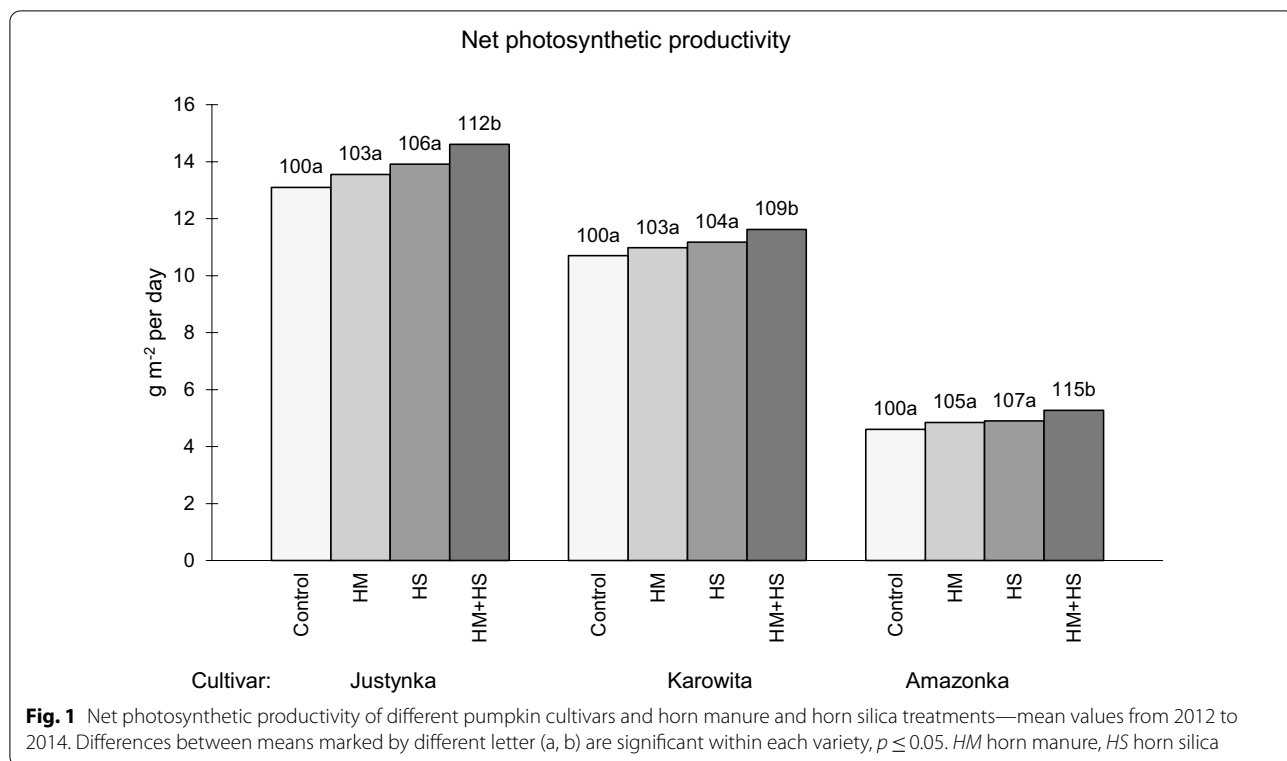| Spray treatments	Total yield (t ha^{-1})	Marketable yield (t ha^{-1})	Non-marketable yield* (t ha^{-1})
Justynka			
Control	46.00[a]	40.25[a]	5.75
HM	58.25[c]	52.21[c]	6.04
HS	54.20[b]	51.30[b]	2.90
HM + HS	54.56[b]	51.15[b]	3.41
Karowita			
Control	34.05[a]	31.95[a]	2.10
HM	36.20[b]	33.00[b]	3.20
HS	36.96[b]	34.65[c]	2.31
HM + HS	38.40[c]	36.00[d]	2.40
Amazonka			
Control	23.55[a]	20.15[a]	3.40
HM	27.63[c]	24.20[c]	3.43
HS	24.32[a]	22.80[b]	1.52
HM + HS	25.60[b]	24.00[c]	1.60

*As difference of total to marketable yield. *HM* horn manure, *HS* horn silica. Differences between means marked by different letter (a, b, c, d) are significant within each cultivar, $p \le 0.05$

The highest proportion of non-marketable pumpkin fruits of all three cultivars resulted from horn manure application. The lowest amounts of non-marketable yield were caused by horn silica application. This ratio was even lower than the non-marketable yield of the control groups of Justynka and Amazonka cvs.

As part of net photosynthetic productivity, the dry mass increase of the control group of Justynka cv. was 24% higher compared to that of Karowita cv. and 181% higher than the respective control of Amazonka cv. (Fig. 1). A slight effect on net photosynthetic productivity was caused by the single application of horn manure or horn silica. The use of both biodynamic preparations (HM + HS) significantly increased photosynthetic productivity of all three cultivars by 9 to 15% (Fig. 1).

