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Distribution of Elements in Durum Wheat Seed and Milling Products, Discrimination of Cultivation Methods by Multivariate Data Treatment

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Abstract: Durum wheat is an important staple food used to obtain several products. At first the wheat is milled to obtain different products: bran, semolina and flour. These products are the base of several artifacts with varying properties both from a nutritional point of view and flavoring characteristics. It is known that most elements concentrate in the outer layers of the wheat seed (pericarp and aleurone) so that the content of the elements vary a lot in the ground products. The present study investigates the characterizing elements of the milled products and the effect of cultivation protocol applied. We measured, by ICP-OES, the concentration of 28 elements in the whole seed and in any grinding products; the results show that only few elements characterize each product. Few elements, but different for each product, permit to disclose the kind of agricultural method used: organic or conventional protocol. Five elements: B, Cd, Cu, K, Se, are the most important to distinguish between organic and conventional agriculture by PCA and PLS analysis; these elements also permit some differentiation of products.

Keywords: cereals; wheat; ICP-OES; spectroscopy; chemometry; classification

1. Introduction

Cereals are among the most important staple food crops; they are cheap source of calories, protein and elements for the inhabitants worldwide. Among the cereals, Durum wheat (Triticum durum) is the base of many largely used foods like pasta [1]. Durum wheat production, the tenth most important crop worldwide, has important impact on the economy and the environment, it was assessed [2] that these impacts could improve by organic cultivation practice. The cultivation method affects the final products, but high quality pasta has been obtained from organic wheat, in Southern Italy, using selected varieties of wheat[3]. Foods derived from wheat contribute to the body's need for essential elements, however, when polluted [4], they can contribute significantly to overexposure to some elements. Wheat plants exploit the elements [5] present in the soil for their biological needs but the concentration of these elements and their solubility changes in the different soils making their uptake by the plants more or less favored [6]. The available content of the elements is related to the content of clay [7] as shown for Saskatchewan agricultural soils. Another important source of some elements is both atmospheric [8] and soil pollution [9], furthermore in agricultural practices there is extensive use of substances containing potentially toxic elements for humans. Wheat plants will accumulate the elements, therefore, influenced by the species and based on the different exposure to the aforementioned sources. It is important to understand, since the cereal seeds are used for food purposes, how many and which elements are accumulated in the seeds.

The elemental distribution in the kernel is important because going from the outer to the inner of the seed, during the grinding process, we can obtain bran, semolina and flour that are used for

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different kind of food products. The knowledge of their elemental affinity can help to produce food of special characteristic or reduce the impact of environmental pollution on the final food products.

The accumulation of the elements in the seed[1] also depends on the plant genotype, the environment, the yearly rain amount.

The correlation between the genotypes of common wheat (*Triticum aestivum*) cultivated in Cina and the Mineral element concentrations of grain was investigated [10] with the goal of selecting those genotypes having the higher content of Fe and Zn. Brizio et al. studied the correlation of the metals with cereals species in Italy [11] paying attention to micronutrient and toxic elements. A study on French soft-wheat showed a tie between the topsoil characteristics and the content of metals of the common wheat seeds [6]. A comparison of the elements measured in several varieties of bread and durum wheats grown in Turkey [12] showed a high variability of the concentrations.

Geographical traceability of durum wheat was studied combining the elemental analysis with the Sr isotopic ratio in an Italy versus world study [13] and to characterize the Tyrol cereals[14] by $^{87}Sr/^{86}Sr$ ratio.

Multivariate analysis applies to several food materials in order to easy their authentication and fraud prevention [15], some studies involve cereals for which infrared spectroscopy [16] coupled to calibration methods permit to measure chemical composition (e.g. protein, moisture, oil) but the same spectroscopic techniques combined with pattern recognition and/or discriminant techniques were used for the authentication and traceability of cereals. Some works consider the volatiles substances, chromatographically determined, as the base of the multivariate methods for classification on the base of wheat cultivation area and species [17] or authenticate the Italian pasta [18] or even correlate the characteristics to the cultivation altitude [19]. There are studies in which the chemometric methods are coupled to the measured elemental contents for authentication purpose [20][21].

The present work aims to broaden the knowledge about the content of the elements contained in different products obtained from the milling process of the durum wheat seed. The study includes many varieties cultivated under organic or conventional protocol so we will check if a difference exists, at grinding product level, between these two cultivation protocols. To the goal some chemometric methods will need to analyze the measurements database.

2. Materials and Methods

2.1. *The samples*

The seeds of different varieties of durum wheat were sampled after their harvesting in July 2022, they were stored in a refrigerator till their grinding. The wheats were cultivated in the experimental fields of the AMAP in Jesi, two fields not far each other devoted one to the organic cultures the other for conventional agricultural procedure. These are clay soils; each field is divided into parcels of 7x1.4 m in each of which a different variety of wheat is grown; every variety is triplicate on three parcels. The seed harvest is carried out by keeping the seeds of each plot divided. The seeds were milled to obtain 4 products of each variety: whole seed, bran, semolina, flour as below detailed. There are some additional samples of seed that were not milled, Table 1 details the samples.

Table 1. Details of the samples.

Wheat variety	Code of the variety	Code of the Organic samples	Code of the Conventional samples	seed	bran	semolina	Flour
Saragolla new	01	01_2	01_1	Yes	NO	NO	NO
San Carlo	02	NO	02_1	Yes	NO	NO	NO
Fuego grown in a large plot	03	03_2	03_1	Yes	NO	NO	NO

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EVOLDUR evolutionary population harvest 2021	04	04_2	NO	Yes	NO	NO	NO
Evoldur grown in a large plot	04	NO	04_1	Yes	NO	NO	NO
Senatore Cappelli	05	05_2	NO	Yes	NO	NO	NO
Saragolla old	06	06_2	NO	Yes	NO	NO	NO
Fuego grown in the edge near the railway.	07	07_2	NO	Yes	NO	NO	NO
Antalis	08	08_2	08_1	Yes	Yes	Yes	Yes
Bering	09	09_2	09_1	Yes	Yes	Yes	Yes
Casteldoux	10	10_2	10_1	Yes	Yes	Yes	Yes
Claudio	11	11_2	11_1	Yes	Yes	Yes	Yes
Fuego	12	12_2	12_1	Yes	Yes	Yes	Yes
Idefix	13	13_2	13_1	Yes	Yes	Yes	Yes
Iride	14	14_2	14_1	Yes	Yes	Yes	Yes
Marakas	15	15_2	15_1	Yes	Yes	Yes	Yes
Marco Aurelio	16	16_2	16_1	Yes	Yes	Yes	Yes
Monastir	17	17_2	17_1	Yes	Yes	Yes	Yes
Platone	18	18_2	18_1	Yes	Yes	Yes	Yes
RGT Natur	19	19_2	19_1	Yes	Yes	Yes	Yes
Tito Flavio	20	20_2	20_1	Yes	Yes	Yes	Yes

In the following every product sample is coded with the following syntax: CC_ntt where CC is the code indicated in the second column of Table 1, n indicate the agricultural method: 1 means conventional, 2 stands for organic. The two characters tt are absent in codes of seeds while they are: Cr for bran, Se for semolina, Fa for flour.

2.2. Milling

The durum wheat seed samples were conditioned in order to reach 17% humidity by adding water in two stages 16 h and 3 h before grinding. The seeds are then milled with a CD2 Chopin Technologies mill then passed through the Chopin purifier to get three fractions:

flour: ≤ 160 microns
semolina: 160 < semolina ≤ 560 micron
bran: > 560 microns

2.3. Mineralization

The samples of whole seed, bran, semolina and flour were dried in an oven at 60 °C for 24 hours; seeds were washed with ultrapure 18.2 MΩ water from a Milli-Q (Millipore, USA) before drying. About one gram of each dry sample (whole seed, bran, semolina, flour) was added with 8 ml ultrapure HNO_3 65% and 2 ml ultrapure HCl 37% then digested by a microwave assisted instrument (ultraWAVE, Milestone Srl, Sorisole (BG), Italy) for 40 minutes. The digested were recovered with ultrapure water and diluted to 50 ml. All the reagents are high purity Merck products for Inductively Coupled Plasma Spectroscopy (ICP).

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2.4. ICP analysis

The mineralized solutions were analyzed by a ThermoFisher Scientific iCAP PRO X Duo ICP-OES for the elements in Table 2. Quantification occurred with the use of calibration lines from 0.001 to 10 mg/L, in decadic steps. Calibrations were obtained by means of the Multi Element Standards Ultra Scientific IQC-026 except for P (Sigma Aldrich 207357) and Sn (Merck 43922907). LOD and LOQ were automatically computed from the calibration lines by the instrumental software. Samples outside the calibration range were suitably diluted to fall within the calibration.

Table 2. Elements determined and wavelengths used for their quantification. The parameters are computed on all the product samples as a whole: seed, bran, semolina and flour in a unique set. (**) indicates the elements, whose means, give a significant t-test comparison (α=0.05) between organic products and the conventional ones.

