Comparative Investigation of Concentrations of Major and Trace Elements in Organic and Conventional Danish Agricultural Crops. 1. Onions (*Allium cepa* Hysam) and Peas (*Pisum sativum* Ping Pong)

Vagn Gundersen,* Iben Ellegaard Bechmann, Annette Behrens,† and Stefan Stürup

Plant Biology and Biogeochemistry Department, Risø National Laboratory, PBK-124, P.O. Box 49, DK-4000 Roskilde, Denmark

210 samples of onions (*Allium cepa* Hysam) from 11 conventionally and 10 organically cultivated sites and 190 samples of peas (*Pisum sativum* Ping Pong) from 10 conventionally and 9 organically cultivated sites in Denmark were collected and analyzed for 63 and 55 major and trace elements, respectively, by high-resolution inductively coupled plasma mass spectrometry. Sampling, sample preparation, and analysis of the samples were performed under carefully controlled contamination-free conditions. Comparative statistical tests of the element concentration mean values for each site show significantly ($p < 0.05$) different levels of Ca, Mg, B, Bi, Dy, Eu, Gd, Lu, Rb, Sb, Se, Sr, Ti, U, and Y between the organically and conventionally grown onions and significantly ($p < 0.05$) different levels of P, Gd, and Ti between the organically and conventionally grown peas. Principal component analysis (PCA) applied to the 63 elements measured in the individual onion samples from the 21 sites split up the sites into two groups according to the cultivation method when the scores of the first and third principal components were plotted against each other. Correspondingly, for peas, a PCA applied to the 55 elements measured as mean values for each site split up the 19 sites into two groups according to the cultivation method when the scores of the third and fourth principal component were plotted against each other. The methodology may be used as authenticity control for organic cultivation after further method development.

Keywords: *Onion (Allium cepa Hysam); pea (Pisum sativum Ping Pong); major elements; trace elements; organic; conventional; comparison; authenticity; multi-element analysis; principal component analysis; PCA; HR−ICPMS*

INTRODUCTION

Since the beginning of the 20th century the use of agricultural chemicals such as fertilizers and pesticides has escalated in conventional farming. As early as the 1920s it was claimed that food crops grown using agricultural chemicals were detrimental to the health of animals and humans consuming them. During the last 30 years, the organic cultivated area in Denmark has increased every year. Today, only 17% of the adult consumers in Denmark never buy organic foods, and 46% of the organic consumers point out that the primary reason for buying organic foods is their health (Danish Gallup, 1999). It has been shown that a growing number of consumers feel that conventional food is less healthy than organic food. Despite this, very little research has been performed to clarify how cultivation practices (conventional vs. organic) effect human health through food (Woese et al., 1997; Worthington, 1998).

It has been postulated by Tjell and Lamm (1969) that “the causes of the ‘diseases of civilization’ would be found in the practice of fertilization generally conducted in technically highly developed communities for the purpose of ensuring optimal plant growth. The intense and one-sided use of fertilizers in agriculture involves an increasing risk of deficient supply of some essential elements to man and domestic animals via the food.”

Which elements we name essential, neutral, or toxic is still changing (Markert, 1993); which (among other things) is caused by evolution of analytical techniques with lower detection limits. Compared with the fact that the human body's uptake of some elements via food may be subject to competitive uptake of other elements, it is necessary to look at more than a few elements when comparing the nutritional aspects of organic crops to those of conventional crops. Elements named as neutral in nutritional relations may have an important role as a potential essential element in the future or as a competitive element by the uptake of other elements. This calls for a comparative investigation of as many elements as possible in Danish agricultural crops organically and conventionally cultivated.

It is possible to characterize some agricultural products with regard to their geographic origin by comparison of the elemental concentration profiles by multivariate analysis. This has been done by Schwartz and Hecking (1991) for orange juice, and pistachio and macadamia nuts; by Marcos et al. (1998) for tea; by Haswell and Walmsley (1998) for wine and coffee; and by Baxter et al. (1997) and Thiel and Danzer (1997) for wines, in relation to region of production. The variation in elemental profiles in wine has been attributed to variation in soil components and climate at the cultivation site (Lizama et al., 1997).

* To whom correspondence should be addressed. Telephone: +45 4677 4272. Fax: +45 4677 4260. E-mail: vagn.gundersen@risoe.dk.

† DHI, Water & Environment, Agern Allé 11, DK-2970 Hørsholm, Denmark

10.1021/jf0009652 CCC: $19.00
Published on Web 11/22/2000

The elemental uptake of a specific crop is a function of the environment and the genetics. The environment is affected by the character of the soil, the climate, the supply of agricultural chemicals and manure, the microbiological activity in the soil, and the biological attacks on the plant from fungi, insects, etc. The soil character includes texture, pH, organic carbon and its characterization, major and micronutrients (more or less available), and the rest of the total elemental profile. The climate includes light, temperature, and precipitation, and their intensities and variations throughout the period of growth. In the same geographical area there is only a little variation in the climate but the variation in soil character from site to site may be considerable. However, the soil character may not necessarily be the most important parameter in plants' uptake of all elements. The other parameters affecting the environment and mentioned above are all very much related to the cultivation practice. In conventional farming, with an intensive use of fertilizers and pesticides, the biological activity in the soil and the biological attacks from fungi and insect may be very different from those in organic farming, with the use of farmyard manure and frequent rotation of crops but no use of pesticides. In light of these facts it will be of great importance to verify if it is possible, by multivariate analyses of elemental concentration profiles, to separate crops of uniform genetics (same sort) cultivated organically and conventionally in the same geographic area, by the cultivation practices.

The Danish Food Technology and Development Program (FØTEK) financed this study to carry out a comprehensive survey of the influence of different cultivation practices on the elemental concentration profiles in different types of vegetables (leafy, root, and legume) and various grains. The elements measured are those for which a routine high-resolution−inductively coupled plasma mass spectrometry (HR−ICPMS) method could be applied. The different cultivation methods are organic cultivation in accordance with Danish law (1987) and EEC Council Regulation (1991) and conventional cultivation with use of fertilizers and pesticides. In this study the investigated vegetables are onions and peas, which are both important ingredients in Danish human diets.

MATERIALS AND METHODS

A detailed description of site selection, harvesting, sampling, sample preparation, chemical analysis, cleaning procedures, instrumental conditions, and quality control measurements is given in Bibak et al. (1998a) for onions and in Bibak et al. (1998b) for peas. A brief description of these issues is given below.

Site Selection. Onion samples were collected from 11 conventional and 10 organic production areas. Pea samples were collected from 10 conventional and 9 organic production areas. All production areas for both crops are located on Funen and Jutland in Denmark and the six most widespread soil types are represented in each cultivation practice as evenly as possible. Some properties of the surface soil from the sites are summarized in Tables 1 and 2. The cultivation and harvest were performed in 1995. The cultivation practices, including crop rotation, application of fertilizers and farmyard manure for the last 6 years, and the use of pesticides in the growing year, were registered for all sites. Simplified information for the growing year is given below. All organic crops were cultivated on fields with organic practice for more than 2 years. Five of the sites with onions and seven sites with peas were organically cultivated for more than 5 years. On all organically cultivated sites except two with peas, different types of farmyard manure were used. One of the conventional sites with peas was cultivated without any use of fertilizers and pesticides in the year of growing. All other conventional sites were cultivated with use of pesticides and fertilizers except one site with peas where no fertilizer was used and one site with peas that had application of cattle slurry. Two of the conventional sites with onions had both fertilizer and farmyard manure application.