Horn manure application had no significant effect on dry matter content overall (Fig. 2). Horn silica application resulted partly in significantly higher values compared to control and horn manure application (e.g. peel of Amazonka, flesh of Justynka and Amazonka cvs.). The combination of horn manure and horn silica led to significantly higher dry matter contents of peel and flesh in all cultivars.

Independent of cultivar and treatment variant, higher contents of macroelements were analysed in fruit peel than in flesh (Table 2). Compared to control, potassium

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Fig. 1 Net photosynthetic productivity of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014. Differences between means marked by different letter (a, b) are significant within each variety, $p \le 0.05$. *HM* horn manure, *HS* horn silica

Fig. 2 Dry matter content of pumpkin peel and flesh of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014. Differences between means marked by different letter (a, b, c) are significant, $p \le 0.05$. *HM* horn manure, *HS* horn silica

contents in peel and flesh were slightly increased by the biodynamic preparations, but without significant differences overall. Phosphor, calcium and magnesium contents were increased by horn silica application with or without horn manure. The single horn manure application resulted in predominantly lower contents than those of the respective control groups. Significant effects on phosphor in the peel of Justynka cv. and in the flesh of Amazonka cv. resulted from horn silica use. Calcium values were significantly increased mainly by horn silica only in pumpkin peel of all varieties. Magnesium was significantly increased mainly by horn silica in the peel of Justynka cv. and in the flesh of Amazonka cv. and in both analysed plant parts of Karowita cv. In total, horn manure had no clearly distinct impact on macroelement contents. Compared to the combination with horn manure, the single application of horn silica led to at least equal or increased values of macroelements in all 24 results (4 macroelement contents × 3 varieties × 2 plant parts).

The analysis of pumpkin fruits resulted in higher total carotenoid contents in the peel than in the flesh by on average 51% (Fig. 3). Horn manure had no significant impact on total carotenoid contents, both in the peel and flesh of all three cultivars. The application of horn silica with or without horn manure resulted in significantly higher values in the peel and flesh of all three cultivars. Horn silica increased total carotenoid content in the peel by on average 15.14% and in the flesh by 11.14%. The use

of both preparations led to increasing carotenoid contents in the peel by on average 16.67% and in the flesh by 12.52%.

Analytical results of single carotenoids are shown in Fig. 4a–c. The control groups of Justynka and Amazonka cvs. contained lower amounts of the carotenoids lutein and zeaxanthin and ß-carotene in pumpkin fruit flesh compared to the respective control group of Karowita cv. (Fig. 4a, c). In case of low values of the control groups (Justynka and Amazonka cvs.) of lutein, zeaxanthin and ß-carotene, the application of horn manure led to a significant increase between 14 and 30%. Horn silica application resulted in a significant increase of lutein, zeaxanthin and ß-carotene between 33 and 72%. The significantly highest values of the single carotenoids were caused by the combination of both preparations (+78% up to 109%) (Fig. 4a, c). At higher initial values of lutein, zeaxanthin and ß-carotene in the control groups (Karowita cv.), horn manure application resulted in slightly decreasing amounts (Fig. 4a, c). Horn silica application led to increasing values, partly significant and in case of significance, the amount was higher than the combination of both preparations. In total, if the control values of lutein, zeaxanthin and ß-carotene were higher (Karowita cv.), a lower increase caused by horn silica with or without horn manure was observable (+2% up to 18%) (Fig. 4a, c). For lycopene, the contents of the three cultivar control groups were similar. Horn manure application

Table 2 Contents of macroelements in peel and flesh of pumpkin fruits of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014

Spray treatments	K		P		Ca		Mg	
	% DM							
	peel	flesh	peel	flesh	peel	flesh	peel	flesh
Justynka								
Control	0.22[a]	0.19[a]	0.94[a]	0.82[a]	0.25[a]	0.16[a]	0.30[a]	0.17[a]
HM	0.23[a]	0.20[a]	1.02[ab]	0.81[a]	0.26[a]	0.16[a]	0.28[a]	0.14[a]
HS	0.25[a]	0.22[a]	1.24[b]	0.88[a]	0.47[b]	0.23[a]	0.51[b]	0.25[a]
HM+HS	0.25[a]	0.22[a]	1.16[ab]	0.86[a]	0.36[b]	0.21[a]	0.51[b]	0.21[a]
Karowita								
Control	0.23[a]	0.19[a]	1.44[a]	0.87[a]	0.27[a]	0.18[a]	0.33[a]	0.14[ab]
HM	0.24[a]	0.20[a]	1.42[a]	0.86[a]	0.33[a]	0.17[a]	0.32[a]	0.13[a]
HS	0.26[a]	0.23[a]	1.52[a]	1.02[a]	0.46 b	0.23[a]	0.54[b]	0.33[b]
HM+HS	0.25[a]	0.23[a]	1.50[a]	0.99[a]	0.41[ab]	0.21[a]	0.48[ab]	0.25[b]
Amazonka								
Control	0.21[a]	0.19[a]	1.46[a]	0.88[a]	0.26[a]	0.19[a]	0.54[a]	0.14[a]
HM	0.22[a]	0.20[a]	1.43[a]	0.86[a]	0.28[b]	0.18[a]	0.52[a]	0.14[a]
HS	0.25[a]	0.22[a]	1.55[a]	1.28[b]	0.39[b]	0.24[a]	0.58[a]	0.26[b]
HM+HS	0.24[a]	0.22[a]	1.52[a]	1.02[a]	0.30[b]	0.22[a]	0.56[a]	0.22[ab]