				All together			Organic			Conventional		
Element	**Wavelength (nm)**	**LOD (mg/Kg)**	**LOQ (mg/Kg)**	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**
Ag (**)	328.068	7E-4	0.002	0.003 ± 0.003	0.003	LOD - 0.013	0.003 ± 0.002	LOQ	LOD - 0.007	0.004 ± 0.003	0.003	LOD - 0.013
Al (**)	396.152	3E-4	0.001	5.514 ± 4.505	4.701	1.447 - 44.802	6.940 ± 5.875	5.311	1.788 - 44.802	4.089 ± 1.517	4.117	1.447 - 7.705
As	189.042	0.002	0.007	0.078 ± 0.051	0.066	0.020 - 0.363	0.069 ± 0.029	0.067	0.021 - 0.182	0.086 ± 0.066	0.065	0.020 - 0.363
B (**)	249.773	8E-4	0.003	1.538 ± 0.845	1.589	LOD - 3.185	1.182 ± 0.890	1.250	LOD - 2.502	1.894 ± 0.624	1.844	1.109 - 3.185
Ba	493.409	3E-5	1E-4	0.718 ± 0.385	0.600	0.266 - 2.057	0.748 ± 0.367	0.626	0.285 - 1.953	0.688 ± 0.403	0.584	0.266 - 2.057
Be	313.042	2E-5	6E-5	0.001 ± 0.001	0.001	LOD - 0.006	0.001 ± 0.001	0.001	LOD - 0.006	0.001 ± 0.001	0.001	LOD - 0.003
Ca	393.366	0.007	0.024	458.658 ± 34.798	459.547	372.960 - 531.502	460.897 ± 31.085	460.871	391.647 - 519.332	456.418 ± 38.296	457.874	372.960 - 531.502
Cd (**)	214.438	1E-4	4E-4	0.021 ± 0.010	0.019	0.008 - 0.053	0.024 ± 0.011	0.021	0.010 - 0.053	0.018 ± 0.008	0.017	0.008 - 0.036
Co	228.616	0.001	0.003	LOQ ± 0.003	LOQ	LOD - 0.012	LOQ ± 0.003	LOQ	LOD - 0.011	LOQ ± 0.003	LOQ	LOD - 0.012
Cr	267.716	7E-4	0.002	0.086 ± 0.067	0.076	0.024 - 0.615	0.090 ± 0.054	0.083	0.034 - 0.382	0.081 ± 0.077	0.072	0.024 - 0.615
Cu (**)	327.396	8E-4	0.003	3.822 ± 1.335	3.604	2.000 - 7.457	3.579 ± 1.131	3.535	2.000 - 5.848	4.065 ± 1.481	3.964	2.138 - 7.457
Fe	259.940	4E-4	0.001	23.056 ± 10.306	24.206	7.844 -	24.698 ± 10.022	25.202	9.983 -	21.413 ± 10.410	22.031	7.844 -

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						48.132			48.132			41.784
K	769.896	0.003	0.011	2756.460 ± 926.361	3009.755	1377.052 - 4540.521	2679.148 ± 882.771	2968.295	1377.052 - 4242.568	2833.772 ± 969.482	3072.834	1623.165 - 4540.521
Mg	279.553	1E-4	4E-4	458.369 ± 93.674	494.552	300.566 - 603.480	463.610 ± 91.025	504.347	300.566 - 603.480	453.129 ± 96.758	488.754	314.100 - 603.324
Mn	259.373	1E-4	3E-4	26.592 ± 18.062	28.919	6.734 - 73.585	26.278 ± 16.906	29.341	6.734 - 61.671	26.906 ± 19.292	28.613	7.456 - 73.585
Mo	202.030	7E-4	0.002	0.974 ± 0.398	0.889	0.539 - 4.341	0.953 ± 0.502	0.852	0.539 - 4.341	0.994 ± 0.260	0.935	0.659 - 1.644
Na (**)	588.995	0.001	0.004	32.454 ± 19.676	25.360	15.214 - 141.929	28.037 ± 10.448	25.428	16.225 - 74.832	36.871 ± 25.143	25.317	15.214 - 141.929
Ni	231.604	0.001	0.004	0.161 ± 0.119	0.141	0.027 - 0.920	0.161 ± 0.138	0.139	0.027 - 0.920	0.161 ± 0.098	0.143	0.041 - 0.580
P	177.495	0.001	0.004	2764.478 ± 1136.910	3082.185	1286.050 - 4985.480	2725.118 ± 1078.984	3082.185	1286.050 - 4748.610	2803.837 ± 1200.185	3065.615	1337.920 - 4985.480
Pb	220.353	0.004	0.012	0.073 ± 0.032	0.067	0.026 - 0.263	0.076 ± 0.027	0.072	0.036 - 0.148	0.070 ± 0.036	0.066	0.026 - 0.263
Sb	217.581	0.005	0.015	LOD ± 0.003	LOD	LOD - 0.020	LOD ± 0.004	LOD	LOD - 0.019	LOD ± 0.003	LOD	LOD - 0.020
Se (**)	196.090	0.001	0.005	0.182 ± 0.051	0.182	0.077 - 0.290	0.217 ± 0.038	0.217	0.151 - 0.290	0.148 ± 0.039	0.155	0.077 - 0.222
Si	251.611	8E-5	3E-4	21.539 ± 13.823	18.368	4.997 - 85.435	23.961 ± 14.109	21.451	6.314 - 85.435	19.116 ± 13.210	15.465	4.997 - 69.089
Sn	189.989	3E-4	9E-4	0.008 ± 0.007	0.006	0.002 - 0.059	0.007 ± 0.003	0.006	0.003 - 0.016	0.008 ± 0.010	0.005	0.002 - 0.059
Ti (**)	323.452	4E-4	0.001	0.041 ± 0.051	0.028	0.007 - 0.484	0.051 ± 0.068	0.032	0.007 - 0.484	0.031 ± 0.019	0.025	0.007 - 0.099
Tl	190.856	5E-4	0.002	0.004 ± 0.008	LOD	LOD - 0.043	0.003 ± 0.007	LOD	LOD - 0.033	0.004 ± 0.009	LOD	LOD - 0.043
V (**)	292.402	6E-4	0.002	0.023 ± 0.058	0.010	LOQ - 0.520	0.012 ± 0.011	0.010	LOQ - 0.075	0.035 ± 0.080	0.010	LOQ - 0.520

Zn	202.548	6E-4	0.002	23.597 ± 12.000	24.810	8.952 - 47.971	23.564 ± 11.829	25.262	8.952 - 45.948	23.629 ± 12.273	24.680	9.035 - 47.971

Complementary analysis on soil samples were executed as described in the appendix A.

3. Results

Table 2 shows the average, standard deviation and range of each element in the whole of samples (seed, bran, semolina and flour used as a unique set) both considered all together and grouped by cultivation protocol. T-tests were used to compare the mean values of each element in the organic and conventional samples. The elements that test significant are indicated, in the table, with a double asterisk. Similar analysis were performed on each product, the results are summarized in Table 3 for seeds, Table 4 for bran, Table 5 semolina and Table 6 flour.

Table 3. Comparison of seeds. (**) indicates the elements, whose means, give a significant t-test comparison (α=0.05) between the organic product and the conventional one.

	All seeds			Organic seeds			Conventional seeds		
Element	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**
Ag	0.003 ± 0.002	0.003	LOD - 0.009	0.003 ± 0.002	0.003	LOD - 0.006	0.003 ± 0.002	0.003	LOD - 0.009
Al	4.530 ± 1.711	5.164	1.533 - 6.741	4.811 ± 1.522	5.235	1.788 - 6.583	4.216 ± 1.897	4.824	1.533 - 6.741
As	0.074 ± 0.037	0.070	0.020 - 0.218	0.073 ± 0.031	0.068	0.024 - 0.156	0.075 ± 0.043	0.071	0.020 - 0.218
B (**)	2.381 ± 0.514	2.285	0.526 - 3.185	2.107 ± 0.405	2.179	0.526 - 2.502	2.686 ± 0.453	2.916	1.999 - 3.185
Ba	0.801 ± 0.322	0.714	0.358 - 1.631	0.842 ± 0.357	0.790	0.358 - 1.631	0.756 ± 0.282	0.699	0.394 - 1.359
Be	0.000 ± 0.000	LOD	LOD - 0.001	0.000 ± 0.001	LOD	LOD - 0.001	0.000 ± 0.000	LOD	LOD - 0.001
Ca	478.232 ± 30.116	480.896	392.386 - 531.502	482.951 ± 23.743	489.331	432.194 - 519.332	472.957 ± 35.970	469.289	392.386 - 531.502
Cd (**)	0.026 ± 0.009	0.023	0.013 - 0.045	0.029 ± 0.010	0.028	0.016 - 0.045	0.022 ± 0.007	0.022	0.013 - 0.036
Co	0.005 ± 0.003	0.005	LOD - 0.011	0.005 ± 0.003	0.005	LOD - 0.011	0.005 ± 0.003	0.005	LOQ - 0.009
Cr	0.096 ± 0.090	0.081	0.064 - 0.615	0.084 ± 0.010	0.083	0.072 - 0.115	0.109 ± 0.131	0.075	0.064 - 0.615
Cu (**)	4.336 ± 0.590	4.212	3.433 - 5.814	4.091 ± 0.534	4.040	3.433 - 5.226	4.610 ± 0.537	4.462	3.851 - 5.814
Fe (**)	26.857 ± 2.758	27.006	21.324 - 34.350	28.111 ± 2.702	28.021	23.929 - 34.350	25.455 ± 2.115	25.047	21.324 - 29.221
K	3243.369 ± 170.998	3253.807	2933.319 - 3697.111	3203.997 ± 176.816	3149.462	2933.319 - 3697.111	3287.373 ± 157.776	3270.450	3046.468 - 3587.377
Mg (**)	520.287 ± 19.505	522.190	481.189 - 558.077	526.603 ± 18.206	526.692	481.189 - 558.077	513.229 ± 18.949	509.408	484.819 - 553.452
Mn	35.138 ± 4.469	35.383	28.101 - 42.808	35.561 ± 4.322	35.409	28.101 - 42.808	34.665 ± 4.715	35.357	28.301 - 41.289
Mo	0.963 ± 0.255	0.881	0.566 - 1.644	0.929 ± 0.215	0.839	0.566 - 1.327	1.001 ± 0.296	0.903	0.659 - 1.644
Na	26.690 ± 6.451	25.109	18.349 - 48.180	27.683 ± 7.748	25.300	18.349 - 48.180	25.580 ± 4.584	24.914	20.586 - 37.991
Ni	0.187 ± 0.086	0.158	0.105 - 0.580	0.177 ± 0.059	0.155	0.111 - 0.327	0.198 ± 0.110	0.174	0.105 - 0.580
P	3358.290 ± 254.652	3289.515	2984.810 - 3903.900	3373.664 ± 268.498	3301.550	2984.810 - 3903.900	3341.108 ± 245.271	3257.150	3001.920 - 3889.520
Pb	0.056 ± 0.021	0.055	0.026 - 0.128	0.057 ± 0.018	0.050	0.036 - 0.092	0.054 ± 0.025	0.055	0.026 - 0.128
Sb	LOD ± 0.005	LOD	LOD - 0.020	LOQ ± 0.005	LOD	LOD - 0.019	LOD ± 0.005	LOD	LOD - 0.020

