Journal of Cereal Science 76 (2017) 76–84

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Limitation of multi-elemental fingerprinting of wheat grains: Effect of cultivar, sowing date, and nutrient management

Alfonso Suarez-Tapia [a], Sergey V. Kucheryavskiy [b], Bent T. Christensen [a], Ingrid K. Thomsen [a, *], Jim Rasmussen [a]

[a] Department of Agroecology, Aarhus University, AU-Foulum, DK-8830, Tjele, Denmark
[b] Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, DK-6700, Esbjerg, Denmark

ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form 19 May 2017
Accepted 21 May 2017
Available online 29 May 2017

Keywords:
Animal manure
Mineral fertilisers
Askov-LTE
Multi-element fingerprinting

ABSTRACT

Multi-element fingerprinting demonstrates some potential for tracing the origin of agricultural products but not for discriminating among crop cultivars and nutrient management (source, rate). With principal component analysis (PCA) and univariate statistics, we examined 19 elements in grains from two winter wheat cultivars (Hereford, Mariboss) grown with different rates of animal manure (AM) or mineral fertilisers (NPK) in a long-term field experiment and two sowing dates (early, timely).

Nitrogen, Cd and Mn related to NPK, and Mo and Na to AM. Barium, Fe, and P reflected nutrient rate; these elements increased with nutrient rate regardless of source. Unmanured grains were enriched in Cu. Mariboss was characterized by higher concentrations of Sr, Ba and Sc compared to Hereford with Sr in grain as the main separator. Univariate statistics showed higher concentrations of N, P, Mg, Ba, Cu, Mo and Zn in early sown than in timely sown wheat. Compared with Hereford grains Mariboss was higher in P, Mg, Ba, Cu and Sr but lower in Mn, Mo and Zn. Thus, confounding effects of cultivar, sowing date, nutrient source and rate limits the potential of multi-element analysis in discriminating among agricultural products from different sites and cropping systems.

1. Introduction

Wheat accounts for 60% of the production of small grain cereals in the European Union. In Denmark, winter wheat (*Triticum aestivum* L.) occupies 25% of the arable land, corresponding to 40% of the area cropped with cereals. The majority of cereals grown in Denmark are used for animal feeding. On farms specialized in livestock production high yielding cultivars of autumn sown wheat are preferred and usually grown with application of animal manure. Historically, breeding programmes for wheat have targeted disease resistance, grain yield potentials and crop response to mineral fertilisers (Graham et al., 1999; Rengel et al., 1999) while the elemental composition of grains has been of secondary importance when developing new cultivars.

The element contents in grain have been shown to differ among wheat cultivars (Fan et al., 2008; Svecnjak et al., 2013; Zhao et al., 2009), but cultivar properties may be of minor importance relative to the impact of soil parent material and crop management. Increasing rates of mineral N fertiliser may increase trace element concentrations in wheat grains (Gooding et al., 2012; Kutman et al., 2011; Shi et al., 2010; Svecnjak et al., 2013). Moreover, a range of elements has been found to differ in concentration between crops dressed with mineral fertiliser or animal manure (Christensen and Elsgaard, 2014; Fan et al., 2008; Hamnér and Kirchmann, 2015; Kirchmann et al., 2009). However, effects of soil type, climate, cultivar, and management factors are often confounded, and we found little conclusive evidence in the literature on the specific impact of animal manure versus mineral fertilisers on the elemental composition of grains from different wheat cultivars.

Studies based on multi-element analysis and multivariate statistics have been applied to fingerprint grains of different geographical origin (Høgh-Jensen et al., 2006; Husted et al., 2004; Zhao et al., 2011, 2013) and to discriminate among different management systems such as organic versus conventional cropping (Gundersen et al., 2000; Laursen et al., 2011, 2013). Although multi-element fingerprinting demonstrates some potential for authentication of agricultural products, this approach has not yet been successful in discriminating among crop cultivars, nutrient source, and nutrient addition rate as the effect of these factors were confounded.

Abbreviations: PCA, principal component analysis; AM, animal manure; NPK, mineral fertilisers; LTE, long term experiment.

* Corresponding author. Department of Agroecology, Aarhus University, AU-Foulum, Blichers Allé 20, PO Box 50, DK-8830, Tjele, Denmark.
E-mail address: ingrid.thomsen@agro.au.dk (I.K. Thomsen).

http://dx.doi.org/10.1016/j.jcs.2017.05.015
0733-5210/

To examine if these confounding factors violate the concept of multi-element fingerprinting, we analysed for 38 elements (19 elements above detection limits) in grains from two winter wheat cultivars grown on soils with long-term application of different rates of nutrients in either animal manure or mineral fertilisers. The wheat cultivars were planted at two dates spaced one month apart (early and timely sowing). Using multivariate statistics and univariate analyses, our aim was to isolate the effect of sowing date, nutrient source, and nutrient addition rate on the elemental composition of grains from two winter wheat cultivars.

2. Materials and methods

2.1. The Askov Long-Term Experiments (Askov-LTE)

The Askov-LTE was established in 1894 on the Lermarken site at Askov Experimental Station, Denmark (55° 28′ N, 09° 07′ E). The soil is a light sandy loam derived from glacial deposits and classified as Ultic Hapludalf (USDA Soil Survey Staff). The topsoil (0–20 cm) has 10% clay (<2 μm), 12% silt (2–20 μm), 43% fine sand (20–200 μm) and 35% coarse sand (200–2000 μm). Soil pH is maintained in the range 5.5–6.5 by addition of magnesium-enriched lime every four years. Sulphur is applied annually at a rate of 12.5 kg S ha^{-1}. Annual average precipitation and temperature is 862 mm and 7.7 °C, respectively (1961–1990 averages).