HM horn manure, *HS* horn silica, *DM* dry matter. Spray treatments: different letters (a, b, c) are significant within each cultivar, $p \leq 0.05$

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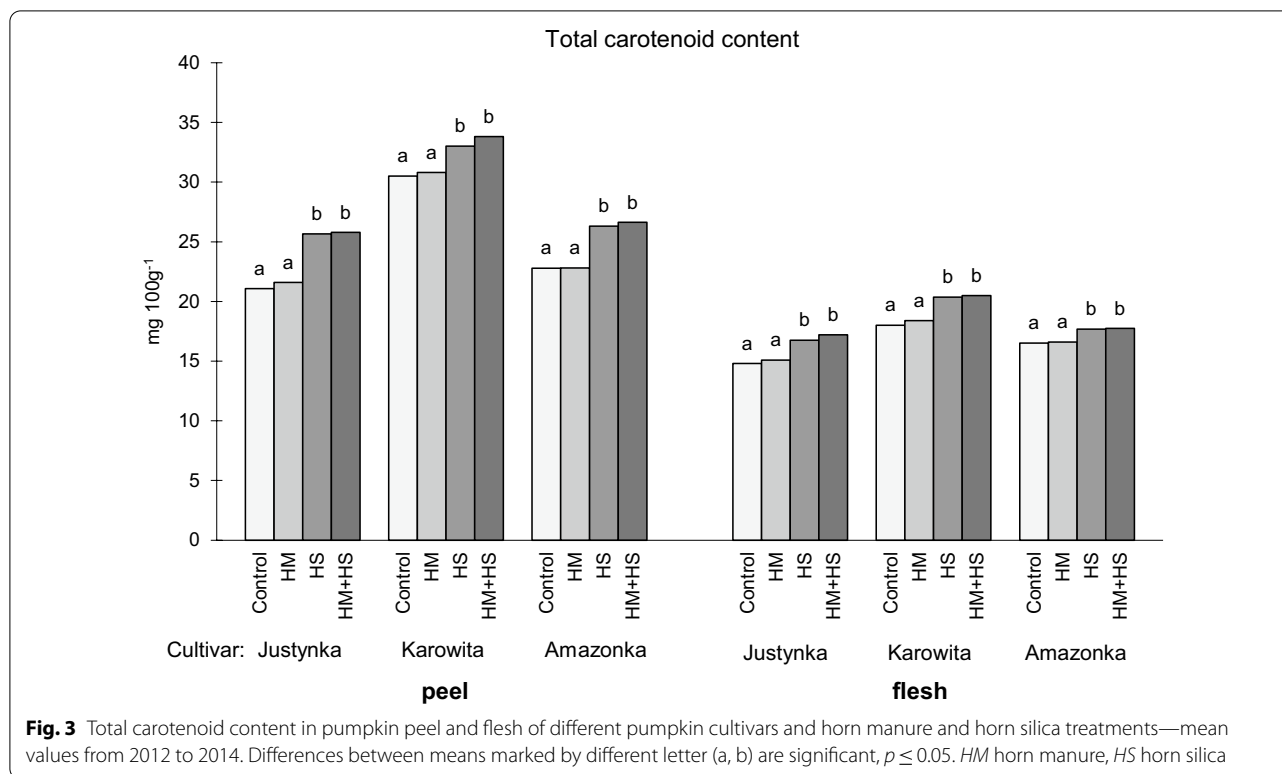
Fig. 3 Total carotenoid content in pumpkin peel and flesh of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014. Differences between means marked by different letter (a, b) are significant, $p \leq 0.05$. *HM* horn manure, *HS* horn silica

led to a slight decrease or significant increase of 13–15% (Fig. 4b). Horn silica application resulted in a significant increase between 11 and 30%. Highest amounts of lycopene were caused by the combination of horn manure and horn silica in all three varieties with an increase of 22% up to 43% (Fig. 4b). Antioxidant contents of lutein and zeaxanthin, lycopene and β-carotene were increased by on average 9% by horn manure, 32% by horn silica and 44% by both preparations.