Table 1. Properties of the Surface Soil (0−25 cm) at 21 Sites where Onions were Grown[a]

	field texture of soil surface				
site	sand (%)	silt (%)	clay (%)	pH ($CaCl_2$)	organic C (%)
FE	60.0	26.3	11.5	6.3	1.31
GE	66.3	25.0	6.0	6.0	1.57
HE	88.2	4.9	4.2	5.1	1.60
IE	61.0	25.2	11.7	6.4	1.26
JE	61.2	28.0	7.7	6.3	1.83
KE	76.0	14.9	5.9	6.0	1.89
LE	88.0	5.2	4.7	6.4	1.18
ME	88.6	6.7	3.2	5.1	0.89
ZE	89.6	3.8	3.1	5.9	2.05
ÆE	77.0	12.7	7.3	5.9	1.79
OC	71.5	21.1	5.4	7.0	1.19
PC	68.6	23.6	5.9	5.6	1.09
QC	88.8	5.4	4.2	6.1	0.88
RC	58.1	29.1	10.8	6.6	1.16
SC	85.6	5.7	4.3	5.5	2.60
TC	66.1	13.5	7.7	7.6	2.10
UC	63.1	26.2	8.5	6.8	1.26
VC	64.2	25.7	8.2	7.0	1.09
XC	64.8	24.6	8.2	6.4	1.42
YC	66.1	21.8	10.1	6.6	1.17
ØC	50.3	42.3	4.2	7.2	1.27

[a] Soil texture: sand (0.063−2.0 mm), silt (0.002−0.063 mm), and clay (<0.002 mm). Sites with second letter E are organic and sites with second letter C are conventional.

Table 2. Properties of the Surface Soil (0−25 cm) at 19 Sites where Peas were Grown[a]

	field texture of soil surface				
site	sand (%)	silt (%)	clay (%)	pH ($CaCl_2$)	organic C (%)
FE	73.7	18.6	4.8	5.3	1.71
GE	88.5	4.8	4.1	6.0	1.49
HE	66.4	21.2	9.4	5.7	1.77
IE	63.5	23.1	10.5	5.7	1.71
JE	76.6	15.2	5.4	4.5	1.62
LE	47.8	30.8	18.4	6.6	1.73
ME	50.6	31.3	13.8	6.0	2.60
NE	78.3	13.9	7.3	6.0	0.32
OE	87.1	6.2	4.2	4.5	1.47
PC	65.5	22.8	9.9	5.1	1.06
QC	45.6	35.9	16.0	4.8	1.41
RC	62.1	27.2	7.5	5.7	1.89
SC	66.2	21.2	10.4	5.6	1.29
TC	55.7	27.6	14.7	6.9	1.13
UC	67.8	19.9	10.6	5.4	1.00
VC	79.9	9.7	6.7	6.5	2.20
XC	62.7	24.2	10.7	6.5	1.41
YC	81.3	10.2	7.3	7.1	0.69
ZC	72.0	15.6	10.6	6.2	1.04

[a] Soil texture: sand (0.063−2.0 mm), silt (0.002−0.063 mm), and clay (<0.002 mm). Sites with second letter E are organic and sites with second letter C are conventional.

Sampling. All samples were handled with Nitrile gloves (Nitrile, powder free, Ansell Edmont).

Onions were harvested manually and allowed to weather for 1 week on the soil surface. Ten undamaged, healthy, average sized and normal shaped onions (*Allium cepa*, Hysam) were sampled evenly across each site and were send to a drying room with shelter from the rain but open to the wind to maintain natural drying conditions, where they were dried for

Table 3. Elements in Onions: Major Components (mg/kg, fresh weight)

	Organic						Conventional						Comparative tests	
element	N_{total}	N_{farmer}	mean (mg/kg)	std. dev. (mg/kg)	minimum (mg/kg)	maximum (mg/kg)	N_{total}	N_{farmer}	mean (mg/kg)	std. dev. (mg/kg)	minimum (mg/kg)	maximum (mg/kg)	*p* value (*F* test)	*p* value (*t* test)
Ca	91	10	142	55.6	67.7	400	98	11	197	84.1	87.5	407	0.0819	**0.0213**
Fe	95	10	3.2	0.746	1.88	5.35	101	11	2.65	0.798	1.33	5.21	0.6961	0.0948
K	90	10	1830	1180	691	4530	105	11	1640	935	200	3680	0.8642	0.6440
Mg	96	10	109	18.2	68.4	164	106	11	102	16.9	68.8	166	0.1655	**0.0377**
Na	94	10	159	54.4	83.6	376	98	10	214	111	78.1	496	0.6704	0.6376
P	94	10	421	80.4	287.0	685	108	11	439	74.7	239	660	0.1333	0.8248
S	95	10	1210	542	289	2470	108	11	1230	478	558	2450	0.7819	0.4105
Si	84	10	8.99	3.39	3.79	16.5	97	11	11.0	7.11	3.19	35.9	**0.0068**	0.3202
Zn	95	10	3.5	1.16	1.68	8.31	101	11	3.36	1.42	1.45	9.97	0.9575	0.6657

more than 2 weeks. This drying procedure is similar to the normal drying procedure for basket onions in Denmark.

A total of 20 undamaged closed pea pods (*Pisum sativum* Ping Pong) from 10 healthy pea plants (2 pea pods per plant) was nipped evenly across each site and packaged in poly(ethylene terephthalate) (PET) bags for shipment. To avoid decomposition, the peas were refrigerated in the laboratory until sample preparation.

Sample Preparation. To minimize the risk of contamination, all sample preparations were performed under controlled conditions in three rooms with lock-gate connections. The rooms were classified as R1 (ordinary condition), R2 (fairly clean), and R3 (clean, class 1000 room) (Bibak et al., 1998a,b).

All sample preparations (after the cleaning procedures) were carried out in the class 1000 environment (R3). Disposable surgical latex gloves (Gammex, sterile and powder free, Ansell Edmont) and disposable full laboratory dress (Tyvek) were worn throughout the procedure. Laboratory wares were stored in a clean air environment (R3).

After the cleaning procedure, each onion was quartered and homogenized in a blender (EVA, type 267732, DK) modified with a nitride-hardened titanium cutter. From each homogenized sample of one onion, a subsample was taken out and digested with redistilled nitric acid in a microwave oven (MDS 2000, CEM Co., Matthews, NC) equipped with 12 closed perfluoroalkoxy (Teflon PFA) digestion vessels (CEM Co.). The clear, light yellow digest without any residue was then cooled to room temperature and transferred quantitative with double-deionized water to a polyethylene flask and stored at 5 °C until analysis.