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Se (**)	0.210 ± 0.044	0.208	0.131 - 0.290	0.238 ± 0.036	0.252	0.151 - 0.290	0.178 ± 0.029	0.165	0.131 - 0.222
Si	22.371 ± 6.684	21.451	13.517 - 48.981	22.694 ± 5.045	23.307	13.869 - 32.932	22.010 ± 8.297	20.815	13.517 - 48.981
Sn	0.005 ± 0.002	0.004	0.002 - 0.011	0.005 ± 0.002	0.004	0.003 - 0.009	0.005 ± 0.003	0.005	0.002 - 0.011
Ti	0.021 ± 0.012	0.019	0.007 - 0.064	0.021 ± 0.013	0.017	0.007 - 0.064	0.020 ± 0.011	0.021	0.007 - 0.039
Tl	0.007 ± 0.010	LOQ	LOD - 0.043	0.004 ± 0.007	LOD	LOD - 0.022	0.010 ± 0.013	0.005	LOD - 0.043
V	0.010 ± 0.002	0.010	0.006 - 0.015	0.009 ± 0.002	0.010	0.006 - 0.011	0.010 ± 0.002	0.010	0.006 - 0.015
Zn	29.532 ± 4.976	28.053	22.541 - 45.556	30.130 ± 5.757	27.744	22.541 - 45.556	28.863 ± 3.997	28.361	24.495 - 40.231

Table 4. Comparison of brans. (**) indicates the elements, whose means, give a significant t-test comparison (α=0.05) between the organic product and the conventional one.

	All brans			Organic Brans			Conventional Brans		
Element	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**
Ag	0.006 ± 0.003	0.006	LOQ - 0.013	0.005 ± 0.002	0.005	LOQ - 0.007	0.007 ± 0.003	0.007	LOQ - 0.013
Al (**)	5.093 ± 2.009	4.460	2.579 - 12.263	5.916 ± 2.512	4.884	3.784 - 12.263	4.270 ± 0.797	4.214	2.579 - 5.757
As	0.079 ± 0.041	0.073	0.021 - 0.236	0.070 ± 0.025	0.071	0.021 - 0.118	0.088 ± 0.052	0.075	0.045 - 0.236
B (**)	1.712 ± 0.331	1.806	0.578 - 2.092	1.504 ± 0.340	1.593	0.578 - 1.890	1.919 ± 0.139	1.900	1.561 - 2.092
Ba	1.137 ± 0.405	1.096	0.587 - 2.057	1.093 ± 0.379	1.029	0.603 - 1.953	1.181 ± 0.440	1.172	0.587 - 2.057
Be	0.001 ± 0.000	0.001	0.001 - 0.003	0.001 ± 0	0.001	0.001 - 0.001	0.001 ± 0.001	0.001	0.001 - 0.003
Ca (**)	476.057 ± 26.534	476.740	412.787 - 525.940	465.731 ± 23.447	466.287	412.787 - 490.929	486.383 ± 26.193	479.765	447.286 - 525.940
Cd	0.028 ± 0.010	0.026	0.017 - 0.053	0.031 ± 0.012	0.027	0.017 - 0.053	0.024 ± 0.007	0.021	0.017 - 0.035
Co	0.005 ± 0.003	0.004	LOD - 0.012	0.004 ± 0.002	0.004	LOD - 0.008	0.006 ± 0.003	0.006	LOQ - 0.012
Cr	0.108 ± 0.018	0.109	0.072 - 0.140	0.104 ± 0.020	0.099	0.072 - 0.140	0.112 ± 0.015	0.113	0.087 - 0.137
Cu (**)	5.546 ± 0.962	5.486	3.456 - 7.457	4.925 ± 0.712	4.913	3.456 - 5.848	6.168 ± 0.763	6.012	5.078 - 7.457
Fe	36.678 ± 4.824	36.994	25.335 - 48.132	36.673 ± 6.217	37.872	25.335 - 48.132	36.684 ± 3.136	36.834	30.115 - 41.784
K (**)	3954.524 ± 482.432	4082.236	2561.379 - 4540.521	3702.171 ± 516.030	3912.625	2561.379 - 4242.568	4206.877 ± 283.921	4260.125	3511.909 - 4540.521
Mg	567.520 ± 28.755	571.690	462.179 - 603.480	558.948 ± 36.235	569.114	462.179 - 603.480	576.091 ± 15.827	581.517	547.554 - 603.324
Mn	50.742 ± 10.443	50.736	24.625 - 73.585	46.950 ± 9.574	49.176	24.625 - 61.671	54.534 ± 10.216	53.746	35.295 - 73.585
Mo (**)	1.122 ± 0.236	1.100	0.733 - 1.598	0.980 ± 0.166	0.898	0.733 - 1.258	1.264 ± 0.213	1.283	0.973 - 1.598
Na (**)	42.709 ± 16.512	39.489	24.952 - 97.760	35.491 ± 9.294	32.918	24.952 - 61.069	49.927 ± 19.203	41.321	34.075 - 97.760
Ni	0.232 ± 0.076	0.232	0.086 - 0.419	0.223 ± 0.090	0.218	0.086 - 0.419	0.241 ± 0.063	0.233	0.147 - 0.343
P (**)	4247.483 ± 558.857	4335.040	2544.200 - 4985.480	3974.312 ± 596.423	4099.390	2544.200 - 4748.610	4520.654 ± 365.057	4438.640	3664.670 - 4985.480
Pb (**)	0.089 ± 0.025	0.090	0.055 - 0.135	0.100 ± 0.027	0.108	0.055 - 0.135	0.077 ± 0.016	0.072	0.055 - 0.106
Sb	LOD ± 0.001	LOD	LOD - LOD	LOD ± 0.001	LOD	LOD - LOD	LOD ± 0.001	LOD	LOD - LOD

Se (**)	0.204 ± 0.044	0.193	0.141 - 0.275	0.242 ± 0.024	0.247	0.188 - 0.275	0.166 ± 0.015	0.164	0.141 - 0.195
Si	34.462 ± 13.098	32.302	14.746 - 69.089	33.757 ± 12.361	33.056	14.746 - 56.431	35.167 ± 14.267	32.148	19.242 - 69.089
Sn (**)	0.006 ± 0.003	0.005	0.003 - 0.016	0.007 ± 0.003	0.006	0.004 - 0.016	0.005 ± 0.001	0.005	0.003 - 0.009
Ti	0.053 ± 0.032	0.045	0.019 - 0.153	0.061 ± 0.040	0.048	0.019 - 0.153	0.046 ± 0.021	0.042	0.023 - 0.099
Tl	0.007 ± 0.009	0.004	LOD - 0.033	0.007 ± 0.010	LOQ	LOD - 0.033	0.007 ± 0.007	0.005	LOD - 0.021
V	0.008 ± 0.006	0.007	LOQ - 0.021	0.009 ± 0.006	0.008	LOQ - 0.021	0.008 ± 0.006	0.005	LOQ - 0.018
Zn	39.233 ± 5.628	40.157	22.804 - 47.971	37.297 ± 6.372	38.188	22.804 - 45.948	41.169 ± 4.154	41.491	33.005 - 47.971

Table 5. Comparison of Semolina. (**) indicates the elements, whose means, give a significant t-test comparison (α=0.05) between the organic product and the conventional one.