Irrespective of cultivar and spray treatment, higher contents of leuco-anthocyanins were analysed in pumpkin peel than in flesh (Fig. 5b). There were no clear differences between peel and flesh of catechin and total phenolic contents (Fig. 5a, c).
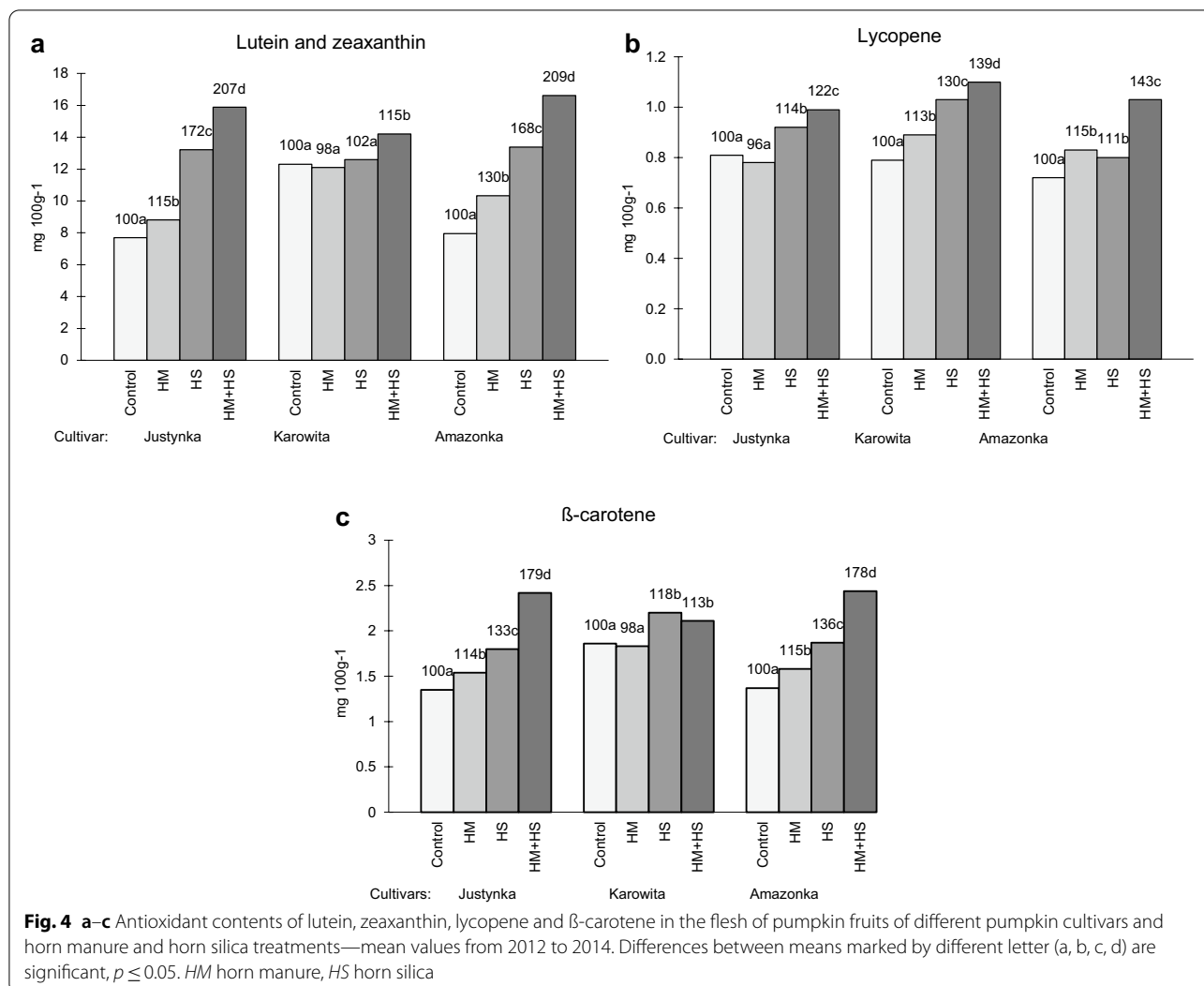
The application of horn manure had a mainly decreasing effect on antioxidant contents of all three cultivars compared to control (Fig. 5). In 17 out of 18 comparisons, significantly highest contents of all three antioxidants and of all cultivars resulted from single horn silica application. The combination of horn silica and horn manure led to antioxidant amounts between those of horn silica application and control. All results of horn silica application significantly varied to horn manure application and/or control group.

Horn silica application (with or without horn manure) increased on average the content of leuco-anthocyanin in the peel by 15% and in the flesh by 13%, of catechins in

the peel by 9% and in the flesh by 7%, of total phenolics in the peel by 22% and in the flesh by 18% compared to control.

Relatively low contents of anthocyanins were analysed in seeds of giant pumpkin fruits without significant differences between spray treatments or cultivars (see μg unit in Table 3). Horn manure application decreased the values of anthocyanin contents of all three cultivars. This decreasing effect was also observable at leuco-anthocyanin contents of Justynka cv. and Amazonka cv., whereas leuco-anthocyanins of Karowita cv. were significantly increased by horn manure application. Total carotenoid contents were increased, partly significant, by horn manure. Single horn silica application had an increasing effect on all types of antioxidants analysed in the seeds of all cultivars. Single horn silica application led to significantly highest contents of leuco-anthocyanins and total carotenoids in giant pumpkin seeds of all cultivars. Compared to control, mean values of leuco-anthocyanins and total carotenoids were increased by single horn silica application by 32% and 46%, respectively. The combination of horn manure and horn silica had a balancing effect with contents of leuco-anthocyanins and total carotenoids ranged between those of the control groups and horn silica application (Table 3).