The sample preparation of peas was performed similar to the procedure for onions. The 20 pea pods collected from a field were prepared for analysis as follows. Two frozen pea pods from one pea plant, representing one sample for analysis, were opened manually by applying finger pressure to the sides of the pods. Then two normal-sized pea seeds were freed from each pod by plastic tweezers: one nearest the stalk and one from the middle of the pod from the first pod, and one farthest from the stalk and one from the middle of the pod from the second pod. The four seeds were put directly into a Teflon digestion vessel and were mashed with a Teflon stave for acceleration of digestion. The homogenized samples were digested in a microwave oven and stored as described above for onions.

Multielement Determination. The sample solutions were diluted with double-deionized water, and HR−ICPMS (PlasmaTrace2, Micromass, Manchester, U.K.) was used to determine as many elements in the sample solutions and blanks that we found possible in a routine HR−ICPMS method. In the onion samples 63 elements were determined: Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, P, Pb, Pr, Pt, Rb, Re, Ru, S, Sb, Sc, Si, Sm, Sn, Sr, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, and Zr. In the pea samples 55 elements were determined: Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Mn, Mo, Nb, Nd, P, Pb, Pd, Pr, Pt, Re, Rh, Sb, Sc, Se, Si, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr.

Statistical Analysis. Principal component analysis (PCA) was performed using Unscrambler (CAMO A/S ver. 7.5, Oslo, Norway). Univariate comparisons on mean differences between the two farming methods were studied by Student's *t* test on mean data (SAS ver. 6.12, Cary, NC).

RESULTS AND DISCUSSION

Onions. The 63 element concentrations in organically and conventionally grown onions are given in Tables 3 and 4. The levels of major components are reported in Table 3 in mg/kg fresh weight, and the levels of minor components are reported in Table 4 in μg/kg fresh weight. For some elements it was necessary to remove outliers from the data. For this reason the number of samples may differ for each element. The total number of samples (N_{total}), the number of farmers (N_{farmer}), mean values, standard deviations, and the range of variation (minimum and maximum values) for each of the 63 elements measured for onions are shown. The mean values of the elements measured in organically and conventionally grown onions do not represent the true mean values of these elements in Danish onions because the variations in the soil and cultivation practices in each group did not cover all variations in Danish soil textural classes and cultivation practices. Furthermore, the representation of sorts, different cultivation practices, and soil classes in the experiment are not balanced with their representation in Danish onion cultivation.

Comparative Statistical Tests. The effect of the two farming methods on the elemental content in onions was examined by comparing the mean values for each farmer of each of the 63 elements for the two farming methods. The means are compared by Student's *t* test, testing the hypothesis $H_0{:}\mu_1 = \mu_2$ against the alternative $H_0{:}\mu_1 \neq \mu_2$. For each element an *F* test is performed to test for equality of the variances of the groups. The results of the test of equality of variances show that for part of the elements the variances of the two populations are unequal at the 5% level (indicated by boldfaced *p* value). For these elements the *t* test is based on an approximative test. The results of the *F* test and the *t* test are given in Tables 3 and 4 together with the basic statistics of the onion data.

It appears that the levels of Ca, Mg, B, Bi, Dy, Eu, Gd, Lu, Rb, Sb, Se, Sr, Ti, U, and Y differ significantly ($p < 0.05$) between the organically and conventionally grown onions (indicated by boldfaced *p* value).

It should be noted that it is possible that a few of the 63 elements will show significance in the *t* test at the 5% level without representing a real difference between the means. However, with 15 elements showing significance at the 5% level a clear difference between the organically grown and conventionally grown onions is present.

Table 4. Elements in Onions: Minor Components (μg/kg, fresh weight)

