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INFLUENCE OF ORGANIC FERTILIZERS ON GRAIN QUALITY OF *TRITICUM MONOCOCCUM* L., *TRITICUM DICOCCUM* SCH., AND *TRITICUM SPELTA* L.

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Abstract

Organic farmers require cultivars that can be multiplied and grown in the organic farming system with no undue negative effect on the health and function of the agroecosystem. The objective is to research the impact of different types of fertilizers on the grain quality for species of wheat (*Triticum dicoccum* Sch., *Triticum monococcum* L., and *Triticum spelta* L.) cultivated under the conditions of organic farming.

The experiment was conducted in 2014-2017 at the Agroecological Center at the Agricultural University - Plovdiv (Bulgaria) by the block method in three replications with a size of the experimental plot of 10.5 m^2. The organic fertilizers: Amalgerol, Lithovit, Baikal EM, Tryven - for foliar application, and one soil fertilizer - Agriorgan pellet were used in the study. Indicators measured and methods used were: Mass of 1000 grains (g)- weight method; Separation of grain from chaff (%); Hectolitre weight (kg/100 L grain) - BSS method; raw protein and fats according to the BSS 11374 method; starch according to the polarimetric method, BSS EN 13040; wet gluten - method adopted for low-gluten foods (%).

The results show that all organic fertilizers increase the 1,000 seeds weight in all wheat species. Higher percentage differences were observed for *Tr. spelta* when treated with Tryven - 17.8%, Amalgerol - 9.6% over control. In *Tr. dicoccum* in the variants treated with Baikal EM (3.9%), Amalgerol - 2.9%, and for *Tr. monococcum* the variant treated with Amalgerol - 8.4%, Baikal EM and Tryven. Treatment with Baikal EM and Lithovit increased the hectoliter mass in *Triticum dicoccum* by 1.8% and 1.2% above the control, respectively, and in *Triticum monococcum* - Baikal EM by 3.5 and Amalgerol by 2.3%. In *Triticum spelta*, an increase in the indicator above the control is observed after treatment with Baikal EM - by 3.8%. The amount of crude protein, starch and fat in the grain varies within a narrow range between the different fertilization options and the control within species. In organic farming specific conditions all three wheat types show a low gluten content. The highest wet gluten content was reported after Tryven treatment at *Tr. dicoccum* - 2.9% and with Amalgerol- 3% at *Tr. spelta*.

The studied organic fertilizers have a positive effect on the physical and biochemical parameters of the grain can be successfully used in the production of healthy foods in organic farming.

***Key words**: Organic agriculture, Grain quality, Biofertilizers, Triticum dicoccum Sch., Triticum monococcum L., Triticum spelta L., Wet gluten.*

1. Introduction

Organic farmers require cultivars that can be multiplied and grown in the organic farming system with no undue negative effect on the health and function of the agroecosystem [1]. Lacko- Bartošová *et al.*, [2] quoted Macák, [3], according to who the organic growing system offers an alternative way for the environment to be friendly and to satisfy crop production. Organic agricultural systems have already proved their capacity to produce food with the best sensory quality, high nutritional values, and high quality standards [4]. Wheat is very important world-wide, being cultivated in more than 100 countries throughout the world [5], it is the principal source of energy, protein, and dietary fiber for a major portion of the world's population [6]. In Europe, wheat cultivars for organic farming have to fulfill the requirements of farmers and consumers of organic products [7], and because of its great adaptability, it is very suitable for organic production [8, 9]. The group of alternative cereal grains comprises some old and nearly forgotten wheat species, such as spelt (*Triticum spelta* L.), emmer (*Triticum dicoccum* Schrank), and einkorn (*Triticum monococcum* L.) [10]. "Ancient" wheat species were cultivated historically but are today only grown on small areas [11]. Hulled wheat species (einkorn, emmer and spelt) are among the most ancient cereal crops of the Mediterranean region [12], which belong to the family *Poaceae* [13, 14]. Einkorn wheat was one of the first crops domesticated approximately 12,000 years ago in the Near East, alongside emmer wheat (*Triticum dicoccum*) [15]. Einkorn, a small soft-seeded diploid wheat (*Triticum monococcum* L.), contains elevated levels of protein and yellow pigments [16] and is rich in lutein [17]. Dinu *et al.*, [18], point out that wild einkorn is widely adapted with respect to both soil and climate. Typically, einkorn was cultivated on marginal agricultural land, being able to survive in harsh environments and poor soils where other species of wheat could not survive. Emmer wheat (*Triticum dicoccon* Schrank, *Triticum dicoccum* Schübl) is one of the oldest crops in the world [19]. Emmer wheat as functional food is an excellent source of several functional compounds and is very useful in the organic farming practice [20] has high total antioxidant activity, total phenolics, ferulic acid and flavonoids [21]. Emmer is considered healthy food due to its high protein level and antioxidant capacity [22]. High grain quality, first of all high protein content, predetermines emmer wheat for preparation of healthy dishes in bio quality [23]. *Triticum dicoccum* Schübl contains special carbohydrates, which are important factors for blood clotting and the stimulation of the human immune system. It is also a superb fiber resource and has a large amount of B-complex vitamins [24]. *Triticum spelta* represents a hexaploid series of the *Triticum* genome constitution, which is characterized by a great adaptation to a wider range of environments [18]. The nutritive value of *Triticum spelta L.* is high, as it contains all basic components people need, such as sugars, proteins, lipids, vitamins and minerals [25]. The spelt grain may have higher contents of protein and fiber, minerals, β-carotene [26]. Proteins make the second most abundant group of compounds in wheat kernels, right after starch. The protein level is one of the most important chemical indicators according to which wheat is classified into quality classes [10]. In Bulgaria einkorn and emmer are grown on a quite limited area mostly on soils with low fertility not suitable for other types of wheat, in mountainous regions and near some river valleys. The emmer and einkorn wheat have fewer requirements in terms of soil and climatic conditions, rarely suffer from plant diseases, and overall fewer pesticides are needed [21]. Organic farmers and producers have become more and more interested in marginal crops such as the tetraploid emmer wheat because of its suitability for organic farming [23].