	All Semolina			Organic Semolina			Conventional Semolina		
Element	Mean±StdDev	Median	Min - Max	Mean±StdDev	Median	Min - Max	Mean±StdDev	Median	Min - Max
Ag	LOQ ± 0.002	LOQ	LOD - 0.007	LOQ ± 0.001	LOQ	LOD - 0.005	0.003 ± 0.002	LOQ	LOD - 0.007
Al (**)	4.816 ± 2.592	4.343	1.447 - 13.392	6.147 ± 2.871	4.771	3.461 - 13.392	3.662 ± 1.678	3.821	1.447 - 7.705
As	0.077 ± 0.064	0.058	0.024 - 0.363	0.070 ± 0.038	0.059	0.027 - 0.182	0.083 ± 0.081	0.056	0.024 - 0.363
B (**)	0.855 ± 0.624	1.133	LOD - 1.851	0.288 ± 0.432	0.013	LOD - 0.951	1.346 ± 0.188	1.325	1.109 - 1.851
Ba	0.435 ± 0.123	0.412	0.279 - 0.767	0.482 ± 0.125	0.479	0.321 - 0.767	0.395 ± 0.109	0.359	0.279 - 0.635
Be	0.001 ± 0.000	0.001	0.001 - 0.002	0.001 ± 0	0.001	0.001 - 0.001	0.001 ± 0.000	0.001	0.001 - 0.002
Ca	434.047 ± 29.811	429.169	390.838 - 486.215	437.659 ± 28.502	425.796	404.677 - 486.215	430.917 ± 31.543	432.541	390.838 - 479.139
Cd (**)	0.014 ± 0.005	0.013	0.008 - 0.031	0.017 ± 0.006	0.015	0.010 - 0.031	0.012 ± 0.004	0.011	0.008 - 0.018
Co	LOD ± 0.002	LOD	LOD - 0.009	LOD ± 0.002	LOD	LOD - 0.009	LOD ± 0.000	LOD	LOD - LOD
Cr	0.049 ± 0.036	0.040	0.024 - 0.226	0.057 ± 0.051	0.041	0.034 - 0.226	0.042 ± 0.009	0.039	0.024 - 0.061
Cu (**)	2.487 ± 0.288	2.466	2.034 - 3.228	2.341 ± 0.226	2.297	2.034 - 2.745	2.614 ± 0.281	2.591	2.138 - 3.228
Fe (**)	11.859 ± 2.504	10.928	7.844 - 19.124	13.298 ± 2.761	12.756	9.983 - 19.124	10.612 ± 1.404	10.546	7.844 - 13.894
K (**)	1738.047 ± 142.878	1760.645	1377.052 - 2009.210	1657.293 ± 134.168	1655.897	1377.052 - 1864.274	1808.033 ± 112.416	1801.074	1623.165 - 2009.210
Mg	345.022 ± 19.921	345.146	300.566 - 383.394	349.486 ± 24.248	348.558	300.566 - 383.394	341.154 ± 15.061	342.398	314.100 - 365.416
Mn	9.156 ± 1.174	8.988	7.173 - 11.745	8.885 ± 0.868	8.853	7.173 - 10.532	9.391 ± 1.373	9.666	7.456 - 11.745
Mo	0.834 ± 0.170	0.818	0.539 - 1.249	0.810 ± 0.210	0.813	0.539 - 1.249	0.855 ± 0.131	0.862	0.702 - 1.101
Na	26.765 ± 17.695	20.731	15.214 - 95.377	21.091 ± 4.336	20.374	16.225 - 32.199	31.682 ± 23.065	21.728	15.214 - 95.377
Ni	0.082 ± 0.085	0.056	0.027 - 0.411	0.074 ± 0.102	0.049	0.027 - 0.411	0.088 ± 0.071	0.062	0.041 - 0.330
P	1544.900 ± 131.448	1583.585	1286.050 - 1710.380	1506.895 ± 135.215	1528.190	1286.050 - 1710.380	1577.838 ± 123.078	1623.870	1337.920 - 1710.160
Pb	0.073 ± 0.040	0.062	0.038 - 0.263	0.069 ± 0.014	0.063	0.054 - 0.102	0.077 ± 0.054	0.061	0.038 - 0.263
Sb	LOD ± 0.002	LOD	LOD - LOQ	LOD ± 0.001	LOD	LOD - LOD	LOD ± 0.002	LOD	LOD - LOQ

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Se (**)	0.152 ± 0.047	0.156	0.077 - 0.240	0.191 ± 0.025	0.188	0.155 - 0.240	0.118 ± 0.032	0.114	0.077 - 0.212
Si (**)	8.985 ± 3.597	7.457	4.997 - 19.054	11.120 ± 3.979	10.245	6.314 - 19.054	7.135 ± 1.866	6.526	4.997 - 11.171
Sn	0.008 ± 0.010	0.006	0.004 - 0.059	0.007 ± 0.002	0.006	0.005 - 0.010	0.010 ± 0.014	0.005	0.004 - 0.059
Ti	0.030 ± 0.018	0.022	0.008 - 0.077	0.033 ± 0.018	0.024	0.016 - 0.077	0.028 ± 0.018	0.021	0.008 - 0.077
Tl	LOD ± 0.001	LOD	LOD - 0.003	LOD ± 0.000	LOD	LOD - LOD	LOQ ± 0.001	LOD	LOD - 0.003
V	0.028 ± 0.097	0.007	LOQ - 0.520	0.008 ± 0.006	0.006	LOQ - 0.020	0.046 ± 0.132	0.007	LOQ - 0.520
Zn	11.213 ± 1.042	11.374	8.952 - 12.972	10.907 ± 0.955	11.075	8.952 - 12.198	11.478 ± 1.072	11.556	9.035 - 12.972

Table 6. Comparison of flour. (**) indicates the elements, whose means, give a significant t-test comparison (α=0.05) between the organic product and the conventional one.

	All flour samples			Organic flours			Conventional flours		
Element	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**	**Mean±StdDev**	**Median**	**Min - Max**
Ag	LOQ ± 0.002	LOQ	LOD - 0.007	LOQ ± 0.002	LOD	LOD - 0.006	LOQ ± 0.003	LOQ	LOD - 0.007
Al (**)	8.051 ± 8.316	5.605	2.295 - 44.802	11.869 ± 10.517	7.930	5.225 - 44.802	4.233 ± 1.379	4.141	2.295 - 7.391
As	0.083 ± 0.063	0.062	0.036 - 0.305	0.063 ± 0.020	0.061	0.036 - 0.095	0.103 ± 0.084	0.065	0.036 - 0.305
B (**)	0.932 ± 0.651	1.149	LOD - 2.030	0.400 ± 0.467	0.071	LOD - 1.106	1.464 ± 0.230	1.381	1.192 - 2.030
Ba	0.488 ± 0.138	0.479	0.266 - 0.784	0.530 ± 0.142	0.531	0.285 - 0.784	0.446 ± 0.126	0.461	0.266 - 0.641
Be	0.001 ± 0.001	0.001	0.001 - 0.006	0.002 ± 0.001	0.001	0.001 - 0.006	0.001 ± Non un numero reale	0.001	0.001 - 0.001
Ca	440.659 ± 27.472	443.842	372.960 - 486.933	447.069 ± 28.188	452.268	391.647 - 486.933	434.249 ± 26.244	437.667	372.960 - 480.070
Cd	0.015 ± 0.005	0.014	0.008 - 0.030	0.017 ± 0.006	0.016	0.010 - 0.030	0.013 ± 0.004	0.012	0.008 - 0.020
Co	LOD ± 0.002	LOD	LOD - 0.010	LOQ ± 0.003	LOD	LOD - 0.010	LOD ± 0.001	LOD	LOD - 0.004
Cr	0.089 ± 0.073	0.063	0.037 - 0.382	0.117 ± 0.092	0.080	0.046 - 0.382	0.061 ± 0.029	0.051	0.037 - 0.149
Cu	2.824 ± 0.431	2.778	2.000 - 3.861	2.724 ± 0.509	2.739	2.000 - 3.861	2.924 ± 0.326	2.937	2.401 - 3.426
Fe (**)	16.227 ± 6.721	14.701	10.950 - 45.423	19.134 ± 8.469	17.476	11.067 - 45.423	13.320 ± 2.018	12.975	10.950 - 17.896
K (**)	1980.966 ± 174.635	1970.042	1664.408 - 2303.912	1910.892 ± 172.820	1879.192	1664.408 - 2246.599	2051.040 ± 151.758	2020.542	1832.890 - 2303.912
Mg	385.553 ± 27.592	389.810	329.942 - 426.213	390.328 ± 30.020	395.837	329.942 - 424.400	380.778 ± 25.209	376.573	343.350 - 426.213
Mn	9.388 ± 1.475	8.989	6.734 - 12.084	9.434 ± 1.645	8.885	6.734 - 11.814	9.343 ± 1.349	9.092	7.660 - 12.084
Mo	0.990 ± 0.712	0.832	0.551 - 4.341	1.105 ± 1.003	0.816	0.551 - 4.341	0.875 ± 0.144	0.873	0.666 - 1.180
Na	36.308 ± 30.047	24.581	17.774 - 141.929	28.049 ± 14.513	24.313	17.849 - 74.832	44.567 ± 39.018	24.848	17.774 - 141.929

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Ni	0.139 ± 0.167	0.098	0.039 - 0.920	0.161 ± 0.234	0.092	0.039 - 0.920	0.117 ± 0.047	0.104	0.062 - 0.229
P	1772.662 ± 200.208	1791.390	1335.880 - 2159.440	1746.273 ± 213.606	1787.770	1335.880 - 2115.440	1799.051 ± 190.710	1795.010	1517.020 - 2159.440
Pb	0.081 ± 0.032	0.074	0.036 - 0.186	0.088 ± 0.025	0.081	0.062 - 0.148	0.074 ± 0.037	0.067	0.036 - 0.186
Sb	LOD ± 0.001	LOD	LOD - LOD	LOD ± 0.001	LOD	LOD - LOD	LOD ± 0.001	LOD	LOD - LOD
Se (**)	0.156 ± 0.042	0.164	0.091 - 0.230	0.186 ± 0.023	0.181	0.158 - 0.230	0.127 ± 0.036	0.108	0.091 - 0.200
Si (**)	20.982 ± 16.716	15.869	10.116 - 85.435	28.860 ± 20.680	18.165	15.683 - 85.435	13.105 ± 4.473	11.285	10.116 - 27.071
Sn	0.013 ± 0.008	0.011	0.005 - 0.035	0.011 ± 0.003	0.011	0.005 - 0.016	0.015 ± 0.011	0.010	0.006 - 0.035
Ti	0.069 ± 0.093	0.044	0.018 - 0.484	0.104 ± 0.122	0.063	0.027 - 0.484	0.034 ± 0.018	0.025	0.018 - 0.080
Tl	LOQ ± 0.004	LOD	LOD - 0.019	0.002 ± 0.005	LOD	LOD - 0.019	LOD ± 0.000	LOD	LOD - LOQ
V (**)	0.051 ± 0.064	0.021	0.003 - 0.235	0.021 ± 0.018	0.018	0.003 - 0.075	0.082 ± 0.079	0.045	0.007 - 0.235
Zn	13.078 ± 1.762	12.731	9.403 - 16.102	12.892 ± 1.982	12.796	9.403 - 16.102	13.264 ± 1.569	12.666	11.396 - 15.950