	Organic						Conventional						Comparative tests	
elements	N_{total}	N_{farmer}	mean (μg/kg)	std. dev. (μg/kg)	minimum (μg/kg)	maximum (μg/kg)	N_{total}	N_{farmer}	mean (μg/kg)	std. dev. (μg/kg)	minimum (μg/kg)	maximum (μg/kg)	*p* value (*F* test)	*p* value (*t* test)
Ag	92	10	0.589	0.286	0.194	1.74	100	11	0.65	0.35	0.0962	1.87	**0.0248**	0.0864
Al	96	10	131	80.3	50.2	660	98	11	254	235	49.2	1230	**0.0024**	0.0646
Au	93	10	0.679	0.369	0.151	2.29	91	10	1.35	1.54	0.196	11.7	**0.0000**	0.1144
B	96	10	888	324	119	1660	102	11	1590	806	336	3810	**0.0134**	**0.0056**
Ba	96	10	104	64.2	20.8	360.0	102	11	114	89.4	18.2	450	**0.0163**	0.4828
Be	78	10	0.0618	0.0654	b > c[a]	0.36	86	11	0.0329	0.0327	b > c	0.141	0.4880	0.2870
Bi	90	10	0.159	0.086	0.0153	0.412	100	11	0.086	0.0511	0.0112	0.288	0.0984	**0.0294**
Cd	96	10	15.3	10.0	3.63	50.1	100	10	21.7	13.6	4.88	84.4	0.0583	0.3104
Ce	93	10	0.667	1.71	b > c	6.72	100	11	2.32	2.33	b > c	8.02	0.4784	0.0669
Co	93	10	1.34	0.939	0.0672	4.78	100	11	1.84	1.09	0.119	5.05	**0.0428**	0.1600
Cr	90	10	9.17	4.32	3.2	28.6	102	11	9.61	6.29	1.86	46.9	0.8362	0.8077
Cs	84	9	0.555	0.638	b > c	3.3	81	9	0.211	0.341	b > c	0.982	0.0970	0.1169
Cu	96	10	623	157	253	1060	102	11	501	143	202	1010	0.7430	0.0512
Dy	96	10	0.0459	0.0469	b > c	0.186	102	11	0.017	0.0257	b > c	0.143	0.0737	**0.0010**
Er	95	10	b > c	0.0261	b > c	0.0904	102	11	0.000653	0.0201	b > c	0.0537	0.9289	0.4751
Eu	84	9	0.14	0.499	b > c	3.64	82	9	0.321	0.606	b > c	3.56	0.1311	**0.0276**
Ga	74	8	0.172	0.245	b > c	1.22	99	11	0.222	0.263	0.00596	1.7	0.4060	0.9979
Gd	95	10	0.0846	0.078	b > c	0.334	101	11	0.0501	0.0544	b > c	0.283	0.1377	**0.0209**
Ge	77	8	9.48	4.42	0.622	20.6	62	7	11.5	5.95	0.484	25.9	0.5667	0.9043
Hf	82	9	0.433	0.472	b > c	2.09	71	9	0.84	1.5	b > c	10.0	0.1232	0.4949
Ho	96	10	0.00232	0.00532	b > c	0.022	102	11	0.00253	0.00496	b > c	0.026	0.1216	0.2139
In	92	10	0.297	0.493	b > c	2.88	98	11	0.421	0.345	b > c	2.5	0.4509	0.4466
Ir	86	9	0.0115	0.0324	b > c	0.129	93	11	0.0336	0.0615	b > c	0.321	0.0554	0.1526
La	85	9	0.134	0.0937	0.0395	0.58	85	10	0.213	0.206	0.026	0.931	0.0737	0.6084
Li	72	8	b > c	0.598	b > c	2.3	89	10	0.644	1.46	b > c	5.2	**0.0167**	0.1929
Lu	96	10	0.00166	0.00857	b > c	0.0294	102	11	b > c	0.00609	b > c	0.0345	0.0751	**0.0252**
Mn	95	10	1510	427	730	3830	102	11	1570	622	493	3580	0.6510	0.7499
Mo	87	10	21.4	39.4	b > c	233.0	78	10	7.99	7.99	b > c	32.2	**0.0003**	0.3276
Nb	90	10	1.19	0.977	0.00004	5.86	90	11	1.4	1.36	b > c	6.94	**0.0110**	0.2829
Nd	95	10	0.0983	0.0827	b > c	0.557	101	11	0.174	0.197	b > c	1.02	**0.0017**	0.1832
Pb	94	10	6.01	3.34	1.09	14.9	99	11	6.23	3.65	1.22	19.7	0.4966	0.8488
Pr	95	10	0.0233	0.0407	b > c	0.141	101	11	0.0337	0.0568	b > c	0.289	0.4777	0.7987
Pt	86	9	0.15	0.157	b > c	0.947	88	10	0.203	0.293	b > c	1.74	**0.0253**	0.4963
Rb	95	10	667.0	210.0	278.0	1130.0	102	11	378.0	170.0	96.0	878.0	0.3993	**0.0021**
Re	91	10	0.0089	0.0223	b > c	0.0873	98	11	0.0143	0.0277	b > c	0.124	0.5830	0.8719
Ru	95	10	0.108	0.151	b > c	0.49	99	11	0.161	0.209	b > c	1.1	0.2884	0.7818
Sb	95	10	1.04	0.618	0.286	3.77	102	11	1.79	1.03	0.162	4.56	0.1430	**0.0055**
Sc	92	10	1.06	0.696	0.286	3.76	97	11	1.66	0.927	0.216	5.04	0.9159	0.0808
Se	95	10	52.5	17.1	23.2	99.4	92	10	66.7	20.4	34.0	126.0	0.9897	**0.0247**
Sm	95	10	0.0378	0.0515	b > c	0.253	102	11	0.0444	0.0454	b > c	0.204	0.2984	0.3876
Sn	92	10	3.59	2.1	0.605	12.2	98	10	3.53	1.66	0.897	8.38	0.9687	0.5714
Sr	94	10	551	261	192	1550	102	11	912	508	191	3090	**0.0001**	**0.0230**
Tb	96	10	0.00143	0.00817	b > c	0.0244	102	11	0.00687	0.0106	b > c	0.0512	**0.0017**	0.0808
Te	86	9	1.94	1.07	0.226	7.28	83	9	2.18	0.924	0.517	4.94	**0.0335**	0.4064
Th	84	10	1.32	1.77	0.257	8.74	98	11	1.05	0.865	0.287	5.2	0.2956	0.7178
Ti	96	10	388	255	90.5	1390	99	11	908	561	105	2500	**0.0101**	**0.0073**
Tl	94	10	0.783	0.407	0.236	2.27	101	11	0.696	0.574	0.0892	2.57	0.4539	0.4647
Tm	96	10	0.00413	0.00572	b > c	0.0303	102	11	0.00478	0.00416	b > c	0.0177	0.6718	0.9550
U	89	10	0.0221	0.0174	b > c	0.0735	90	11	0.0466	0.0473	0.00002	0.264	0.0000	0.0440
V	83	9	0.534	0.309	0.139	1.59	82	9	0.441	0.154	0.111	1.03	0.1082	0.5421
W	88	10	18.8	8.15	4.67	45.3	64	8	16.7	7.88	6.32	39.1	0.5353	0.8997
Y	95	10	0.114	0.0626	0.0334	0.306	99	11	0.221	0.147	0.0273	0.738	**0.0386**	**0.0299**
Yb	94	10	0.0022	0.0144	b > c	0.0551	102	11	0.00484	0.0116	b > c	0.0591	0.2962	0.7242
Zr	84	10	3.38	2.99	0.668	16.9	82	11	3.14	2.46	0.316	10.9	0.7301	0.8020

[a] b > c, element concentration below the mean of 10 blanks.

Principal Component Analysis. Principal component analysis (PCA) was applied to the 63 elements measured in the individual onion samples from the 21 farmers to investigate the relevant and interpretable structure in the data. The data set consisted of a table containing the results of the elemental analysis performed on the 204 samples; i.e., 204 objects and 63 variables. The variables were weighted with the inverse of the standard deviation of all objects before the PCA. This was done to compensate for the different scales of the variables. It was found that three principal components (PCs) explained only 29% of the variation in the data set (PC1, 13%; PC2, 9%; and PC3, 7%). However, as it appears from Figure 1, the onion samples split up into groups according to the farming method when the scores of the first and third principal components (PCs) are plotted against each other. In Figure 1 the samples named FE, GE, HE, IE, JE, LE, ME, NE, OE, and ÆE represent organically grown samples and the samples named PC, QC, RC, SC, TC, UC, VC, XC, YC, and ZC represent conventionally grown samples.

It appears that the organic farmer marked ZE is located together with the conventional farmers. An explanation of this is not present in the cultivation history. Some observation may perhaps be of interest. Only ZE (and ME) has had potatoes in the field of observation in the year before the experiment. Clover grass was the most common crop before onion cultivation among the other organic farmers. The soil at ZE differs markedly from the soils at the other organic farmers' sites. ZE has the sandiest soil with the highest content of organic carbon and with the lowest potassium