The objective is to research the impact of different types of organic fertilizers on the grain quality from species wheat *Triticum dicoccum Sch., Triticum monococcum* L., and *Triticum spelta* L., cultivated under the conditions of organic farming.

2. Materials and Methods

2.1 Materials

The experiment with hulled (einkorn, emmer and spelt) wheats was carried out in 2014 - 2017 on the fields of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria), which are a part of the certified Demonstration organic farm, on the soil type Mollic Fluvisols in the FAO classification [27]. The sowing material is provided by an organic sowing-seed and by the Institute for Plant Genetic Resources 'K. Malkov' - Sadovo. The sowing was conducted in mid-October using a block method in three repetitions with a size of the reported parcel of 10.5 m^2 with a sowing rate of 500 germinating seeds (g.s) per square meter (m^2) (g.s./m^2), following a pepper (*Capsicum annuum* L.) precursor. Biofertilizer Agriorgan Pellet was introduced into the soil, in the form of basic fertilization on the entire experimental area before sowing in the dosage 1,000 kg/ha. The experimental factors were as follows: A. the vegetation year: a1: 2014 - 2015, a2: 2015 - 2016 and a3: 2016 - 2017; B. 3 wheat species emmer, spelt and eincorn: b1: *Triticum dicoccum* Sch., b2: *Triticum spelta* L. and b3: *Triticum monococcum* L.; C. foliar biofertilizers: c1: no import of foliar fertilizers, c2: Amalgerol, c3: Lithovit, c4: Baikal EM, and c5: Tryven. Three types of ancient wheats (*Triticum dicoccum* Sch., *Triticum spelta* L. and *Triticum monococcum* L.), which are grown on small areas in Bulgaria, were tested. All biofertilizers were applied foliarly through two vegetation sprinkling, respectively at the stages of tillering and stem elongation, in the following concentrations: Amalgerol - 2 L/ha (tillering), and 5 L/ha (stem elongation); Lithovit - 1.5 kg/ha (tillering), and 1.5 kg/ha (stem elongation); Baikal EM - 0.1% solution (tillering), and a solution of 0.1% (stem elongation); Tryven - 4 L/ha (tillering) and 4 L/ha (stem elongation).

The active ingredients of all used biofertilizers are among permitted substances list of the Regulation [28] concerning organic farming in the European Union), or: Amalgerol - is liquid emulsion concentrate, containing extracts of seaweeds, distilled paraffin oil, vegetable oil, distilled herbal extracts; Lithovit is a high-quality nanotech product created via tribodynamic activation and micronization. Its contents is following: $CaCO_3$ - 79.19%; $MgCO_3$ - 4.62%; and Fe - 1.31%; Baikal EM are effective microorganisms, mixed cultures of useful microorganisms. Its contents is following: organic carbon - 0.15%; total nitrogen - 0.01%; P_2O_2 - 0.001%; K_2O - 0.02%; pH - 3.2, and secondary microflora; Tryven contents is following: Total nitrogen (N) - 24.4%; Ammonium nitrogen - 2.60%; Nitrate nitrogen < 0.01%; Organic nitrogen - 17.3%; P_2O_5 - 17.2%; K_2O - 7.42%; Agriorgan Pellet which consist of sheep manure enriched with microorganisms and a supplement of microelements. Contents is following: Total nitrogen (N) - 3.0%; Organic nitrogen - 2.5%; P_2O_5 - 3%; K_2O - 1%; Fe - 1.5%; Organic carbon (C) - 28.5%; Humic acids - 6%; C/N ratio - 11.4%; It contains 70% organic matter (Ca, Mg, S), its humidity is 10 - 14%, and pH - 6.

2.2 Methods

2.2.1 Physical indicators of the grain

Mass of 1000 grains (g) was determined according to the weight method; Separation of grain from husk (%) by determination of the percentage share of hulled grain to the husk in a sample of 5 g manually peeled grain, and hectolitre mass (HLM) (kg/100 L grain) - according to the BSS- based methods.