In summary, the results in this chapter indicate that horn manure and horn silica preparations affected yield

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Fig. 4 **a**–**c** Antioxidant contents of lutein, zeaxanthin, lycopene and ß-carotene in the flesh of pumpkin fruits of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014. Differences between means marked by different letter (a, b, c, d) are significant, *p* ≤ 0.05. *HM* horn manure, *HS* horn silica

and nutritive values of different pumpkin cultivars. The efficacy varied depending on cultivar, analysed parameter and used preparation.

Discussion

The results of the analysed parameters of this 3-year investigation varied between the three cultivars Justynka F_1, Karowita and Amazonka. Cultivar and genotype are indicated as main factors for the different amounts of bioactive compounds in pumpkin fruits [20, 34]. Especially among pumpkin varieties of *Cucurbita maxima* D., contents of dry matter, free sugars, carbohydrates, minerals, antioxidants and phytochemicals (e.g. vitamins, carotenoids and polyphenols) differ significantly [20, 35, 36].

Differences in yield or food quality of the three cultivars without the application of biodynamic preparations were analysed in several investigations (Amazonka cv. vs.

Karowita cv.: [30, 35, 37, 38]; Justynka F_1 vs. Karowita cv.: [39]; Justynka F_1 vs. Amazonka cv.: [36]; Justynka F_1 vs. Amazonka vs. Karowita cvs.: [40]).

Environmental impacts during growing season such as weather conditions or cultivation methods including fertiliser type are further important impacts on yield and ingredient composition of pumpkin fruits [20, 30, 39]. These influences were limited because all analysed pumpkins grew under the same environmental conditions, except the application of the biodynamic preparations horn manure and horn silica. The effects of single and combined biodynamic preparations are discussed below.

Yield

The differences in yield between cultivar's control groups correspond to the results reported by Biesiada et al. [30] for Karowita and Amazonka cvs. In the present study,

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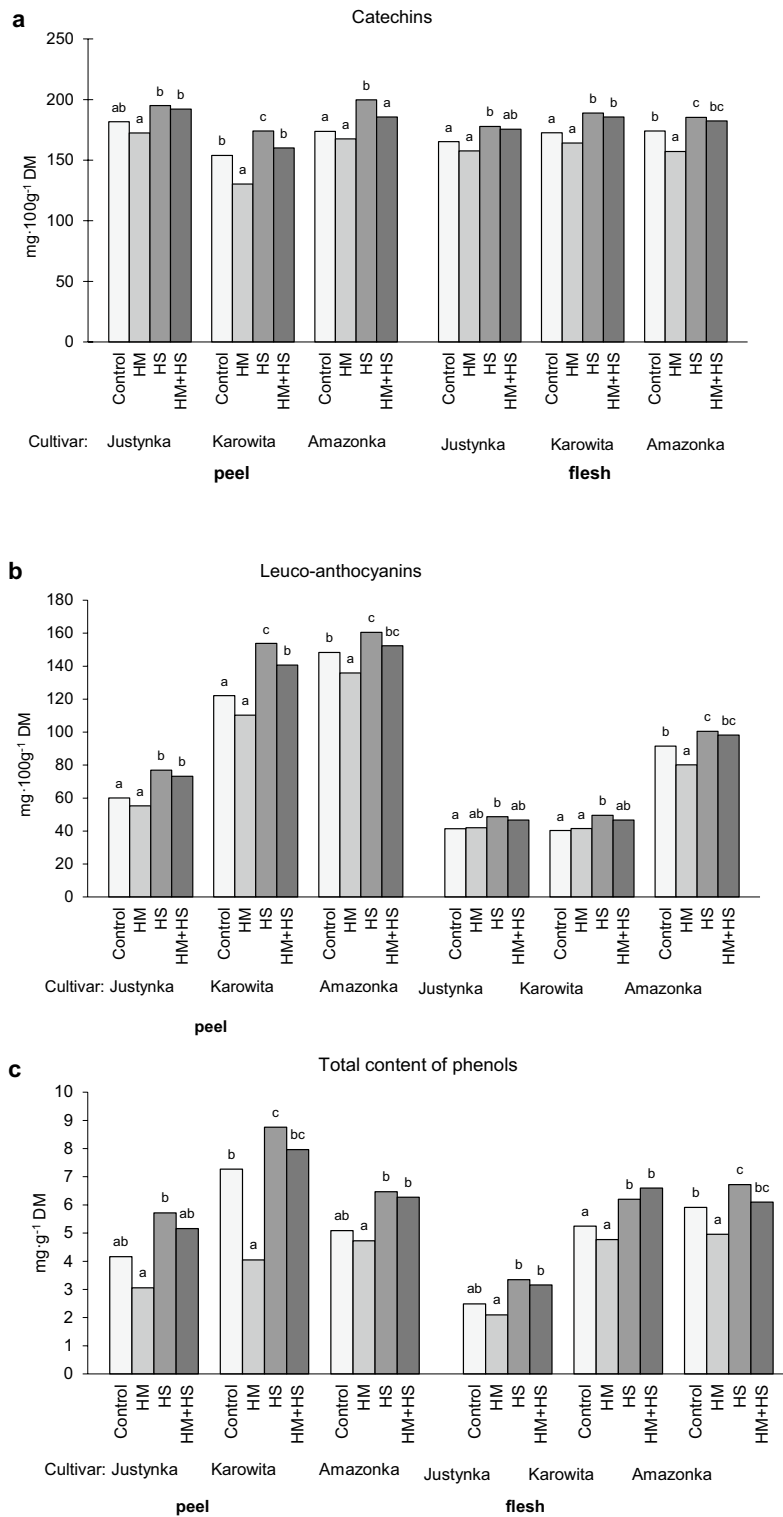
Fig. 5 a–c Antioxidant content of catechin, leuco-anthocyanins and total phenolic in peel and flesh of pumpkin fruits of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014. Differences between means marked by different letter (a, b, c, d) are significant, $p \leq 0.05$. *HM* horn manure, *HS* horn silica