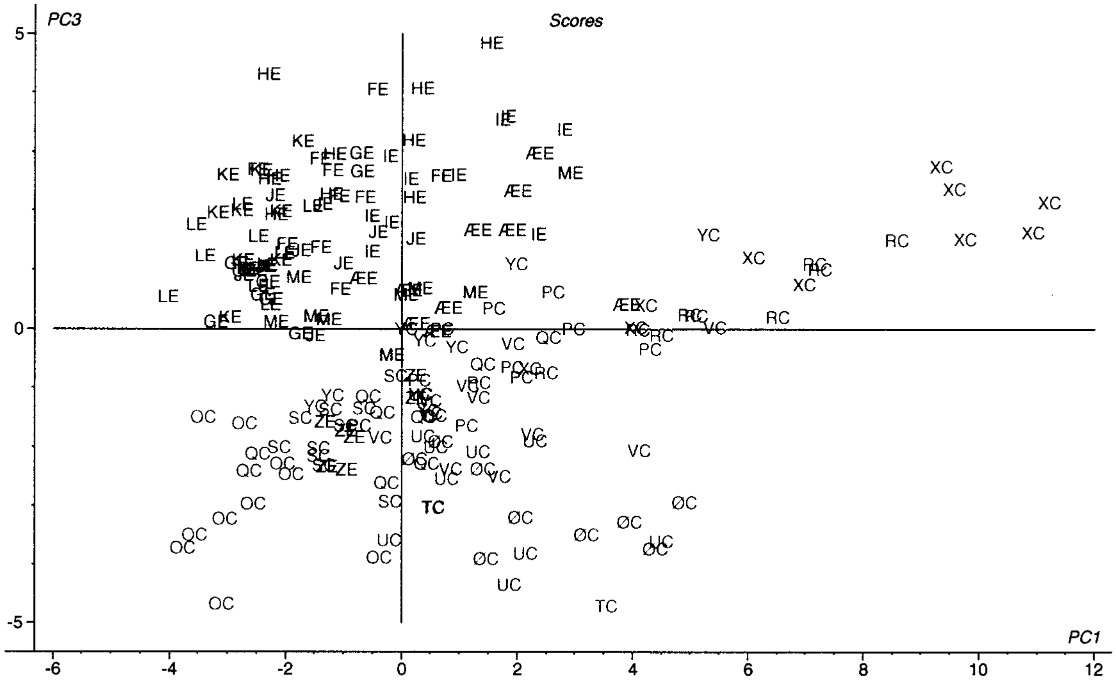

Figure 1. Scores plot for the first and third principal component of the PCA model for individual onion samples. Sites with second letter E are organic and sites with second letter C are conventional.

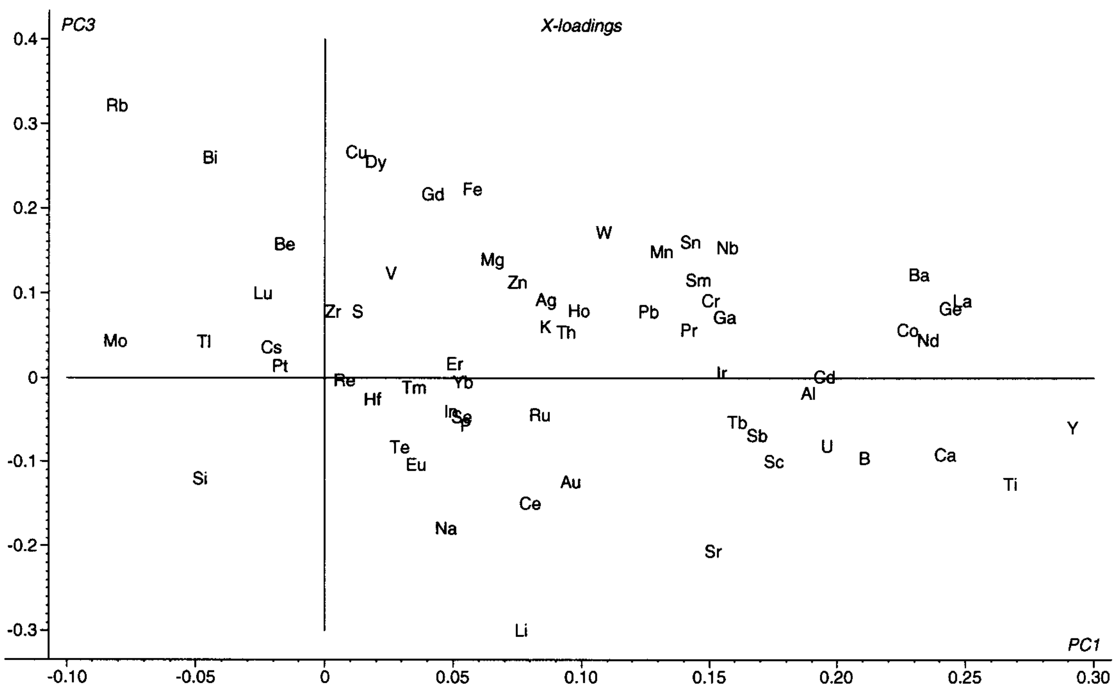

Figure 2. Scatter plot of loadings for the first and third principal component in the PCA model for onions corresponding to Figure 1.

and highest phosphorus contents, but there is no obvious explanation of how those conditions could effect the trace element profile.

The model is based on individual samples from each farmer and thus includes the variations between the individual samples from each farmer. In case of authority control of onions, this would be an advantage because it would be possible to identify even a few conventionally grown onions in a batch of organically grown onions samples. A model based on mean values for each farmer was not found to improve the separation of the two farming methods. Models based on a reduced number of elements also did not improve the separation.

In Figure 2 the corresponding scatter plot of the loadings for PC1 and PC3 is shown. In general, the elements with high numerical loadings are the elements being most important for the model. By combining the plots of scores and loadings it is possible to interpret sample properties and variable relationships simultaneously. It appears that elements such as Rb, Bi, Mo, Cu, Dy, Be, Lu, Gd, and Fe are found in higher levels in organically grown onions than in conventionally grown onions, but elements such as Y, Ti, Ca, B, Sr, U, Sc, Ba, La, Nd, Co, and Ge are found to have higher levels in conventionally grown onions than in organically grown onions. It is remarkable that the elements

Table 5. Elements in Peas: Major Components (mg/kg, fresh weight)

	organic						conventional						comparative tests	
elements	N_{total}	N_{farmer}	mean (mg/kg)	std. dev. (mg/kg)	minimum (mg/kg)	maximum (mg/kg)	N_{total}	N_{farmer}	mean (mg/kg)	std. dev. (mg/kg)	minimum (mg/kg)	maximum (mg/kg)	*p* value (*F* test)	*p* value (*t* test)
B	85	9	12.9	3.41	5.35	24.5	93	10	12.2	4.35	1.12	26.4	0.4034	0.5599
Ca	89	9	219	57.9	140	535	98	10	231	96	15.2	645	**0.0026**	0.4841
Fe	90	9	11.5	2.03	6.01	15.1	96	10	10.2	2.93	5.06	20.8	0.1540	0.1696
P	87	9	1820	639	1100	3290	94	10	1290	245	801	1790	**0.0022**	**0.0444**
Si	80	9	9.35	5.48	1.46	30.8	85	10	8.73	5.17	2.54	27.5	0.9506	0.6739
Zn	89	9	9.99	3.12	5.57	19.4	95	10	7.93	2.49	4.21	16.3	0.5455	0.0719