2.2.2 Biochemical indicators of the grain

Contents of raw protein and fats was determined according to the BSS 11374 method, starch according to the polarimetric method determined for the dry substance based on BSS EN 13040, and contents of wet gluten according to the method adopted for low-gluten foods (%).

2.2.3 Statistics

The statistical processing of the experimental data was performed using SPSS V. 9.4 for Microsoft Windows [29], Microsoft Office Excell 2007 [30].

3. Results and Discussion

3.1 Agroclimatic characteristics

During the study period 2014 - 2017, the conditions characterized the years as relatively warm, with temperature values above the norm for the long-term period except for January 2017 (Table 1). Rainfall was unevenly distributed throughout the vegetation. Sowing of the wheat was carried out in October. In 2015, spring vegetation started in March in heavy rainfall conditions (138 mm/m^2). This situation had a positive effect on the panicle structural elements formation which structuring is in March - April. From March 2016 until the end of the vegetation, temperatures are slightly above normal and rainfall is near long-term values. Rainfall values in May (64.7 mm/m^2) twice exceed these for the long period, which allows the normal grain filling and feeding during the milk-waxy maturity phase. The growing season 2016 - 2017 is characterized as relatively warm. Low temperatures below normal were recorded in January, but no frost in plants was recorded. The year has an uneven distribution of rainfall, but relatively favorable for high yields. Overall, the weather conditions during the study period 2014 - 2017 are favorable for all three wheat species. The study period is characterized by being warm and well stocked with moisture. These conditions allow formation of big grain with good physical and biochemical parameters.

3.2 Physical parameters of the grain

3.2.1 1,000 seeds weight

The parameter 1,000 seeds weight is strongly influenced by genotype, weather conditions and the nutrients, available to the plant during its vegetation. In tracking this indicator, the impact of the different fertilizers on the plant is multifaceted (Table 2). The different wheat

Table 1. Average monthly air temperatures and monthly amount of rainfall for the study period (2014-2017)

	Months									
	IX	X	XI	XII	I	II	III	IV	V	VI
	Temperature (^{0}C)									
2014 - 2015	18.1	12.8	7.9	5.1	3.1	3.7	6.7	12.4	19.3	21.1
2015 - 2016	21	12.8	11.3	5.1	-0.1	8	9.3	15.5	17	23.3
2016 - 2017	19.6	12.7	6.6	2.2	-3.9	3.2	9.7	12.7	17.6	22.8
Average monthly temperature-norm 1965 - 1995	18.3	12.6	7.4	2.2	-0.4	2.2	6	12.2	17.2	20.9
	Rainfall (mm/m^2)									
2014 - 2015	195.7	121.1	49.5	93	17.4	76.6	138	14	69.5	76.7
2015 - 2016	100.6	70.3	39.6	3.6	69.6	24.4	33.9	30.7	64.7	59.7
2016 - 2017	2.3	19.7	32.9	2.4	70.1	11.1	47.9	26.1	52.7	15.4
Amount of rainfall-norm 1965 - 1995	65	47	35	36	40	48	44	39	32	36

species also differ from each other. In 2015, treatment with Baikal EM and Tryven increased the weight of 1,000 seeds in *Tr. dicoccum* Sch. from 2.2% (35.13 g) to 2.8% (35.34 g) compared to the control variant - 34.37 g, and for *Tr. spelta* L. treatment with Lithovit - 40.23 g (1.85% over the control). For both types of wheat, these differences are not statistically proven.

A stronger overall effect of fertilizers was observed in *Tr.monococcum* L., where all variants of fertilization exceeded the control and these differences are statistically proven. This is most pronounced when fertilizing with Baikal EM - 28.78 g (12.91% above control), followed by Amalgerol - 27.51 g and Lithovit - 27.11 g. In 2016, the impact of biofertilizers was stronger and there were statistical differences in fertilization variants in all three types of wheat.

Under the climatic conditions of 2016, the treatment of *Tr. dicoccum* with Amalgerol increases the weight of 1,000 seeds by 12.7% over the results obtained for the control variant, which is statistically proven. At *Tr. spelta*, Tryven and Amalgerol proved an increase the seed weight, which range from 26.3% to 26.8%, respectively. The reaction to applied biofertilizers was most pronounced in *Tr. monococcum*. All fertilized variants have statistically proven higher values than the control variant. Values ranged from 4.3% above control with Amalgerol treatment to 10.1% with Lithovit treatment. In 2017 again statistically proven differences were observed in the different fertilization variants compared to controls plants. At *Tr. dicoccum* Sch. proven highest values were reported for variants fertilized with Baikal EM (35.2 g) and Lithovit (34.93 g) compared to 33.66 g for the control variant. Fertilizing with Tryven at *Tr.spelta* L. exceed by 42% the control variant, and the results obtained are statistically proven. All other fertilized variants also have proven higher values than the control. At *Tr. monococcum* L., all fertilizer variants have proven higher values than the control variant. The highest value was obtained for Tryven fertilization - 28.33 g and Amalgerol - 28.23 g. On average over the study period, in *Tr. dicoccum*, the highest grain mass was obtained by fertilizing with Baikal EM - 32.85 g, 3.9% above control, and in variants with Amalgerol - 32.51 g, by 2.9% above control. In *Tr. spelta*, the highest value of grain mass was reported with Tryven treatment - 32.97 g with 17.8% over control and Amalgerol - 30.68 g. Other fertilizer variants also have values higher than controls. In *Tr. monococcum*, the strongest influence on increasing 1,000 grains weight was recorded in the Amalgerol treated variant - 25.45 g (8.4% above control), followed by the variants treated with Baikal EM - 25.27 g and Tryven - 25.22 g. On average over the study period, differences from controls are not proven, but all fertilized variants increase the 1000 seeds weight in all three wheat species to varying degrees over the years. This indicates that the foliar biofertilizers applied, contribute to a better nutrition of the grain and a greater mass of seeds under the specific agro-environmental conditions of the year.