Table 3 Antioxidant content of anthocyanins, leuco-anthocyanins, total carotenoid content in the seeds of pumpkin fruits of different pumpkin cultivars and horn manure and horn silica treatments—mean values from 2012 to 2014

Cultivation variant	Anthocyanins (µg·100g⁻¹ FM)	Leuco-anthocyanins (mg·100g⁻¹ FM)	Total carotenoid content (mg·100g⁻¹ FM)
Justynka			
Control	0.09[a]	4.50[a]	10.20[a]
HM	0.06[a]	4.20[a]	18.70[b]
HS	0.10[a]	5.70[b]	23.70[b]
HM + HS	0.09[a]	4.80[a]	21.50[b]
Karowita			
Control	0.10[a]	2.40[a]	23.90[a]
HM	0.06[a]	4.80[c]	24.50[a]
HS	0.11[a]	4.90[c]	32.20[b]
HM + HS	0.10[a]	4.20[b]	29.60[ab]
Amazonka			
Control	0.10[a]	6.50[a]	32.20[a]
HM	0.07[a]	6.30[a]	35.00[ab]
HS	0.11[a]	7.10[b]	40.70[b]
HM + HS	0.10[a]	6.70[ab]	38.00[ab]

HM horn manure, *HS* horn silica, *FM* fresh mass. Spray treatments: different letters (a, b, c) are significant within each cultivar, $p \leq 0.05$

highest yield increase (significant total and marketable yield) of two out of three cultivars were caused by single horn manure application, followed by the combination of horn manure and horn silica, single use of horn silica and control in descending order. A similar highest increasing effect by the individual horn manure application and a lower increasing effect by the combination of horn manure with horn silica were shown for sugar beet leaves [12]. Compared to control, the yield of Karowita cv. was enhanced lowest by horn manure application. Horn silica application resulted in a further increase and the combination of both preparations had the significantly highest impact. These different effects on varieties might depend on cultivar's genotype. In other field trials, horn silica application had a slightly yield increasing effect on lettuce in combination with organic fertiliser [41] or compensated for the yield reduction due to later potato planting dates [42]. However, the combination of both preparations was beneficial compared to control (soybeans: [43]; rice: [44]) or single horn manure use (sugar beet roots, wheat: [12]; cumin: [45]). Microbial contents of horn silica preparations were discussed to have pathogen controlling properties and therefore enhance yield and crop quality [46]. In this study, the lowest proportion of non-marketable yield resulting from the single horn silica use might indicate the quality-enhancing effect. In chemical analyses of horn manure preparation, bioactive substances and microorganism were detected which might stimulate soil nutrient processes and plant growth

[47–50]. This growth-promoting effect of horn manure used individually or in combination with horn silica enhanced yield and might be partly reasonable for the highest part of non-marketable yield at all pumpkin cultivars compared to the lowest part caused by single horn silica application. A stronger yield increasing effect of horn manure vs. horn silica was also shown by Spieß [12].

Net photosynthetic productivity
The net photosynthetic productivity of pumpkin plants was slightly increased by the single use of horn manure and horn silica in ascending order. Significant effects resulted from the combination of both biodynamic preparations. Similar results were reported for three potato cultivars. The single application of horn manure or horn silica resulted in slightly increasing values without significant effects as well. Highest values, significantly in two out of three cultivars, resulted from the combination of horn manure and horn silica [51].