Table 6. Elements in Peas: Minor Components (μg/kg, fresh weight)

	organic						conventional						comparative tests	
elements	N_{total}	N_{farmer}	mean (μg/kg)	std. dev. (μg/kg)	minimum (μg/kg)	maximum (μg/kg)	N_{total}	N_{farmer}	mean (μg/kg)	std. dev. (μg/kg)	minimum (μg/kg)	maximum (μg/kg)	*p* value (*F* test)	*p* value (*t* test)
Ag	82	9	0.624	0.435	0.153	2.59	84	10	0.587	0.451	0.116	2.76	0.5316	0.8137
Al	82	9	99.3	49.8	34.2	268.0	85	9	88.6	45.6	32.3	271.0	0.1331	0.5519
Au	59	6	0.955	0.609	0.308	3.7	84	9	0.713	0.389	0.119	2.96	0.0910	0.1967
Ba	85	9	213.0	156.0	47.0	693.0	94	10	202.0	138.0	30.2	692.0	0.7363	0.9549
Be	86	9	0.137	0.122	b > c[a]	0.617	86	10	0.139	0.156	b > c	1.07	0.7391	0.8301
Bi	87	9	0.0919	0.092	0.0093	0.589	91	10	0.0894	0.0759	0.0095	0.368	0.5025	0.8192
Cd	90	9	9.35	4.16	3.37	22.9	95	10	12.1	4.91	5.03	29.9	0.7250	0.0938
Ce	77	8	0.935	2.05	b > c	6.84	77	8	b > c	0.661	b > c	1.53	**0.0003**	0.2047
Co	88	9	5.06	2.72	1.37	14.5	93	10	5.48	3.73	0.574	17.4	0.3306	0.686
Cr	85	9	4.15	1.72	1.44	9.19	86	9	3.28	1.42	0.452	6.68	0.5772	0.1377
Cu	88	9	1210	307	647	2180	95	10	1390	278	769	2140	0.0681	0.1032
Dy	88	9	0.00613	0.0126	b > c	0.0423	96	10	0.0116	0.0214	b > c	0.0915	**0.0090**	0.2542
Er	87	9	0.0148	0.0237	b > c	0.0729	96	10	0.00484	0.0211	b > c	0.0508	0.5424	0.2006
Eu	88	9	0.639	0.624	0.0632	3.51	95	10	0.371	0.442	b > c	2.18	0.6094	0.3252
Ga	87	9	0.324	0.148	0.105	0.87	95	10	0.435	0.196	0.0889	0.982	0.1683	0.1154
Gd	88	9	0.102	0.0455	0.0264	0.2	93	10	0.0553	0.0354	0.0082	0.163	0.4491	**0.0033**
Ge	88	9	85.4	18.8	47.4	148.0	93	10	78.7	19.0	38.6	125.0	0.3431	0.2515
Hf	73	8	0.38	0.512	b > c	3.12	84	9	0.303	0.393	b > c	2.03	0.2224	0.5527
Ho	88	9	0.0023	0.00277	b > c	0.0089	95	10	0.00143	0.00373	b > c	0.0177	**0.0059**	0.4664
Ir	86	9	0.00657	0.0543	b > c	0.355	84	9	b > c	0.0223	b > c	0.0878	**0.0099**	0.6121
La	87	9	0.235	0.118	0.0696	0.582	95	10	0.221	0.118	0.0496	0.662	**0.0348**	0.8613
Lu	87	9	b>c	0.00392	b>c	0.0057	94	10	b>c	0.00377	b>c	0.006	0.2255	0.4097
Mn	90	9	2360	769	1370	5630	96	10	2210	582	1260	4240	0.7811	0.6581
Mo	87	9	295	233	41.5	1090	96	10	253	268	26.4	1480	0.6572	0.6187
Nb	72	8	1.48	1.34	0.0332	5.69	78	9	1.16	0.661	0.178	3.25	**0.0230**	0.5263
Nd	86	9	0.152	0.0847	0.0301	0.436	96	10	0.139	0.1	0.0189	0.521	**0.0299**	0.6735
Pb	83	9	4.39	2.95	0.719	15.6	91	10	5.64	3.44	0.44	18.0	0.6677	0.2548
Pd	78	8	3.8	1.94	0.917	10.2	57	6	4.95	3.51	0.569	14.3	0.0722	0.3434
Pr	87	9	0.0538	0.0481	b > c[a]	0.173	96	10	0.0288	0.0467	b > c	0.177	0.9263	0.2435
Pt	79	8	0.14	0.186	b > c	1.04	86	9	0.0754	0.175	b > c	1.38	0.0684	0.2778
Re	86	9	0.00368	0.0188	b > c	0.116	92	10	0.0102	0.0354	b > c	0.231	0.0744	0.1706
Rh	90	9	0.368	0.168	0.0777	1.04	95	10	0.441	0.318	0.0606	1.82	**0.0285**	0.4241
Sb	88	9	1.32	0.923	0.189	4.44	95	10	0.952	0.65	0.199	3.16	0.1317	0.181
Sc	89	9	1.59	0.731	0.744	4.99	57	6	2.24	1.09	0.649	5.92	0.3761	0.0671
Se	86	9	71.9	22.5	37.6	142.0	93	10	79.7	31.4	23.1	159.0	0.2307	0.5011
Sm	89	9	0.0234	0.0196	b > c	0.0848	94	10	0.0287	0.0252	b > c	0.122	**0.0402**	0.2475
Sr	88	9	505	131	237	887	76	8	480	189	179	1120	**0.0198**	0.7926
Ta	82	9	0.681	0.928	0.0996	7.92	83	10	0.981	0.655	0.0179	4.03	0.1132	0.1314
Tb	89	9	0.00118	0.00594	b > c	0.0157	93	10	0.00305	0.00739	b > c	0.0223	0.5138	0.3268
Te	88	9	2.24	1.53	0.352	6.69	96	10	1.64	0.754	0.103	4.65	**0.0012**	0.2738
Th	66	7	0.564	0.742	0.0313	4.71	76	9	0.324	0.248	0.0102	1.4	**0.0077**	0.3221
Ti	85	9	625	155	291	1070	96	10	1030	390	420	1920	**0.0012**	**0.0051**
Tl	77	8	0.413	0.407	0.0001	1.71	95	10	0.344	0.265	b > c	1.53	0.1233	0.6593
Tm	84	9	0.00341	0.00282	0.0	0.0111	96	10	0.00255	0.00227	0.0	0.0108	0.1094	0.2277
U	87	9	0.0515	0.071	0.0005	0.616	91	10	0.0449	0.0398	b > c	0.171	0.0901	0.5949
V	87	9	0.295	0.147	0.0541	0.696	94	10	0.307	0.148	0.0771	0.793	0.8291	0.6254
Y	89	9	0.18	0.0507	0.0947	0.346	96	10	0.242	0.119	0.0931	0.663	**0.0001**	0.0974
Yb	87	9	0.00132	0.0077	b > c	0.0322	95	10	0.00113	0.00768	b > c	0.0282	0.0856	0.9597
Zr	80	9	3.01	3.50	0.464	29.9	75	10	2.20	1.08	0.421	5.29	**0.0332**	0.1124

[a] b > c, element concentration below the mean of 10 blanks.

that showed significant difference between the two farming methods in the *t* test are only a subset of the elements that showed high loadings in the PCA model. A PCA model including only the significant elements from the *t* test performed more poorly than the model with all elements. This illustrates the importance of studying all measured elements together using multivariate data analysis in order to not disregard interactions between the elements.