3.2.2 Husk of grain

The presence of husk on grains after harvest is a characteristic feature of ancient wheat species, which

Table 2. Influence of biofertilizers on 1,000 seeds weight (excluding husk) in wheat species

Treatments	2015		2016		2017		Average (g)	%
	Average (g)	%	Average (g)	%	Average (g)	%		
				Emmer wheat- (*Triticum dicoccum* Sch.)				
Control	34.37 [a]	100	26.75 [c]	100	33.66b [c]	100	31.59 [a]	100
Amalgerol	33.93 [a]	98.7	30.16 [a]	112.7	33.46 [c]	99.4	32.51 [a]	102.9
Lithovit	34.47 [a]	100.3	25.91 [d]	96.9	34.93 [a]	103.8	31.77 [a]	100.6
Baikal EM	35.13 [a]	102.2	28.22 [b]	105.5	35.20 [a]	104.6	32.85 [a]	103.9
Tryven	35.34 [a]	102.8	25.72 [d]	96.1	34.26 [b]	101.8	31.77 [a]	100.6
				Spelt wheat - (*Triticum spelta* L.)				
Control	39.50 [a]	100	24.88 [b]	100	19.60 [c]	100	27.99 [a]	100
Amalgerol	39.24 [a]	99.3	31.55 [a]	126.8	21.27 [b]	108.5	30.68 [a]	109.6
Lithovit	40.23 [a]	101.8	24.93 [b]	100.2	21.42 [b]	109.3	28.86 [a]	103.1
Baikal EM	39.58 [a]	100.2	25.14 [b]	101.0	21.60 [b]	110.2	28.77 [a]	102.8
Tryven	39.58 [a]	100.2	31.43 [a]	126.3	27.89 [a]	142.3	32.97 [a]	117.8
				Einkorn wheat- (*Triticum monococcum* L.)				
Control	25.50 [b]	100	19.76 [c]	100	25.20 [c]	100	23.48 [a]	100
Amalgerol	27.51 [ab]	107.9	20.61 [b]	104.3	28.23 [a]	112.0	25.45 [a]	108.4
Lithovit	27.11 [ab]	106.4	21.76 [a]	110.1	25.86 [b]	102.6	24.91 [a]	106.1
Baikal EM	28.78 [a]	112.9	21.32 [a]	107.9	25.70 [b]	101.9	25.27 [a]	107.6
Tryven	25.89 [ab]	101.6	21.43 [a]	108.4	28.33 [a]	112.4	25.22 [a]	107.5

Legend: *Means followed by the same letter are not statistically different (P < 0.05) by Duncan's multiple range test.

includes the species studied in the experiment. The presence of husk reduces the yield and increases the cost of the grain itself. The data in Table 3 shows that the percentage of husk in the grain is a relatively constant indicator that is not significantly affected by the fertilizers applied.

Differences in the percentage distribution of hulled grain - husk are mainly observed at the level of wheat type. The percentages of hulled grain - husk vary over the years in all variants of fertilization. The average for the study period with the highest proportion of hulled grain to husk was *Tr. dicoccum*, followed by *Tr. monococcum* and *Tr. spelta*. A higher percentage of hulled grain, as a result of the applied biofertilizers was reported in *Tr. dicoccum*, treated with Lithovit (79.92% hulled grain and 20.08% husk), with *Tr. spelta* treated with Amalgerol (71.59% hulled grain against 28.41% husk), *Tr. monococcum* when treated with Amalgerol (72.54 hulled grain and 27.46% husk), and Tryven (71.88% hulled grain and 28.12% husk). Greater differences of hulled grain to husk compared the control were observed with variants treated with Amalgerol, Tryven and Lithovit.

3.2.3 Hectoliters of grain

In monitoring the effect of fertilization on the hectoliter weight, an increase in values of indicators was observed to a different extent in all variants compared to the control, but this effect was not statistically proven (Table 4).