The elements were measured on four products for each wheat: whole seed, bran, semolina and flour. The Sb has values ≤LOD in 93% of the samples, 97% of them are ≤LOQ. The Tl is not present in semolina and flour (~93% of samples <LOD). Tl has values >LOD in 50% of seed samples and 58% of the bran samples but most of the positive samples have concentration close to LOD. Co was under the detection limit (43%<LOD, 58%<LOQ) especially due to the absence in the most of semolina (96%<LOD) and flour (81%<LOD) samples; most of the organic samples contain a bit less than the conventional ones. Be was not detected on 67% of the whole seed samples but trace of it are present in bran, semolina and flour. B is not detectable in half of the organic semolina (46%<LOD) and in some of the organic flour (~8%<LOD) but it is measurable in organic seeds, bran and even in all conventional samples. Ag, when detectable, has values close to its LOQ; it is present in bran, in some samples of seeds (39% of seeds <LOQ) and in few samples of semolina (43%<LOD) and flour (46%<LOD); this element has no statistically meaningful difference between organic and conventional products. The measurement of V are <LOD in about 15% of bran and semolina samples. All other elements were determined in all the samples. Comparing by a t-test the organic products versus the conventional ones give poor information; most of the elements show no difference, some of them test positively for some product as highlighted in Table 3 to Table 6 where the main parameters are also reported. Ba, Be, Co, Cr, Mn, Ni and Zn that are significantly different in soils (Table A1 in Appendix A) have no difference in the products while V that has significant test in soil has significant difference only in Semolina. The other elements: Al, Cd, Cu, Fe that give significant test for soil have significant difference also in most of the products.

As shown in Table 2 there are low difference between values obtained from organic samples and those from the conventional cultivation. As, in our samples, is a little bit higher than the values reported by Cubadda et other [22]. B content is similar to what measured on Austrian wheats [23] but lower than the measured values on wheat grown in Saskatchewan [7]. The range of values we obtained for Cd, Cu and Zn are similar to those reported for wheats grown in Marche [24].

Table 7, shows the correlation existing between elements within the products. Strong correlations are evaluated for Zn, Mg, Mn, P, K and other elements some of which connected to one or more products.

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Table 7. Correlation coefficient of the elements in the different products used without distinction between organic and non-organic products. Letters close to the number stand for a=all samples together, s= Seeds, b=Brans, m=Semolina, f=Flours.

	Ag	Al	As	B	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	Sb	Se	Si	Sn	Ti	Tl	V	Zn
Ag	1a; 1s; 1b; 1m; 1f																											
Al		1a; 1s; 1b; 1m; 1f							0.74f			0.91f											0.75m		0.89a;0.78b; 0.97f		0.68b	
As			1a; 1s; 1b; 1m; 1f			0.88m																		0.63a;0.65s; 0.89m			0.87m	
B				1a; 1s; 1b; 1m; 1f							0.67b		0.63a;0.71b	0.61a;0.62b					0.71b									0.64b
Ba					1a; 1s; 1b; 1m; 1f						0.64a	0.65a	0.71a	0.68a	0.68a				0.7a									0.71a
Be			0.88m			1a; 1s; 1b; 1m; 1f								0.66s			0.68b	0.9f	0.67s					0.96m			1m	0.66s
Ca							1a; 1s; 1b; 1m; 1f							0.65a;0.61s					0.6a									
Cd								1a; 1s; 1b; 1m; 1f						0.64a	0.64a				0.61a									0.65a
Co		0.74f							1a; 1s; 1b; 1m; 1f	0.98m	0.67a;0.68b	0.69a;0.73f	0.67a	0.68a	0.69a;0.62b			0.8m	0.68a						0.79f			0.68a
Cr									0.98m	1a; 1s; 1b; 1m; 1f	0.61b	0.79b	0.71b	0.72b	0.7b			0.82s;0.81m	0.74b				0.7b					0.81b
Cu				0.67b	0.64a				0.67a;0.68b	0.61b	1a; 1s; 1b; 1m; 1f	0.87a	0.94a;0.8b	0.9a;0.67b;0.64f	0.93a;0.73b; 0.7m;0.73f				0.94a;0.8b;0.68f									0.93a;0.79b; 0.63m;0.73f
Fe		0.91f			0.65a				0.69a;0.73f	0.79b	0.87a	1a; 1s; 1b; 1m; 1f	0.9a;0.64b	0.92a;0.76b	0.91a;0.71b				0.92a;0.74b			0.63m	0.7a;0.61b		0.93f			0.91a;0.84b
K				0.63a;0.71b	0.71a				0.67a	0.71b	0.94a;0.8b	0.9a;0.64b	1a; 1s; 1b; 1m; 1f	0.96a;0.86b; 0.64f	0.97a;0.8b				0.99a;0.96b; 0.73f				0.6b					0.96a;0.84b
Mg				0.61a;0.62b	0.68a	0.66s	0.65a;0.61s	0.64a	0.68a	0.72b	0.9a;0.67b;0.64f	0.92a;0.76b	0.96a;0.86b; 0.64f	1a; 1s; 1b; 1m; 1f	0.95a;0.64s; 0.77b;0.82f				0.97a;0.86b; 0.73m;0.86f									0.96a;0.64s; 0.83b;0.81f
Mn					0.68a			0.64a	0.69a;0.62b	0.7b	0.93a;0.73b; 0.7m;0.73f	0.91a;0.71b	0.97a;0.8b	0.95a;0.64s; 0.77b;0.82f	1a; 1s; 1b; 1m; 1f				0.98a;0.73s; 0.86b;0.84f									0.97a;0.66s; 0.87b;0.84f
Mo																1a; 1s; 1b; 1m; 1f							0.77f					
Na						0.68b											1a; 1s; 1b; 1m; 1f			0.73m								
Ni						0.9f			0.8m	0.82s;0.81m								1a; 1s; 1b; 1m; 1f										
P				0.71b	0.7a	0.67s	0.6a	0.61a	0.68a	0.74b	0.94a;0.8b;0.68f	0.92a;0.74b	0.99a;0.96b; 0.73f	0.97a;0.86b; 0.73m;0.86f	0.98a;0.73s; 0.86b;0.84f				1a; 1s; 1b; 1m; 1f				0.62b					0.98a;0.87s; 0.92b;0.85f
Pb																	0.73m			1a; 1s; 1b; 1m; 1f								
Sb																					1a; 1s; 1b; 1m; 1f							
Se												0.63m										1a; 1s; 1b; 1m; 1f						
Si		0.75m								0.7b		0.7a;0.61b	0.6b			0.77f			0.62b				1a; 1s; 1b; 1m; 1f					
Sn			0.63a;0.65s; 0.89m			0.96m																		1a; 1s; 1b; 1m; 1f			0.8a;0.96m	
Ti		0.89a;0.78b; 0.97f							0.79f			0.93f													1a; 1s; 1b; 1m; 1f			
Tl																										1a; 1s; 1b; 1m; 1f		
V		0.68b	0.87m			1m																		0.8a;0.96m			1a; 1s; 1b; 1m; 1f	
Zn				0.64b	0.71a	0.66s		0.65a	0.68a	0.81b	0.93a;0.79b; 0.63m;0.73f	0.91a;0.84b	0.96a;0.84b	0.96a;0.64s; 0.83b;0.81f	0.97a;0.66s; 0.87b;0.84f				0.98a;0.87s; 0.92b;0.85f									1a; 1s; 1b; 1m; 1f

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The comparisons of the content of the various elements reported in Table 2 to Table 6 show that some difference exists between grains grown with conventional or organic cultivation methods. However, the difference of the amount of the elements, also if present, confound with the high variability of the values so that univariate analysis of the data does not permit a clear differentiation both of the materials: seed, bran, semolina and flour neither of the cultivation method: organic and conventional. A multivariate approach, therefore, could simplify the interpretation of the results.

To this goal the measured values were $\log_{10}$ transformed because of the high concentration difference among the elements, then autoscaled before applying PCA, some comparison of data treatment without the logarithmic transform was performed that had similar or worst results. Since Sb and Tl are not present in most of the samples, they were not included in the data treatment.

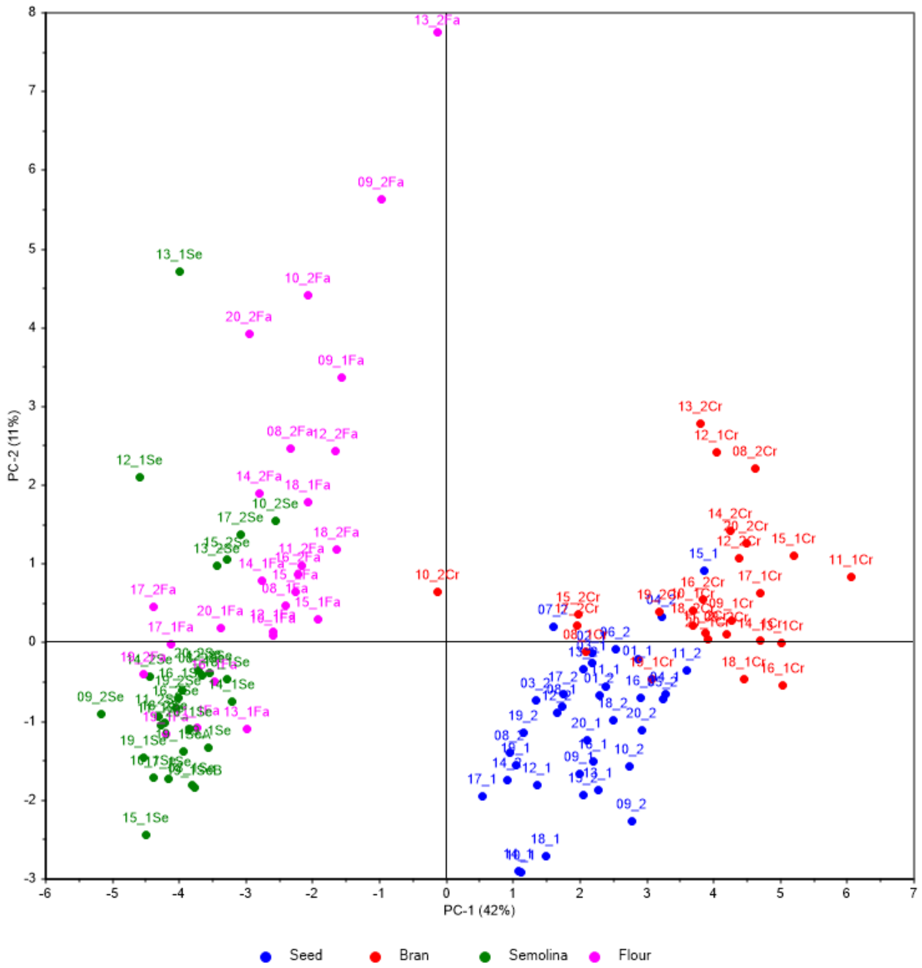

Figure 1. Scores projection of the first two components computed on $\log_{10}$ transformed and autoscaled data.