Peas. The 55 element concentrations in organically grown and conventionally grown peas are given in Table 5 (major components in mg/kg fresh weight) and Table 6 (minor components in μg/kg fresh weight). As for the onion samples, the number of samples (N_{total}) may be different for each element because of removal of outliers. The total number of samples (N_{total}), the number of farmers (N_{farmer}), mean values, standard deviations, and the range of variation (minimum and maximum values)

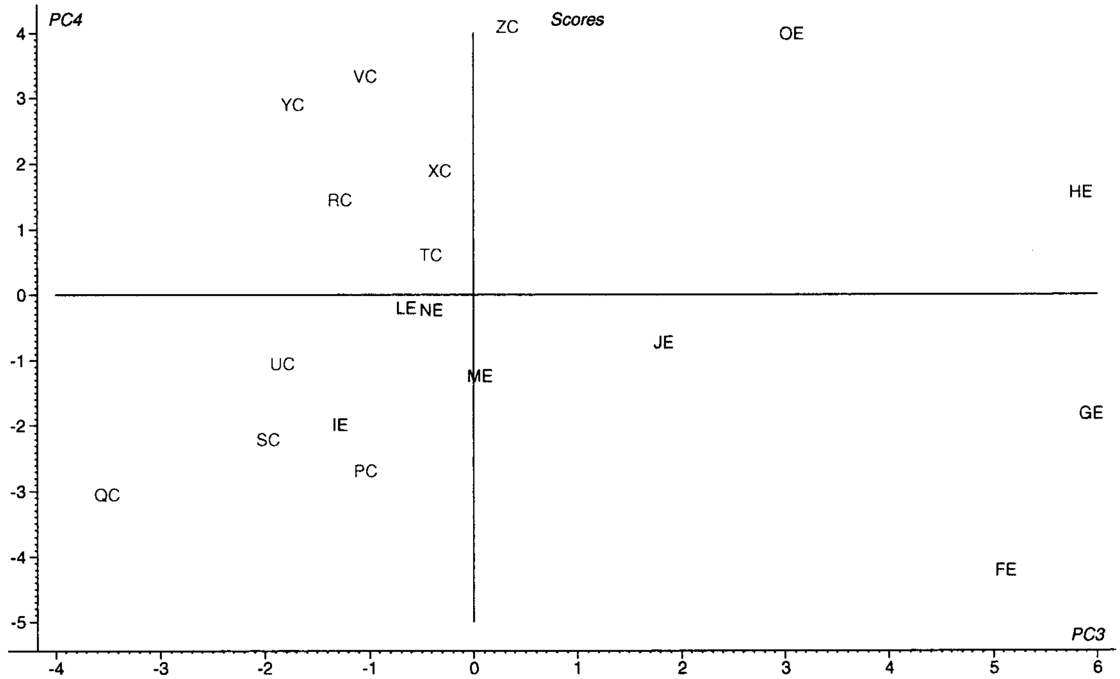

Figure 3. Scores plot for the third and fourth principal component of the PCA model for peas. The model is based on mean values from each site. Sites with second letter E are organic and sites with second letter C are conventional.

of each of the 55 elements measured for peas are shown. The mean values of the elements measured in organically grown and conventionally grown peas do not represent the true mean values of these elements in Danish peas for the same reasons as mentioned for onions.

Comparative Statistical Tests. The effect of the two farming methods on the trace element content in peas was examined by comparison of the mean values for each farmer of each of the 55 elements for the two farming methods. The means are compared by the Student's *t* test, testing the hypothesis $H_0{:}\mu_1 = \mu_2$ against the alternative $H_0{:}\mu_1 \neq \mu_2$. For the elements with unequal variances the *t* test is based on an approximate test (indicated by boldfaced *p* value). The results of the *F* test and the *t* test are given in Tables 5 and 6.

Only the levels of P, Gd, and Ti were found to differ significantly ($p < 0.05$) between the organically and conventionally grown peas (indicated by boldfaced *p* value). Compared to onions, the difference between the two farming methods for peas is thus less evident based on the *t* test.

Principal Component Analysis. A PCA model based on 190 samples and 55 elements was made. The variables were weighted with the inverse of the standard deviation of all objects. This model showed only a weak tendency to separate organically grown peas from conventionally grown peas. However, when the variations between the individual samples from each farmer are eliminated and the model is based on mean values for each farmer the separation of the two farming methods is improved.

The first four principal components for the PCA model on mean data (19 farmers and 55 elements) explained 56% of the variation in the data set (PC1, 18%; PC2, 15%; PC3, 8%; and PC4, 11%). Particularly, the variation explained by the third and fourth principal components is found to be related to the farming method. The scores for the third and fourth components of the PCA model are given in Figure 3.

The samples FE, GE, HE, IE, JE, LE, ME, NE, and OE represent organically grown samples and the samples PC, QC, RC, SC, TC, UC, VC, XC, YC, and ZC represent conventionally grown samples.

It appears that the pea samples split up into groups according to the farming method. However, the conventional farmer marked PC is located together with the organic farmers. This farmer has used cattle slurry and is therefore more similar to the organic farmers than the other conventional farmers who use fertilizers (RC, SC, TC, and UC) or nothing (QC, XC, YC, and ZC). Only one organic farmer (IE) has no application of farmyard manure or slurry and is placed close to the group of conventional farmers. Furthermore, IE has a special cultivation history with organic apple cultivation several years before the experiment. LE and NE are placed close to the conventional group, but nothing in the cultivation history can explain it. The soil from LE has the highest content of clay and the soil from NE has the lowest content of organic carbon of all the cultivation sites, but there is no obvious explanation of how this may affect the elemental profiles. QC represents the only conventional peas cultivated without use of pesticides and has an extreme placement with respect to the group of conventional farmers.

The corresponding loading plot is shown in Figure 4. Elements with high positive loading on PC3, i.e., Er, Te, Gd, Zr, Th, Au, Pt, Ce, Tm, Hf, P, Pr, and Re, are found in higher levels in organically grown peas than in conventionally grown peas. Elements with high negative loading on PC3, i.e., Ti, Sc, Y, Cu, Se, Ba, and Cd, exhibit higher levels in conventionally grown peas than in organically grown peas. It is remarkable that the elements (P, Gd, and Ti) that showed significant difference in the *t* test are among the elements that showed high loadings in the PCA model.

Compared to the PCA model for onions, the separation between farming methods for peas is less obvious when the model is based on individual sample data. However, a PCA model based on mean values for each farmer shows differences between the production meth-