There has been no consistent trend in the impact of different fertilizers over the years on different wheat species. This is to some extent due to the specific conditions of the years, which differ strongly from one another and affect the nutrition and development of the plant. Average for the study period 2014 - 2017, with *Tr. dicoccum*, the highest value per hectoliter weight was recorded in the variants, treated with Baikal EM - 52 kg and Lithovit - 51.71 kg. For *Tr. spelta*, the highest hectoliter weight was obtained for Baikal EM treated variant - 38.68 kg and Lithovit - 37.52 kg, and for *Tr. monococcum* when Baikal EM was applied- 43.45 kg and Amalgerol - 42.92 kg.

3.3 Biochemical parameters of the grain

3.3.1 Crude protein, starch and grain fat content

From the data on the biochemical analysis of the grain, it can be seen that during the different years of the study, the values of the crude protein indicator varied between the different fertilization variants and the controls within the species (Table 5).

This can be explained by the specific conditions of the year. The same tendency is observed in the starch and fat content of the grain. On average during the study period, treatment with the studied biofertilizers increased the percentage of crude protein compared to controls in *Tr. dicoccum* and *Tr. spelta*, but not in *Tr. monococcum*. *Tr. dicoccum* had a higher crude protein content compared to the control variant when treated

Table 3. Percentage ratio of hulled grain to husk in the studied wheat species

Treatments	2015		2016		2017		Average	
	Hulled grain (%)	Husk (%)	Hulled grain (%)	Husk (%)	Hulled grain (%)	Husk (%)	Hulled grain (%)	Husk (%)
Emmer wheat- *(Triticum dicoccum* Sch.)								
Control	80.15	19.85	78.60	21.40	77.50	22.49	78.75 [a]	21.25 [a]
Amalgerol	79.70	20.30	79.14	20.86	79.26	20.74	79.36 [a]	20.63 [a]
Lithovit	79.66	20.34	77.54	22.46	82.57	17.43	79.92 [a]	20.08 [a]
Baikal EM	81.59	18.41	79.87	20.13	76.31	23.68	79.25 [a]	20.74 [a]
Tryven	80.05	19.95	79.27	20.73	78.41	21.59	79.24 [a]	20.76 [a]
Spelt wheat - *(Triticum spelta* L.)								
Control	73.90	26.10	68.47	31.53	69.53	30.47	70.63 [a]	29.37 [a]
Amalgerol	74.10	25.90	70.87	29.13	69.79	30.21	71.59 [a]	28.41 [a]
Lithovit	73.05	26.95	64.80	35.20	64.60	35.40	67.48 [a]	32.52 [a]
Baikal EM	71.20	28.80	69.60	30.40	70.20	29.80	70.33 [a]	29.67 [a]
Tryven	74.50	25.50	69.07	30.93	69.13	30.87	70.90 [a]	29.10 [a]
Einkorn wheat- *(Triticum monococcum* L.)								
Control	69.94	30.06	71.27	28.73	66.96	33.03	69.39 [a]	30.61 [a]
Amalgerol	73.81	26.19	70.47	29.53	73.33	26.67	72.54 [a]	27.46 [a]
Lithovit	68.87	31.13	70.20	29.80	68.26	31.74	69.11 [a]	30.89 [a]
Baikal EM	73.43	26.57	69.47	30.53	66.67	33.33	69.86 [a]	30.14 [a]
Tryven	75.00	25.00	69.20	30.80	71.43	28.57	71.88 [a]	28.12 [a]

Legend: *Means followed by the same letter are not statistically different (P < 0.05) by Duncan's multiple range test.

Table 4. Effect of biofertilizers on the hectolitre weight (kg) of the studied wheat species

Treatments	2015		2016		2017		Average	%
	Average	%	Average	%	Average	%		
Emmer wheat - (*Triticum dicoccum* Sch.)								
Control	50.68 [a]	100	53.83 [a]	100	48.75 [a]	100	51.09 [a]	100
Amalgerol	50.80 [a]	100.2	56.33 [a]	104.6	47.50 [b]	97.4	51.54 [a]	100.9
Lithovit	51.02 [a]	100.7	55.76 [a]	103.6	48.33 [a]	99.1	51.71 [a]	101.2
Baikal EM	51.08 [a]	100.8	56.60 [a]	105.1	48.33 [a]	99.1	52.00 [a]	101.8
Tryven	50.58 [a]	99.8	56.00 [a]	104.0	43.75 [c]	89.7	50.11 [a]	98.1
Spelt wheat - (*Triticum spelta* L.)								
Control	36.53 [a]	100	40.17 [a]	100	35.00 [ab]	100	37.23 [a]	100
Amalgerol	36.75 [a]	100.6	41.70 [a]	103.7	32.50 [b]	92.8	36.65 [a]	99.4
Lithovit	37.90 [a]	103.7	41.17 [a]	102.5	33.50 [b]	95.7	37.52 [a]	100.8
Baikal EM	36.72 [a]	100.5	41.83 [a]	104.1	37.50 [a]	107.2	38.68 [a]	103.9
Tryven	37.27 [a]	102.0	41.17 [a]	102.5	33.50 [b]	95.7	37.31 [a]	100.2
Einkorn wheat - (*Triticum monococcum* L.)								
Control	40.24 [c]	100	51.50 [a]	100	34.17 [a]	100	41.97 [a]	100
Amalgerol	43.58 [ab]	108.3	53.17 [a]	103.2	32.01 [bc]	93.7	42.92 [a]	102.3
Lithovit	40.95 [bc]	101.8	53.33 [a]	103.5	31.50 [c]	92.2	41.93 [a]	99.9
Baikal EM	45.63 [a]	113.4	52.67 [a]	102.3	32.05 [bc]	93.8	43.45 [a]	103.5
Tryven	40.67 [c]	101.1	52.50 [a]	102.0	32.16 [b]	94.1	41.78 [a]	99.5