The PCA analysis on the obtained values, even if these are very similar, highlight on the first component the partitioning of two groups due to seed and bran at high values of PC1 and another one for semolina and flour with low values in PC1. With the help of the second PC seed and bran are separated while the distance between semolina and flour is low. We can expect these results because semolina and flour both come from the kernel of the seed and are mainly starch. Bran is the outer layer of the seed where we expect a different elemental content because of the differences in composition with the seed kernel [1]. The PCA evidence that most of the elemental content in bran is very similar to that of the whole seeds, the accumulation of most elements in the bran is widely documented [1].

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Figure 2 highlights the cultivation protocol on the same PCA projection of Figure 1. The figure shows that it exists a difference between samples from organic agricultural protocol and those from the conventional method of cultivation. It seems that the difference is more evident in the milled portions: bran, semolina, flour and less evident in the whole seed.

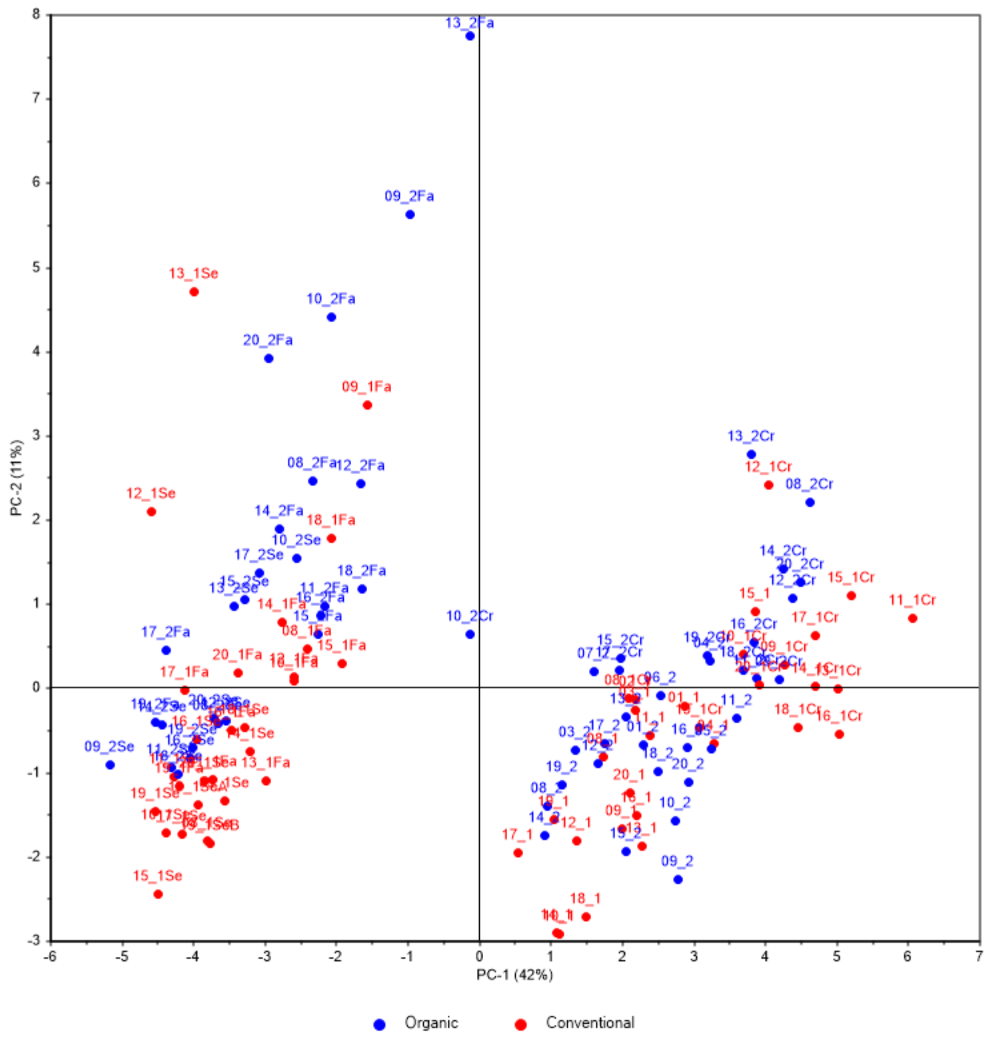

Figure 2. Scores projection of the first two components computed on log_{10} transformed and autoscaled data, colors highlight the cultivation methods.

The Unscrambler® X software (version 10.2, CAMO Software, Oslo, Norway), Matlab® (version R2023a, The MathWorks inc.) and Microsoft Office® Excel softwares were used for data treatment.

Classification methods were used to verify the possibility of discriminating between products from conventional and organic cultivations. The trials consider all the product samples as a whole at the beginning and then we analyzed every single product. For each analysis we optimized the variable selection, Table 8 shows the percentage of variance explained (R2) by the PLS model and the analogous value predicted (Q2) with 5 groups cross-validation moreover an X, in the corresponding row of Table 3, marks the selected elements in each dataset necessary to obtain the optimized classification. The accuracy and precision values are evaluated with the classification toolbox for Matlab [25]. The dependent variables, for PLS-DA, are two dummy variables coded, 0 and 1 as usual, to indicate the belonging or not of the samples to the class associated to the focused dummy variable [26]. The analyses used the data matrix where every column contains the concentration values of a different element, every row the concentrations of the elements in a sample. The PLS-DA analysis uses the log transformed and autoscaled values of the element as predictors, but very good results are obtained also with the measured values simply autoscaled.

Analysing the data by PLS-DA we were able to classify well enough the products with respect to the cultivation methods as shown in Table 8.

We performed the selection of the variables with The Unscrambler [27], that apply the Martens's uncertainty test [28]. Table 8 shows the results of the analysis with the optimized number of variables.

Table 8 highlights that the worst dataset for the classification is that of seeds, especially because it seems to give a less stable model, on the contrary Bran, Semolina and Flour show good differentiation between organic and non; semolina is excellent with its R2 0.91 and Q2 0.89. Both bran and semolina have 100% accuracy and precision while in flour the accuracy in prediction in 96%; in this last case the use of the data without log transform has a worst classification ability. Considering all the samples together an average result is obtained anyway good. Most data treatments need one latent variable for the classification model; only some needs two or more. Comparable discrimination results take place using other discriminant methods. Few elements contribute to the discrimination, but they vary on the base of the product, only B, Cd and Se were always retained, they are enough to differentiate flour with good accuracy and precision and similarly when all the samples are treated together. Cu is always selected except in flour with log transformation.

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Table 8. Comparison of PLS-DA applied on different sets. Precision and accuracy[29] were evaluated by classification toolbox class_gui. The model Row details the pretreatment applied and the elements used in the model. Precision and accuracy were always reported for the two groups, that of conventional samples and that of organic samples. Prediction parameters were estimated by cross-validation.

	All samples together	**All samples together**	**Seed**	**Seed**	**Bran**	**Bran**	**Semolina**	**Semolina**	**Flour**	**Flour**	**Flour**	**Flour**
model	Log10 transform, autoscaled Elements: **B, Cd, Cu, Fe, Se**	autoscaled Elements: **B, Cd, Cu, Se, Si**	Log10 transform, autoscaled Elements: **B, Cd, Cu, Fe, Mg, Se**	autoscaled Elements: **B, Cd, Cu, Fe, Mg, Se**	Log10 transform, autoscaled Elements: **B, Cd, Co, Cu, Fe, K, Na, Pb, Se, Sn**	autoscaled Elements: **B, Cd, Co, Cu, Fe, K, Mo, Na, P, Pb, Se, Si, Sn**	Log10 transform, autoscaled Elements: **Ag, B, Cd, Cu, K, Se, Si**	autoscaled Elements: **Ag, B, Cd, Cu, K, Se**	Log10 transform, autoscaled Elements: **B, K, Se**	Log10 transform, autoscaled Elements: **Al, B, Cd, Cr, Fe, K, Se, Si, V**	autoscaled Elements: **B, K, Se**	autoscaled Elements: **Al, B, Cd, Co, Cr, Cu, Fe, K, Na, Se, Si, V**
Number of elements	5	5	6	6	10	13	7	6	3	9	3	12
Number of LV (computed; optimal)	4; 4	3; 2	2; 1	6; 1	3; 2	2; 2	2; 1	2; 1	3; 1	3; 2	3; 1	12; 1
R^2	0.78	0.74	0.62	0.63	0.89	0.91	0.91	0.89	0.80	0.84	0.81	0.79
Q^2	0.76	0.72	0.59	0.59	0.83	0.86	0.89	0.87	0.73	0.71	0.75	0.75
Precision (conve; org)	0.98; 0.97	0.93; 0.93	1;0.95	1; 0.95	1; 1	1; 1	1; 1	1; 1	0.92; 0.92	1; 1	0.92; 0.92	1; 1
Accuracy	0.97	0.93	0.97	0.97	1	1	1	1	0.92	1	0.92	1
Pred. Precision (Conv; org)	0.96; 0.95	0.93; 0.92	0.88; 0.89	0.88; 0.85	1; 1	1; 1	1; 1	1; 1	0.85; 0.95	1; 0.93	0.92; 0.92	0.93; 1
Pred. accuracy	096	092	0.89	0.86	1	1	1	1	0.85	0.96	0.92	0.96
Ag							X	X				
Al									X	X		X
B	**X**	**X**	X	X	X	X	X	X	X	X	X	X
Cd	**X**	**X**	X	X	X	X	X	X		X		X
Co					X	X						X
Cr									X	X		X
Cu	**X**	**X**	X	X	X	X	X	X				X
Fe	**X**		X	X	X	X			X	X		X
K					X	X	X	X	X	X	X	X
Mg			X	X								
Mo						X						
Na					X	X						X
P						X						