Dry matter content
Similar differences in dry matter contents of the cultivar's control groups were reported by Biesiada et al. [37] and Niewczas et al. [39]. The dry matter content of pumpkin peel as well as flesh of all cultivars was not affected significantly by single horn manure application and was only increased partly significant by single horn silica application. Genetically determined differences as reaction on the biodynamic preparations might be obvious

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at Karowita cv. which was the only cultivar resulting in decreasing contents from single application of horn manure as well as horn silica. The combination of both preparations led to significantly increased dry matter contents of all cultivars. No significant effects of biodynamic preparations on potatoes at all were reported by Vaitkevičienė et al. [52]. Irrespective of the tested potato varieties, a similar effective direction with highest dry matter contents caused by the application of horn silica and horn manure was also recognisable for potatoes. The differences compared to the control groups were not significant [52]. However, the dry matter content of the three cultivars differed significantly [52]. Hence, Vaitkevičienė et al. [52] concluded that potato's genetics had a high influence on dry matter content. In a comparison of sprayed to not sprayed variants in combination with organic or mineral fertiliser, Bacchus [41] also reported significantly higher dry matter contents of lettuce heads sprayed with horn manure und horn silica.

Minerals

Horn manure application had no significant effects on contents of macroelements in peel and flesh of all pumpkin cultivars. Single horn silica application led to highest and partly significant higher contents of macroelements (phosphor, calcium, magnesium) in peel and flesh of all pumpkin cultivars compared to the respective control group. The combination of horn manure und horn silica resulted in macroelement contents ranged between those of the control groups and the single horn silica applications. Vaitkevičienė et al. [52] analysed the impact of biodynamic preparations on macroelement contents (e.g. potassium, phosphorus, magnesium) of potato tubers. Similar to the pumpkin results, single horn silica application led to the highest values of potassium and phosphor contents compared to control, but neither the single use nor the combination of horn manure and horn silica had a significant effect [52]. The combination of biodynamic preparations on potatoes led to intermediate values for potassium [52] which correspond to the pumpkin trial results.

Secondary plant compounds

The application of single horn manure had a mostly decreasing and partly significant effect on the contents of leuco-anthocyanins, catechins and total phenols in flesh and peel of pumpkin fruits compared to the respective control groups. Contents of anthocyanins and leuco-anthocyanins in pumpkin seeds were mainly decreased (not significant) by single horn manure application below the initial values of the respective control groups, except for leuco-anthocyanins of Karowita cv. Total phenolic compounds, total anthocyanins and antioxidant activity

of potato tubers were similarly decreased by single horn manure application compared to the control groups [53]. On the other hand, total carotenoid contents in pumpkin seeds, peel and flesh were not or slightly and in one case significantly increased by horn manure application. The analysis of single carotenoids (lutein + zeaxanthin, lycopene, ß-carotene) in the pumpkin flesh indicated a significant increase in six out of nine values by horn manure use. Overall, the effects of horn manure varied on the levels of secondary plant compounds and were marginal compared to the effects of horn silica treatments.

Highest contents of i) antioxidants in pumpkin seeds and ii) catechins, leuco-anthocyanins and total phenols in peel and flesh of all cultivars were caused by single horn silica application. The total carotenoid contents in pumpkin flesh and peel were quite similar when horn silica was used on its own as well as in combination with horn manure. Highest single carotenoid contents (lutein + zeaxanthin, lycopene, ß-carotene) in pumpkin flesh resulted from the combination of horn silica and horn manure. Overall, horn silica application (with or without horn manure) resulted in highest contents of secondary plant compounds in all test results. The comparison of the biodynamic preparations on the level of cultivars resulted in significant differences between the horn silica treatment (with or without horn manure) and the respective control groups in 35 out of 42 results (total carotenoids in peel and flesh (6); lutein + zeaxanthin, lycopene, ß-carotene in flesh (9); catechins, leuco-anthocyanins, total phenols in peel and flesh (18); anthocyanins, leuco-anthocyanins, total carotenoids in seeds (9)). In the investigation on three potato cultivars by Jarienė et al. [53], the use of horn silica significantly increased total phenols and antioxidant activity, horn manure application had a decreasing effect, and the combination of both preparations had a compensatory effect. In case of high values in the control groups, the use of all biodynamic preparations resulted in lower values, while maintaining the same effective direction of the three preparation variants [53]. These plant reactions of potato cultivars to horn manure and horn silica application correspond to the reactions of pumpkin cultivars (e.g. catechins, leuco-anthocyanins and total phenols in peel and flesh, anthocyanins, leuco-anthocyanins and total carotenoids in seeds).

The total phenolic concentration of white mulberry leaves (*Morus alba* L.) showed opposite results. In one cultivar, the phenolic concentration was reduced by all biodynamic spray treatments (horn manure and/ or horn silica). The phenolic concentration of the second variety was increased by horn manure, also in combination with horn silica. Lowest phenolic concentrations were caused by single horn silica use in

both cultivars, while horn manure application led to the highest values of the three biodynamic spray treatments. The combination of both preparations resulted in intermediate values between those of the two single preparations. The comparison of the three biodynamic spray treatment variants on the flavonoid and chlorogenic acid concentrations also showed the same effects by lowest values by horn silica, highest values by horn manure and intermediate values by the combination of both preparations [54]. This compensatory effect by the combined preparations also appeared on antioxidants (e.g. leuco-anthocyanins in peel, flesh and seeds, catechins and total phenols in peel and flesh and total carotenoid content in seeds) of pumpkin fruits and resulted in values in between those of the single applications. In the investigation on potatoes, Jarienė et al. [53] analysed similar balancing values of each of the three antioxidant parameters of at least one to two potato varieties (out of three varieties) if both preparations were combined.