Legend: *Means followed by the same letter are not statistically different ($P < 0.05$) by Duncan's multiple range test.

Table 5. Crude protein, starch and grain fat content, as % of grain dry matter (2015 and 2016)

Treatments	Crude protein (%)			Starch (%)			Fat (%)		
	2015	2016	Average	2015	2016	Average	2015	2016	Average
Emmer wheat - (*Triticum dicoccum* Sch.)									
Control	10.72	10.81	10.76	69.27	68.23	68.75	2.96	3.48	3.22
Amalgerol	11.20	11.44	11.32	70.77	66.67	68.72	2.75	3.56	3.15
Lithovit	11.85	11.15	11.50	68.44	74.48	71.46	3.33	4.00	3.66
Baikal EM	11.84	11.03	11.43	68.47	71.12	69.79	2.70	3.84	3.27
Tryven	12.68	10.67	11.67	68.44	68.13	68.28	3.05	3.20	3.12
Spelt wheat - (*Triticum spelta* L.)									
Control	12.42	12.40	12.41	67.38	77.97	72.67	2.59	3.45	3.02
Amalgerol	12.48	12.58	12.53	68.06	65.16	66.61	2.78	3.45	3.11
Lithovit	13.52	12.02	12.77	67.31	71.43	69.37	2.68	3.37	3.02
Baikal EM	13.27	13.04	13.15	69.02	66.46	67.74	2.15	3.39	2.77
Tryven	12.23	14.70	13.46	69.65	63.62	66.63	2.47	3.34	2.90
Einkorn wheat - (*Triticum monococcum* L.)									
Control	12.25	11.91	12.08	70.30	64.95	67.62	3.23	4.04	3.63
Amalgerol	12.24	11.61	11.92	69.64	66.25	67.94	3.09	3.17	3.13
Lithovit	13.02	12.06	12.54	73.42	66.73	70.07	2.97	3.29	3.13
Baikal EM	11.25	12.04	11.64	68.87	63.43	66.15	3.36	3.58	3.47
Tryven	11.78	12.12	11.95	72.69	71.10	71.89	3.18	3.95	3.56

with Lithovit - 11.50% and Tryven - 11.67%, with *Tr. spelta* the variants treated with Baikal EM - 13.15% and Tryven - 13.46%, and with *Tr. monococcum*, although minimal, only 12.54% in the Lithovit treated variant. The percentage of starch in the grain is also affected by one of the fertilizing variants. *Tr. dicoccum* showed an increase in starch content compared to control at the Lithovit treated variants - 71.46% and Baikal EM - 69.79%, while in *Tr. monococcum* when treated with Lithovit - 70.07% and Tryven - 71.89 %.

The values of the indicator in *Tr. spelta* are close to those of the control. The grain fat content of the studied wheat species is low and varies within small limits between the variants of fertilization and untreated control. The highest amount of crude protein is found in *Tr. spelta*, followed by *Tr. monococcum* and *Tr. dicoccum*. Most fat content is reported in *Tr. monococcum* and *Tr. dicoccum*, followed by *Tr. spelta*. The studied wheat species are low in fat.

<u>3.3.2 Grain wet gluten content</u>

The gluten content is influenced by both abiotic factors and the applied agricultural techniques. Of particular importance for the gluten content are the meteorological conditions, which are favoured by dry and hot weather in the 'grain filling' phase. The meteorological conditions show that the air temperatures were very suitable between 20 - 25 ^{0}C, with a slight variation between the vegetation years, which in combination with good agricultural techniques affected the normal pouring of the grain.

The quantitative content of gluten is a species /variety trait that is influenced by external factors - most often weather conditions. All three wheat species- Emmer, Spelt and Einkorn are characterized by a very low wet gluten content (Table 6), which makes them particularly suitable for healthy gluten-free foods.

Tr. dicoccum shows a percentage increase in wet gluten content in all fertilizer variants. The highest percentage of wet gluten was reported with Tryven treatment - 2.9%, which was 3.6 times over the control variant (0.8% gluten). When treated with Lithovit, the wet gluten content is 2 times more than control. The reverse trend is observed with *Tr. spelta*.

When treated with biofertilizers, lower values for wet gluten content were observed compared to control. Values ranged from 1.1% (with Tryven treatment) to 2.9% (with Lithovit treatment). A slight increase in gluten content as a result of treatment with Amalgerol and Baikal EM (about 7.7% compared to control) was observed with *Tr. monococcum*.