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Pb					X	X						
Se	**X**	**X**	X	X	X	X	X	X	X	X	X	X
Si		**X**					X		X	X		X
Sn					X	X						
V									X	X		X

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Some elements: As, Ba, Be, Ca, Mn, Ni, Ti and Zn never entered the selected variables, that indicate they are unaffected by the cultivation method, moreover Sb and Tl didn't take part of the analysis as previously written. Mg is selected only when we analyze the seeds without log transform while V and Al enter the selected variables only with flour if treated without log transform also Si enter the group in this condition, but it is selected even when all the samples untransformed are analyzed. The selection of Ag happens only treating semolina without transform. Co, Na, Pb, Sn are tied to bran with the addition of Mo and P if only autoscaling is applied. K is important in all the grinded products.

Few elements, B, Cd, Cu, K, Se are the most meaningful, permit to differentiate the products on the base of the cultivation protocol. PCA with these five elements also reveal a good grouping on the base of the products. Even if the metals are more abundant in the outer layer (pericarp and aleurone) of the seed [30] some are differently absorbed in the kernel of the seed so that it is possible to discriminate even semolina and flour for the cultivation protocol. The large difference of elemental content due to the phenotypes does not affect the discrimination ability with respect to the kind of cultivation protocol.

4. Discussion

The present study focuses on the possibility to recognize, by means of simple analysis and multivariate treatment, the cultivation protocol used for the wheats under investigation. The study used several varieties of wheats cultivated under controlled conditions in a restricted experimental area, this means that some sources of variability are not considered as the season effect, humidity, soils. The study develops a method for protecting foodstuff, but it needs further validation with wide database including the variability sources here not considered.

5. Conclusions

ICP-OES instrumentation is largely available in the analytical laboratory permitting cheap measurements that, despite the sensitivity of the technique, are useful for advanced data treatment.

This study is devoted to characterize the elemental content of milled products of durum wheats grown in Italy. The elemental measurements are also used to verify the possibility of discriminating the ground product of durum wheat versus the cultivation protocol of the cereal. This work permits to define a data treatment methodology for obtaining the discrimination; the results are very good especially for semolina and bran but even flour can be classified with optimal precision and very high accuracy.

An important result is that the discriminations are due to few elements, three at minimum but even the products that need a few more elements can be classified with a lower number of them if we accept a little bit worse classification; in this context B, Cd, Cu, K and Se are the most effective elements.

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Appendix A

Soils were randomly sampled during the year obtaining 40 samples of which 16 from conventional and 24 from organic cultivated parcels. Their pH is about 8.0, table a1 shows the values

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of the elements measured on these soil samples. The mineralization and quantification procedure adopted was the same as the one previously described.

It is remarkable the very low content, less than LOD, of Ag, Sb and Se in the soil, even Tl is minimally present. Relatively high values of Al, Cr, Fe, Cu can be due to some pollution because of the closeness of the field both to railway tracks and mechanical industrial plants. The basic pH can prevent the high amount of Al and Mn from carrying out their toxic effect on wheat plants [31][32]. Comparison, by means of monovariate t-test, of organic and conventional soils shows meaningful difference, at 0.05 significance level, for: Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, V and Zn.

Table A1. soil elemental content. (**) indicates the elements that give a significant t-test comparison (α=0.05) between the average values of soil samples for organic cultivation and those for conventional cultivation.

			All soil samples		Soils from organic cultivation		Soils from conventional cultivation	
	LOD mg/Kg	LOQ mg/Kg	min-max mg/Kg	Median ± std mg/Kg	min-max mg/Kg	median+std mg/Kg	min-max mg/Kg	median+std mg/Kg
Ag	4E-4	0.001	LOQ - LOQ	LOQ ± 0.000	LOQ - LOQ	LOQ ± 0.000	LOQ - LOQ	LOQ ± 0.000
Al (**)	0.001	0.003	1488.550 - 4140.450	1888.480 ± 670.361	1488.550 - 4140.450	1937.785 ± 778.174	0.0 - 3189.510	1841.800 ± 466.345
As	0.001	0.004	1.000 - 3.343	2.363 ± 0.612	1.366 - 3.073	2.337 ± 0.536	0.0 - 3.343	2.483 ± 0.701
B	0.830	2.767	66.027 - 144.156	88.371 ± 18.454	66.027 - 125.625	82.289 ± 17.341	0.0 - 144.156	93.565 ± 18.515
Ba (**)	8E-5	3E-4	140.287 - 233.653	190.821 ± 21.746	140.287 - 228.053	180.543 ± 22.576	0.0 - 233.653	195.709 ± 17.175
Be (**)	3E-5	1E-4	1.249 - 2.093	1.728 ± 0.236	1.249 - 2.003	1.500 ± 0.198	0.0 - 2.093	1.898 ± 0.144
Ca	0.009	0.029	2231.142 - 2993.378	2390.573 ± 176.779	2231.142 - 2993.378	2402.166 ± 185.982	0.0 - 2840.278	2375.122 ± 167.641
Cd (**)	2E-4	6E-4	0.132 - 0.304	0.224 ± 0.041	0.167 - 0.304	0.228 ± 0.033	0.0 - 0.291	0.205 ± 0.047
Cr (**)	4E-4	0.001	53.245 - 96.209	77.855 ± 11.511	53.245 - 93.176	67.709 ± 10.415	0.0 - 96.209	86.225 ± 6.785
Cu (**)	4E-4	0.001	26.933 - 48.164	35.741 ± 4.645	26.933 - 41.021	33.282 ± 3.543	0.0 - 48.164	39.052 ± 4.470
Fe (**)	2E-4	6E-4	13698.050 - 21144.190	17175.520 ± 2128.555	13698.050 - 20820.220	16656.250 ± 1924.222	0.0 - 21144.190	18653.635 ± 1596.296
K	2E-4	8E-4	755.832 - 1222.931	952.072 ± 85.755	755.832 - 1118.040	946.518 ± 67.215	0.0 - 1222.931	982.099 ± 105.488
Mg	0.001	0.005	1990.411 - 2677.165	2234.520 ± 175.033	1990.411 - 2612.228	2191.945 ± 175.439	0.0 - 2677.165	2255.959 ± 170.404
Mn (**)	5E-5	2E-4	693.361 - 1277.959	906.261 ± 147.022	693.361 - 1277.959	829.216 ± 162.902	0.0 - 1147.556	1010.177 ± 84.972
Mo	6E-4	0.002	0.660 - 5.678	0.932 ± 1.012	0.660 - 5.678	0.882 ± 1.058	0.0 - 4.693	1.036 ± 0.968
Na	0.001	0.004	334.815 - 802.104	517.985 ± 95.093	456.723 - 712.040	506.350 ± 74.249	0.0 - 802.104	538.205 ± 121.713
Ni (**)	5E-4	0.002	39.274 - 76.972	52.226 ± 10.004	39.274 - 76.972	46.123 ± 9.669	0.0 - 74.643	59.991 ± 6.668
P	0.004	0.012	98.932 - 871.575	711.963 ± 159.183	103.282 - 871.575	735.635 ± 146.974	0.0 - 786.637	664.361 ± 167.303
Pb	7E-4	0.002	13.623 - 43.143	18.716 ± 4.417	13.623 - 43.143	17.615 ± 5.504	0.0 - 23.817	19.788 ± 1.959

Sb	0.003	0.010	LOQ - LOQ	LOQ ± 0.001	LOQ - LOQ	LOQ ± 0.001	LOQ - LOQ	LOQ ± 0.001
Se	0.003	0.010	LOQ - LOQ	LOQ ± 0.001	LOQ - LOQ	LOQ ± 0.001	LOQ - LOQ	LOQ ± 0.001
Si	0.018	0.060	197.230 - 2826.853	606.368 ± 830.102	218.109 - 2588.145	611.525 ± 796.059	0.0 - 2826.853	606.368 ± 902.656
Sn	0.007	0.023	0.962 - 655.190	2.133 ± 136.265	1.161 - 655.190	2.133 ± 133.045	0.0 - 584.401	2.365 ± 145.189
Tl	0.001	0.003	LOQ - 1.239	0.529 ± 0.338	LOQ - 1.239	0.488 ± 0.361	LOQ - 0.890	0.600 ± 0.307
V (**)	2E-4	5E-4	42.138 - 76.322	58.994 ± 6.575	42.138 - 63.256	54.908 ± 5.130	0.0 - 76.322	62.949 ± 5.165
Zn (**)	7E-5	2E-4	64.296 - 96.585	79.922 ± 9.031	64.296 - 94.897	75.560 ± 8.185	0.0 - 96.585	86.713 ± 6.163