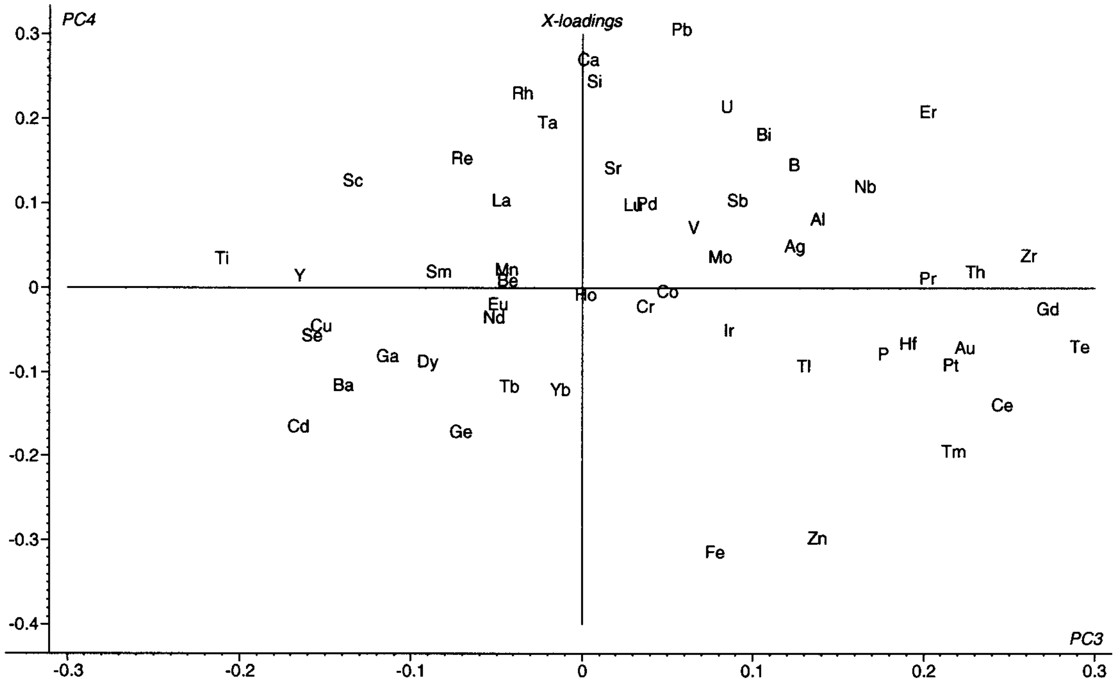

Figure 4. Scatter plot of loadings for the third and fourth principal component in the PCA model for peas corresponding to Figure 3.

ods. This may be explained by the sampling procedure. In the case of onions, a sample is one onion representing the entire plant. From the homogenized onion, it is rather easy to take out a subsample representing the onion and the entire plant. In this way there are comparatively little deviations in elemental concentrations between the mean value and the 10 individual sample values from a site. In the case of peas, a plant is represented by only two pea pods from which four seeds are selected. In this way a pea sample is not as representative for the plant as an onion sample, and the deviations in elemental concentrations between the mean value and the 10 individual sample values from a site are greater than for onions. This is confirmed by comparing onions and peas standard deviations for individual elements concentrations calculated for the 10 samples at each site. Standard deviations for peas are generally greater than for onions in the same group of cultivation. Despite comparatively great deviations between individual sample values, the mean value may still be representative for a site and that may be the reason a PCA model based on individual pea sample data does not separate as well as the model based on mean values.

Comparative statistical tests of element concentrations in organically and conventionally cultivated onions and peas have shown that the cultivation method affects the concentration of some elements in the crops. Principal component analysis (PCA) of the analytical data has convincingly demonstrated that the elemental concentration profiles are different for organically and conventionally grown onions and peas and that it is possible, by multivariate analysis of multi-element concentration data for onions and peas, to separate a crop on the basis of the cultivation method. The limitation of the investigation must be remembered. The crops are represented by only one sort, and conventional cultivation is, in general, restricted to practices where fertilizers and pesticides are commonly used. The methodology used in this study may be developed to use as authenticity control for organic cultivation, but further comprehensive investigations are needed, and it is important to take into account the above-mentioned limitations in future development work.

ACKNOWLEDGMENT

The authors thank Lis Vinther Kristensen, Jette Bruun Nielsen, Trine Frederiksen, and Anette Møller, who did the sample preparation and the analyses. We also thank Leif Knudsen, who organized the selection of farmers, the collection of cultivation history, and soil sampling, Niels B. Isaksen, who collected all the samples, and Allan Bibak and Pernille Gundersen who carefully assisted with the data handling.

LITERATURE CITED

Baxter, M. J.; Crews, H. M.; Dennis, M. J.; Goodall, I.; Anderson, D. The Determination of the authenticity of wine from its trace element composition. *Food Chem.* **1997**, *60* (3), 443−450.

Bibak, A.; Behrens, A.; Stürup, S.; Knudsen, L.; Gundersen, V. Concentrations of 63 major and trace elements in Danish agricultural crops measured by inductively coupled plasma mass spectrometry. 1. Onion (*Allium cepa* Hysam). *J. Agric. Food Chem.* **1998a**, *46*, 6 (8), 3139−3145.

Bibak, A.; Behrens, A.; Stürup, S.; Knudsen, L.; Gundersen, V. Concentrations of 55 major and trace elements in Danish agricultural crops measured by inductively coupled plasma mass spectrometry. 2. Pea (*Pisum sativum* Ping Pong). *J. Agric. Food Chem.* **1998b**, *46*, 6 (8), 3146−3149.

Danish Gallup Institute. Public Opinion Poll for Berlingske Tidende, Newspaper: Copenhagen, 1999, *7 April, 4*, p. 4.

Danish Law. *Lov om økologisk jordbrugsproduktion nr. 363 af 10. juni 1987.* Danish Ministry of Agriculture and Fisheries: Copenhagen, 1987; http://47.29.40.90/DELFIN/A1987/0036330.htm.

EEC Council Regulation. *Council Regulation (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs.* Official Journal L 198 22.07.1991 European Economic Community: Luxembourg, 1991.

Haswell, S. J.; Walmsley, A. D. Multivariate data visualisation methods based on multi-elemental analysis of wines and coffees using total reflection X-ray fluorescence analysis. *J. Anal. At. Spectrom*. **1998**, *13*, 131−134.

Lizama, V.; Aleixandre, J. L.; Alvarez, I.; Garcia, M. J. Differentiation of the three D. O. Valencia Community wines according to their cation content. **1997**, *50* (4), 29−36.

Marcos, A.; Fisher, A.; Gerry, R.; Hill, S. J. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J. Anal. At. Spectrom*. **1998**, *13* (6), 521−525.

Markert, B. Occurrence and distribution of chemical elements in plants—outlook and further research plans. *Toxicol. Environ. Chem*. **1993**, *40*, 31−41.

Schwarts, R. S.; Hecking, L. T. Determination of geographic origin of agricultural products by multivariate analysis of trace element composition. *J. Anal. At. Spectrom*. **1991**, *6* (8), 637−642.

Thiel, G.; Danzer, K. Direct analysis of mineral components in wine by inductively-coupled plasma−optical emission spectrometry (ICP−OES). *Fresenius' J. Anal. Chem*. **1997**, *357* (5), 553−557.

Tjell, J. C.; Lamm, C. G. Human mineral supply in an industrialized community. English abstract in: Menneskets mineralforsyning i et industrialiseret samfund. *Nordisk Medicin* **1969**, *81*, 717−723.

Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventionally grown foods—results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281−293.

Worthington, V. Effect of agricultural methods on nutritional quality: A comparison of organic with conventional crops. *Alternative Therapies* **1998**, *4* (1), 58−69.

Received for review August 1, 2000. Revised manuscript received October 5, 2000. Accepted October 5, 2000. This investigation has been supported financially by the Danish Food and Technology Development (FØTEK) Program. We gratefully acknowledge this support.

JF0009652