In general, all three wheat varieties - *Tr. monococcum*, *Tr. dicoccum* and *Tr. spelta*, grown under current organic farming conditions, are low in wet gluten. This fact is of great importance when these wheats are used for dietary or healthy food by humans.

4. Conclusions

- The organic production system aims to provide nutrients for plants mainly through the natural cycle of feeding and fertilizing the soil. In organic farming, it aims to use local varieties that are well adapted and undemanding to environmental conditions. Ancient wheat has been forgotten, but in recent years it has given them the opportunity through their biological characteristics and requirements to environmental factors to show their adaptive qualities that correspond to the specific agro-ecological area.
- Studied organic fertilizers increase the weight of 1,000 seeds in all three wheat species, although differences are not proven. Higher percentage differences were observed for *Tr. spelta* when treated with Tryven - 17.8% followed by Amalgerol - 9.6% over control. In *Tr. dicoccum* in the variants treated with Baikal EM (3.9%), Amalgerol - 2.9% (over control), and for *Tr.monococcum* the variant treated with Amalgerol - 8.4% over control, followed by Baikal EM and Tryven.
- Highest proportion of hulled grain relative to husk was reported at *Tr. dicoccum* treated with Lithovit (79.92% hulled grain against 20.08% husk), at *Tr. spelta*

Table 6. Content of wet gluten in the grain by variants

Treatments	Wet gluten (%)	Change from control (in times more)
Emmer - (*Triticum dicoccum* Sch.)		
Control	0.8	-
Amalgerol	1.2	1.5
Lithovit	1.6	2.0
Baikal EM	1.4	1.8
Tryven	2.9	3.6
Spelt - (*Triticum spelta* L.)		
Control	3.0	-
Amalgerol	3.0	1.0
Lithovit	2.9	1.0
Baikal EM	1.6	(0.5)
Tryven	1.1	(0.4)
Einkorn - (*Triticum monococcum* L.)		
Control	2.6	-
Amalgerol	2.8	1.1
Lithovit	1.0	(0.4)
Baikal EM	2.8	1.1
Tryven	1.7	(0.7)

treated with Amalgerol (71.59% hulled grain to 28.41% husk), and in *Tr.monococcum* when treated with Amalgerol (72.54% hulled grain to 27.46% husk) and Tryven (71.88% hulled grain to 28.12% husk).

- Increase in hectoliter mass was observed in *Tr. spelta* after treatment with Baikal EM - 38.68 kg and Lithovit - 37.52 kg, and in *Tr. monococcum* after treatment with Baikal EM - 43.45 kg and Amalgerol - 42.92 kg. The amount of crude protein, starch and fat in the grain varies within a narrow range between the different fertilization options and the control within species. Under specific organic farming conditions, all three wheat species - *Tr. monococcum*, *Tr. dicoccum* and *Tr. spelta*, are low in gluten. The highest wet gluten content was reported after treatment with Tryven on *Tr. dicoccum* - 2.9% and on treatment with *Tr. spelta* with Amalgerol - 3% and on treatment with Amalgerol and Baikal EM (2.8%) in *Tr. monococum*.

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5. References

[1] Kunz P., and Karutz C. (1991). *Plant breeding dynamic of the site-specific wheat and spelled varieties* (in German). Research laboratory at the Goetheanum, Dornach, Switzerland, pp. 164.

[2] Lacko-Bartošová M., Korczyk-Szabó J., and Ražný R. (2010). *Triticum spelta - A specialty grain for ecological farming systems*. Res. J. Agric. Sci., 42, (1), pp. 143-147.

[3] Macák M. (2006). *Agri-environmental indicators for agricultural sustainability assessment (1 Ed.)* (in Slovak). Slovak University of Agriculture, Nitra, Slovakia, (35), pp. 118.

[4] Lairon D. (2009). *Nutritional quality and Safety of Organic* Food. Sustain. Agric., (2), pp. 991.

[5] Giraldo P., Benavente E., Manzano-Agugliaro F., and Gimenez E. (2019). *Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis*. Agronomy, 9, pp. 352.

[6] Abdel-Aal E. S. M., Hucl P., and Sosulski F. W. (1995). *Compositional and Nutritional characteristics of Sping Einkorn and Spelt Wheats*. Cereal Chem., 72, (6), pp. 621-624.

[7] Hildermann I. (2010). *Performance of Winter Wheat cultivars in Organic and Conventional Farming Systems*. Inaugural Disseratation, Faculty of Philosophy and Natural Sciences, University of Basel, Switzerland, pp. 119.

[8] Willer H., and Kilcher L. (Eds.). (2011). *The World of Organic Agriculture: Statistics and Emerging Trends* FiBL-IFOAM Report, Frick, Switzerland.

[9] Konvalina P., Capouchová I., and Stehno Z. (2012). *Genetic resources of hulled wheat species in Czech organic farming*. 62 Conference of the Association of Plant Breeders and Seed Merchants in Austria Proceedings, Raumberg-Gumpenstein, Austria, pp. 81- 86.