References

1. *Durum Wheat Chemistry and Technology*; Sissons, M., Abecassis, J., Marchylo, B., Carcea, M., Eds.; second.; AACC International, 2012; ISBN 978-1-891127-65-6.
2. Bux, C.; Lombardi, M.; Varese, E.; Amicarelli, V. Economic and Environmental Assessment of Conventional versus Organic Durum Wheat Production in Southern Italy. *Sustainability (Switzerland)* **2022**, *14*, doi:10.3390/su14159143.
3. Fagnano, M.; Fiorentino, N.; D'Egidio, M.G.; Quaranta, F.; Ritieni, A.; Ferracane, R.; Raimondi, G. Durum Wheat in Conventional and Organic Farming: Yield Amount and Pasta Quality in Southern Italy. *The Scientific World Journal* **2012**, *2012*, doi:10.1100/2012/973058.
4. Vergine, M.; Aprile, A.; Sabella, E.; Genga, A.; Siciliano, M.; Rampino, P.; Lenucci, M.S.; Luvisi, A.; Bellis, L. De Cadmium Concentration in Grains of Durum Wheat (*Triticum Turgidum* L. Subsp. *Durum*). *J Agric Food Chem* **2017**, *65*, 6240–6246, doi:10.1021/acs.jafc.7b01946.
5. Kovarikova, M.; Tomaskova, I.; Soudek, P. Rare Earth Elements in Plants. *Biol Plant* **2019**, *63*, 20–32, doi:10.32615/bp.2019.003.
6. Baize, D.; Bellanger, L.; Tomassone, R. Relationships between Concentrations of Trace Metals in Wheat Grains and Soil. *Agron Sustain Dev* **2009**, *29*, 297–312, doi:10.1051/agro:2008057.
7. Mermut, A.R.; Jain, J.C.; Song, L.; Kerrich, R.; Kozak, L.; Jana, S. Trace Element Concentrations of Selected Soils and Fertilizers in Saskatchewan, Canada. *J Environ Qual* **1996**, *25*, 845–853, doi:10.2134/jeq1996.00472425002500040028x.
8. Ma, C.; Liu, F.; Jin, K.; Hu, B.; Wei, M.; Zhao, J.; Zhang, H.; Zhang, K. Effects of Atmospheric Fallout on Lead Contamination of Wheat Tissues Based on Stable Isotope Ratios. *Bull Environ Contam Toxicol* **2019**, *103*, 676–682, doi:10.1007/s00128-019-02702-1.
9. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K.-H. Heavy Metals in Food Crops: Health Risks, Fate, Mechanisms, and Management. *Environ Int* **2019**, *125*, 365–385, doi:10.1016/j.envint.2019.01.067.
10. Zhang, Y.; Song, Q.; Yan, J.; Tang, J.; Zhao, R.; Zhang, Y.; He, Z.; Zou, C.; Ortiz-Monasterio, I. Mineral Element Concentrations in Grains of Chinese Wheat Cultivars. *Euphytica* **2010**, *174*, 303–313, doi:10.1007/s10681-009-0082-6.
11. Brizio, P.; Benedetto, A.; Squadrone, S.; Curcio, A.; Pellegrino, M.; Ferrero, M.; Abete, M.C. Heavy Metals and Essential Elements in Italian Cereals. *Food Additives & Contaminants: Part B* **2016**, *9*, 261–267, doi:10.1080/19393210.2016.1209572.
12. Harmankaya, M.; Özcan, M.M.; Gezgin, S. Variation of Heavy Metal and Micro and Macro Element Concentrations of Bread and Durum Wheats and Their Relationship in Grain of Turkish Wheat Cultivars. *Environ Monit Assess* **2012**, *184*, 5511–5521, doi:10.1007/s10661-011-2357-3.
13. Monti, C.; Cavanna, D.; Rodushkin, I.; Monti, A.; Leporati, A.; Suman, M. Determining the Geographical Origin of Durum Wheat Samples by Combining Strontium Isotope Ratio and Multielemental Analyses. *Cereal Chem* **2023**, *100*, doi:10.1002/cche.10634.
14. Bacher, F.; Aguzzoni, A.; Chizzali, S.; Pignotti, E.; Puntscher, H.; Zignale, P.; Voto, G.; Tagliavini, M.; Tirler, W.; Robatscher, P. Geographic Tracing of Cereals from South Tyrol (Italy) and Neighboring Regions via 87Sr/86Sr Isotope Analysis. *Food Chem* **2023**, *405*, 134890, doi:10.1016/j.foodchem.2022.134890.
15. *Chemometrics in Food Chemistry*; Marini, F., Ed.; 1st Edition.; Elsevier: AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD PARIS • SAN DIEGO • SAN FRANCISCO • SYDNEY • TOKYO, 2013; Vol. 28; ISBN 978-0-444-59528-7.
16. Cozzolino, D. An Overview of the Use of Infrared Spectroscopy and Chemometrics in Authenticity and Traceability of Cereals. *Food Research International* **2014**, *60*, 262–265, doi:10.1016/j.foodres.2013.08.034.
17. De Flaviis, R.; Sacchetti, G.; Mastrocola, D. Wheat Classification According to Its Origin by an Implemented Volatile Organic Compounds Analysis. *Food Chem* **2021**, *341*, 128217, doi:10.1016/j.foodchem.2020.128217.

18. Cervellieri, S.; Lippolis, V.; Mancini, E.; Pascale, M.; Logrieco, A.F.; De Girolamo, A. Mass Spectrometry-Based Electronic Nose to Authenticate 100% Italian Durum Wheat Pasta and Characterization of Volatile Compounds. *Food Chem* **2022**, *383*, 132548, doi:10.1016/j.foodchem.2022.132548.
19. De Flaviis, R.; Mutarutwa, D.; Sacchetti, G.; Mastrocola, D. Quantitatively Unravelling the Effect of Altitude of Cultivation on the Volatiles Fingerprint of Wheat by a Chemometric Approach. *Food Chem* **2022**, *370*, 131296, doi:10.1016/j.foodchem.2021.131296.
20. Giorgia Potortì, A.; Francesco Mottese, A.; Rita Fede, M.; Sabatino, G.; Dugo, G.; Lo Turco, V.; Costa, R.; Caridi, F.; Di Bella, M.; Di Bella, G. Multielement and Chemometric Analysis for the Traceability of the Pachino Protected Geographical Indication (PGI) Cherry Tomatoes. *Food Chem* **2022**, *386*, 132746, doi:10.1016/j.foodchem.2022.132746.
21. Ruggiero, L.; Fontanella, M.C.; Amalfitano, C.; Beone, G.M.; Adamo, P. Provenance Discrimination of Sorrento Lemon with Protected Geographical Indication (PGI) by Multi-Elemental Fingerprinting. *Food Chem* **2021**, *362*, 130168, doi:10.1016/j.foodchem.2021.130168.
22. Cubadda, F.; Baldini, M.; Carcea, M.; Pasqui, L.A.; Raggi, A.; Stacchini, P. Influence of Laboratory Homogenization Procedures on Trace Element Content of Food Samples: An ICP-MS Study on Soft and Durum Wheat. *Food Addit Contam* **2001**, *18*, 778–787, doi:10.1080/02652030120630.
23. Spiegel, H.; Sager, M.; Oberforster, M.; Mechtler, K.; Stüger, H.P.; Baumgarten, A. Nutritionally Relevant Elements in Staple Foods: Influence of Arable Site versus Choice of Variety. *Environ Geochem Health* **2009**, *31*, 549–560, doi:10.1007/s10653-009-9254-5.
24. Conti, M.E.; Cubadda, F.; Carcea, M. Trace Metals in Soft and Durum Wheat from Italy. *Food Addit Contam* **2000**, *17*, 45–53, doi:10.1080/026520300283577.
25. Ballabio, D.; Consonni, V. Classification Tools in Chemistry. Part 1: Linear Models. PLS-DA. *Analytical Methods* **2013**, *5*, 3790, doi:10.1039/c3ay40582f.
26. Stocchero, M.; De Nardi, M.; Scarpa, B. PLS for Classification. *Chemometrics and Intelligent Laboratory Systems* **2021**, *216*, 104374, doi:10.1016/j.chemolab.2021.104374.
27. CAMO Software Unscrambler® X.
28. Martens, H.; Martens, M. Modified Jack-Knife Estimation of Parameter Uncertainty in Bilinear Modelling by Partial Least Squares Regression (PLSR). *Food Qual Prefer* **2000**, *11*, 5–16, doi:10.1016/S0950-3293(99)00039-7.
29. Ballabio, D.; Grisoni, F.; Todeschini, R. Multivariate Comparison of Classification Performance Measures. *Chemometrics and Intelligent Laboratory Systems* **2018**, *174*, 33–44, doi:10.1016/j.chemolab.2017.12.004.
30. Ficco, D.B.M.; Beleggia, R.; Pecorella, I.; Giovanniello, V.; Frenda, A.S.; Vita, P. De Relationship between Seed Morphological Traits and Ash and Mineral Distribution along the Kernel Using Debranning in Durum Wheats from Different Geographic Sites. *Foods* **2020**, *9*, 1523, doi:10.3390/foods9111523.
31. Foy, C.D. Physiological Effects of Hydrogen, Aluminum, and Manganese Toxicities in Acid Soil. In *Agronomy Monographs*; American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 2015; pp. 57–97.
32. Khabaz-Saberi, H.; Rengel, Z. Aluminum, Manganese, and Iron Tolerance Improves Performance of Wheat Genotypes in Waterlogged Acidic Soils. *Journal of Plant Nutrition and Soil Science* **2010**, *173*, 461–468, doi:10.1002/jpln.200900316.