Effects of biodynamic preparations on vitamins and secondary plant compounds were also analysed in several farming system comparisons. In the second year of a field trial in which the use of horn silica was analysed as an additional factor, the ascorbic acid content of rocket was significantly higher when horn silica application vs. water application was used [5]. Biodynamically produced Batavia lettuce included highest amounts of polyphenols, anthocyanins and flavonoids vs. organic and conventional produced lettuce [55]. Even after 2 months of cold storage, the flesh of red beet (*Beta vulgaris* L. ssp. *vulgaris*) out of the biodynamic production contained significantly higher values of total phenols and antioxidant activity (DPPH radical scavenging method) compared to the conventional system [56]. In five potato cultivars, highest total phenolic contents in potato tubers were analysed for the biodynamic farming system, followed by organic and conventional farming. Contents differed significantly between biodynamically and conventionally produced potatoes. Potato tubers originating from the organic or biodynamic production system contained higher flavonoid contents than those out of conventional production. The sum of carotenoids as well as lutein, zeaxanthin and ß-carotene were significantly higher in biodynamically produced potato tubers. For anthocyanins, significant differences were found between cultivars, but not between the production systems [57].

Compensatory effects of the preparations

In the present pumpkin trial, cultivars with low values vs. high values in the respective control groups showed a more significant increase by the application of the biodynamic preparations. This compensatory effect of the biodynamic preparations was observed for (i) net photosynthetic productivity; (ii) total carotenoid content in pumpkin peel; (iii) lutein + zeaxanthin and ß-carotene in pumpkin flesh and (iv) total carotenoids as well as leuco-anthocyanins in pumpkin seeds. Especially the combination of horn silica and horn manure had a balancing effect. Comparable compensatory effects by the combination of horn manure and horn silica were found by Jarienė et al. [53] and Vaitkevičienė [51] on three potato varieties on net photosynthetic productivity, contents of total phenolics, anthocyanins and antioxidant activity. This compensatory effect of the biodynamic preparations is also described for soil activity [58], yield [12, 45], unfavourable growing conditions [17] and germination of seeds in the following generation [59].

Conclusion

In the present study investigated whether the biodynamic preparations horn manure and horn silica are suitable to improve yield and food quality of pumpkin fruits, based on the content of secondary plant compounds. A plant-based nutrition with vegetables rich in antioxidants is essential for human health. The first hypothesis, that horn manure and horn silica treatments increase pumpkin yield were confirmed by the results of this study. Different plant responses without a clear effective direction did not support the second hypothesis (horn manure reduces the content of secondary plant compounds) and led to its rejection. The hypothesis that single horn silica treatment increases the content of secondary plant compounds was significantly confirmed by i) the contents of macroelements, total carotenoids and antioxidants (catechins, total phenols, leuco-anthocyanins) in peel and flesh; ii) the contents of single carotenoids (lutein + zeaxanthin, lycopene, ß-carotene) in pumpkin flesh and iii) total carotenoids as well as leuco-anthocyanins in seeds. The hypothesis that the combined use of the two preparations increases the secondary plant substances was significantly confirmed by the results of i) total carotenoid contents in pumpkin peel and flesh and ii) the content of lutein + zeaxanthin, lycopene, ß-carotene in pumpkin flesh.

The soil studies of the present trial on pumpkin cultivars are presented in Juknevičienė et al. [28]. Compared to the control, the application of horn manure increased plant-available phosphor, potassium, nitrogen, urease and saccharase activity. Out of the results of the present study, it can be assumed that the increasing soil activity due to the application of horn manure has an important part in the effect of the biodynamic preparations on plant growth and yield formation. The horn silica treatment increased

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the contents of valuable antioxidants, which are important for human nutrition. The results of the present investigation on pumpkin cultivars and the results of the soil investigation [28] confirm the basic statement of organic agriculture—a more active healthy soil leads to a better, more healthy plant growth and a healthy diet. The biodynamic preparations horn manure and horn silica supported a positive development of soil activity and the formation of high-quality plants for nutrition.

It can be summarised, that the pumpkin trial results especially indicated a general growth-promoting effect by horn manure, a quality-enhancing effect by horn silica and a compensatory effect through both preparations on a high yield and qualitative level. Since these effects did not occur in the same way in all plant species, the effect of the biodynamic preparations should be tested in further trials on a plant species-specific basis. Possible influences of environmental factors on the effect of the preparations also need to be investigated more closely.

Abbreviations
DM: Dry matter; FM: Fresh mass; HM: Horn manure; HS: Horn silica.

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Authors' contributions
EJ, HD, EdJ, VZ designed the work and interpreted the results. EdJ carried out the trials and the evaluation. JF, JZ have created the figures and written the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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