[10] Ikanović J., Popović V., Janković S., Dražić G., Pavlović, S., Tatić M., Kolarić L., Sikora V., and Živanovic L. (2016). *Impact of agro-ecological conditions on protein synthesis in hexaploid wheat-spelt (Triticum spelta)*. Biotechnol. Anim. Husb., 32, (1), pp. 91-100.

[11] Melese B., Satheesh N., and Workneh F. S. (2019). *Emmer Wheat - An Ethiopian Prospective: A Short Review*. Ann. Food Sci. Technol., 20, (1), pp. 89-96.

[12] Ünal G. H., (2009). *Some Physical and Nutritional Properties of Hulled Wheat*. Journal of Agricultural Sciences, 15, (1), pp. 58-64.

[13] Peng J., Sun D., and Nevo E. (2011). *Wild emmer wheat, Triticum dicoccoides, occupies a pivotal position in wheat domestication process*. Aust. J. Crop Sci., 5, (9), pp. 1127-1143.

[14] Peña-Chocarro L., Peña L. Z., Urquijo J. E. G., and Estévez J. J. I. (2009). *Einkorn (Triticum monococcum L.) cultivation in mountain communities of the western Rif (Morocco): An ethnoarchaeological project*. Oxbow, Oxford, UK, pp. 103- 111.

[15] Nevo E. (1988). *Genetic resources of wild emmer wheat revisited: Genetic evolution, conservation and utilization*. Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, UK, pp.121-126.

[16] Abdel-Aal E.S.M., Young J.C., Wood P.J., Rabalski I., Hucl P., Falf D., and Frégean-Reid J. (2002). *Einkorn: A potential Candidate for Developing High Lutein Wheat*. Cereal Chem. 79 (3), pp. 455-457.

[17] Serpen A., Gökmen V., Karagöz A., and Köksel H. (2008). *Phytochemical quantification and total antioxidant capacities of emmer (Triticum dicoccon Schrank) and einkorn (Triticum monococcum L.) wheat landraces*. J.bAgric. Food Chem., 56, (16), pp. 7285-7292.

[18] Dinu M., Whittaker A., Pagliai G., Benedettelli S., and Sofi F. (2018). *Ancient Wheat species and Human health: Biochemical and Clinical implications*. J. Nutr. Biochem, pp. 1-33.

[19] Zaharieva M., Ayana N. G., Hakimi A. A. l., Misra S. C., and Monneveux P. (2010). *Cultivated emmer wheat (Triticum dicoccon Schrank), an old crop with promising future: A Review*. Genet. Resour. Crop Evol., 57, pp. 937-962.

[20] Curna V., and Lacko-Bartosova M. (2015). *Indirect Indicators of Baking Quality of Organic Emmer Wheat*. Acta Fytotechn. Zootechn., 18, pp. 1-3.

[21] Mitreva Z., Krasteva V., and Pankov V., (2016). *Method for Evaluation of Soil Conditions Suitable for Growing Emmer and Einkorn Wheat*. Bulg. J. Soil Sci., 1, (2), pp. 147-153.

[22] Lacko-Bartošová M. (2010). *Nutritional quality and antioxidant capacity of Triticum spelta varieties*. J. Ecology Health, 14, (6), pp. 290-294.

[23] Konvalina P., Stehn Z., Capouchová I., Moudrý J., Jůza M., and Moudrý J. (2010*). Emmer Wheat Using and growing in the Czech Republic*. Agronomie, 53, (2), pp. 1-5.

[24] Stefanini S., Alpi A., Guglielminetti L. (2008). *Identification of a commercial emmer (Triticum dicoccum Schübl.) by a proteomic approach*. Quad. Mus. St. Nat. Livorno, 21, pp. 15-20.

[25] Bojňanska T., and Frančáková H. (2002). *The use of spelt wheat (Triticum spelta L.) for baking applications*. Rostlinná Výroba, 48, (4), pp. 141-147.

[26] Petrenko V., Sheiko T., Khudolii L., and Bondar V. (2018). *Evaluation of three wheat species commonly used in organic cropping systems, considering selected*

technological parameters for ethanol production. Proceedings 17th International Scientific Conference "Engineering for Rural Development", Jelgava, Latvia, pp. 451-456.

[27] Popova R., Sevov A. (2010). *Soil characteristics of the experimental field of the Department of Plant Breeding at the Agricultural University - Plovdiv in connection with the cultivation of cereals, technical and fodder crops*. SW of the AU, LV, (1), pp. 151- 156.

[28] EU Commission. (2008). *Regulation No 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.* <URL:https://eur-lex.europa.eu/legal-content/EN/TXT/?uri. Accessed 18 May 2021.

[29] SAS Institute. (1999). Statistical Analysis System, Statistical Methods. SAS Institute, Cary, USA.

[30] Duncan D. (1955). *Multiply range and multiple F-test*. Biometrics, 11, pp. 1-42.