The Askov-LTE encompasses four separate fields (termed B2, B3, B4 and B5) and a four-course crop rotation of winter wheat (*Triticum aestivum* L.), silage maize (*Zea maize* L.), and spring barley (*Hordeum vulgare* L.) undersown with a grass-clover mixture that is cut twice in the subsequent production year. The crop rotation is fixed and each crop is present every year in a separate field. The fields are subject to conventional management and crop protection measures (e.g. pesticides) applied when needed.

The core experimental treatments (established in 1894/1923) of the Askov-LTE are unmanured plots and plots receiving different rates (½, 1, and 1½ times the standard rate for a given crop) of nitrogen (total-N), phosphorus (P) and potassium (K) in animal manure (AM; cattle slurry since 1973) and mineral fertilisers (NPK). Mineral fertiliser N, P and K are added in calcium-ammonium-nitrate, triple-super-phosphate and potassium chloride. Within each field, the nutrient treatments are replicated and randomized. Averaged across the rotation (no additions to the grass-clover crop), 1 AM and 1 NPK represents an annual input of 100 kg total-N ha^{-1}, 20 kg P ha^{-1} and 80 kg K ha^{-1}. Christensen et al. (2006) provide further details on the experimental layout of the Askov-LTE.

2.2. Study of elemental composition of wheat grains

For the present study, we relied on winter wheat grains harvested in 2015 in 3 replicates of the unmanured, 1 AM, 1½ AM, 1 NPK, and 1½ NPK plots in the B3-field. For winter wheat, 1 AM and 1 NPK correspond to 150 kg total-N ha^{-1}, 30 kg P ha^{-1} and 120 kg K ha^{-1}. In the growing season 2014–2015, each nutrient plot in the B3-field was divided into four subplots to accommodate two winter wheat cultivars (Hereford™, Sygenta, CH and Mariboss™, Nordic Seeds, DK) and two sowing dates (early sowing, 20 August 2014; timely sowing, 18 September 2014). This provided a split-split plot experimental design with three replicates of five nutrient treatments, two sowing dates and two cultivars. Hence, 60 samples of grains were retrieved for chemical analyses. The wheat was established after the grass-clover crop which was terminated by herbicide (glyphosate applied 8 August 2014) and ploughing (20 August 2014). In March 2015, AM and NPK was surface-applied onto the emerging wheat crop. The wheat was harvested at physiological maturity on 20 August 2015 and grain samples were subsequently oven-dried at 80 °C before analyses.

2.3. Chemical analyses

Grain samples were ground to <0.5 mm (CT 193 Cyclotec™ Sample Mill, Denmark) and sub-samples analysed for 37 elements (Ag, Al, As, Au, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Te, Th, Ti, Tl, U, V, W and Zn) at Bureau Veritas Mineral Laboratories (Vancouver, Canada). A 1 g split of the ground sample was treated with cold nitric acid and then digested in a hot water bath. Aqua Regia (equal parts of nitric and hydrochloric acid) and distilled water was added and the sample further digested in a heating block. Multi elemental analysis was performed with ICP-MS (Inductively Couple Plasma-Mass Spectrometry) using a NexION 300 (PerkinElmer, USA).

The concentration of N was analysed by dry combustion on a separate subsample of ground wheat grain using a Flash 2000 Organic Elemental Analyser (Thermo Fisher Scientific, USA).

The following 19 elements were found in concentrations above the analytical detection limits (ADL) and used in the subsequent data analyses: Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, Pb, S, Sc, Sr, Ti and Zn. Supplementary Table S1 presents analytical detection limits for the 37 elements and overall mean values for elements with concentrations above detection limits.

2.4. Data analysis

2.4.1. Multivariate analysis

Multivariate analysis was performed using R (version 3.2.4) with the mdatools package (http://dx.doi.org/10.5281/zenodo.59547). Principal component analysis (PCA) was used for unsupervised pattern recognition to determine associations between management factors (nutrient source (AM, NPK), nutrient rate (unmanured, ½, 1, 1½), wheat cultivar, wheat sowing date) and grain elemental composition. The elements related to a given management factor were further subject to response analysis using univariate statistics.

The PCA reduces the number of original variables (X) into fewer latent variables (principal components). The goal of the PCA is to explain as much of the variability as possible with as few principal components. The first principal component (PC1) follows the direction of maximum variance in the data, then the second principal component (PC2) is orthogonal to PC1 alongside the second maximum variance, and so on (Esbensen et al., 2002). There are three main outcomes in PCA: the scores, the loadings and the residuals. The scores show the position of the samples being projected into the PC space and can be used to visualise any form of relationship among the samples (trends, clusters, outliers). The loading are unit vectors in the X space that defines the direction of the principal components, therefore they show which variables are responsible for a particular trend (Bro and Smilde, 2014). Residuals are mainly used to identify samples, which are not well explained by the principal components (e.g. outliers).

2.4.2. Response analysis

The element responses isolated by the PCA and ascribed to different management factors were analysed by univariate statistics using the model:

$$y_{ijkl} = \mu + B_i + Tj + BT_{ij} + P_k + BP_{ik} + TPjk + BTP_{ijk} + V_l + BV_{il} + PV_{kl} + TPV_{jkl} + TV_{jl} + BTV_{ijl} + BPV_{ikl} + BTPV_{ijkl} + \varepsilon_{(ijklm)}$$