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High-dimensional nested analysis of variance to assess the effect of production season, quality grade and steam pasteurization on the phenolic composition of fermented rooibos herbal tea

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ABSTRACT

A nested analysis of variance combined with simultaneous component analysis, ASCA, was proposed to model high-dimensional chromatographic data. The data were obtained from an experiment designed to investigate the effect of production season, quality grade and post-production processing (steam pasteurization) on the phenolic content of the infusion of the popular herbal tea, rooibos, at 'cup-of-tea' strength. Specifically, a four-way analysis of variance where the experimental design involves nesting in two of the three crossed factors was considered.

For the purpose of the study, batches of fermented rooibos plant material were sampled from each of four quality grades during three production seasons (2009, 2010 and 2011) and a sub-sample of each batch was steam-pasteurized. The phenolic content of each rooibos infusion was characterized by high performance liquid chromatography (HPLC)-diode array detection (DAD). In contrast to previous studies, the complete HPLC-DAD signals were used in the chemometric analysis in order to take into account the entire phenolic profile.

All factors had a significant effect on the phenolic content of a 'cup-of-tea' strength rooibos infusion. In particular, infusions prepared from the grade A (highest quality) samples contained a higher content of almost all phenolic compounds than the lower quality plant material. The variations of the content of isoorientin and orientin in the different quality grade infusions over production seasons are larger than the variations in the content of aspalathin and quercetin-3-*O*-robinobioside. Ferulic acid can be used as an indicator of the quality of rooibos tea as its content generally decreases with increasing tea quality. Steam pasteurization decreased the content of the majority of phenolic compounds in a 'cup-of-tea' strength rooibos infusion.

1. Introduction

Typically, the analysis of food and beverages involves a qualitative or quantitative determination of specific chemical components in order to explain changes that occur during processing and loss of quality, amongst others. Recently, the analysis of herbal chromatographic fingerprints and specifically a non-targeted approach has been advocated and applied [1]. The main aims are generally quality control of herbal products and elucidation of the role of various factors on composition taking into account minor and/or unidentified constituents.

The aim of this work was to evaluate the significance of the effect of production season, quality grade and post-production processing (steam pasteurization) on the phenolic content of the infusion of the popular herbal tea, rooibos [2], prepared at 'cup-of-tea' strength using chromatographic fingerprints. Several chemometric methods that combine the statistical properties of analysis of variance (ANOVA) and the advantages of a dimensionality reduction technique like principal component analysis (PCA), ANOVA–PCA or simultaneous component analysis (SCA), ASCA for handling highly correlated multivariate data have been described in the literature [3–6]. To date, all of these supervised chemometric methods have mainly been used to analyze data with fixed effects factors [7]. In this paper, we present a

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methodology based on the ASCA method which allows us to deal with a high dimensional mixed-effects analysis of variance with nesting. Furthermore, a permutation strategy for a test the effect of each particular factor is also presented.

The phenolic composition of rooibos plays an important role in its sensory (color, taste and astringency) and health-promoting properties [8–11]. Joubert et al. [12] have studied the effect of production season and quality grade on the content of 15 selected phenolics of rooibos infusions, which were prepared from steam-pasteurized fermented plant material and showed that the content of some individual phenolic compounds significantly depends on the production season and quality grade. Koch et al. [9] determined that steam pasteurization reduced the color and astringency of fermented rooibos infusions, yet no significant change in the major phenolic compounds with the exception of aspalathin, was observed, indicating that other compounds such as polymers may be important. In the present study, a more comprehensive set containing data from the same rooibos samples used in Joubert et al. [12] and data from sub-samples that were not pasteurized was analyzed using the methodology based on the ASCA approach in order to consider all of the possible sources of variations related to production season, quality grade and steam pasteurization. Specifically, a four-way ANOVA where the experimental design involves nesting in two of the three crossed factors is considered. In contrast with the previous study, which focused on selected compounds, the presented methodology allowed us to work with data in which each sample was characterized by its complete HPLC-DAD signal in order to take into account the entire phenolic profile.

2. Experimental section

2.1. Design of experiments

Individual production batches of unrefined, fermented rooibos plant material, originating from several locations (i.e. farms and areas), were sampled during three production seasons (2009, 2010 and 2011) and classified into four quality grades, A, B, C and D. The quality grading, based on sensory properties, was performed in-house by a rooibos processing and marketing company in accordance with their quality grading system as described by Koch et al. [9]. Samples of 10 batches were then randomly selected from each quality grade material in order to guarantee the representativeness of the samples. Samples were treated as described by Joubert et al. [12]. In short, the samples were sieved to obtain the refined plant material that is sold to the consumer, whereafter a portion of the refined fraction >40 mesh and <10 mesh from each sample was subjected to steam pasteurization following basically the same conditions as those used by the industry (steam exposure ± 96 °C for 2 min followed by drying to ca 10% moisture content). Next, duplicate infusions filtered through Whatman no. 4 filter paper (Whatman International, Ltd., Maidstone, U.K.) were prepared from each sample (both the unpasteurized and pasteurized material) at 'cup-of-tea' strength and aliquots of ca. 1.5 mL were stored at −20 °C. Finally, the HPLC-DAD analysis of each infusion was performed in duplicate.

The design of the experiment is presented schematically in Fig. 1.

A total of 920 HPLC-DAD measurements were recorded for all samples. Each 'X' in Fig. 1 represents one HPLC-DAD measurement registered for one of the two injections from one of the two infusions prepared for each pasteurized or unpasteurized sub-sample. Specifically, 320 measurements were performed during each of the first two production seasons (2009 and 2010). Only 5 sample batches of grade D rooibos plant material were collected during 2011, and therefore, 40 measurements for those infusions are missing (those marked with 'o' in Fig. 1). For the first two seasons, 160 measurements were characteristic for pasteurized plant material and 160 measurements were recorded for infusions of unpasteurized plant material, while only 140 measurements were performed for each of the two processing conditions for the last production season. Considering two replicate measurements for each rooibos infusion originating from each of the ten sample batches and a definite quality grade (A, B, C or D), a total of 40 measurements were recorded for each type (unpasteurized or pasteurized) of plant material per year, with the exception of grade D samples in 2011 for which a total of 20 measurements per type were recorded.

Such a design of the experiments allowed us to evaluate the effects of three main factors–the production season, pasteurization and quality grade–on the phenolic content of rooibos infusion. Sample batches are nested within all of the combinations of the levels of the quality grade and production season factors. Steam pasteurization and quality grade are fixed factors, while the production season and sample batches are considered to be random factors.

2.2. HPLC-DAD methodology

HPLC analysis was carried out as described by Beelders et al. [13] using an Agilent 1200 system (Agilent, Santa Clara, CA) equipped with DAD (standard 13 μL flow cell and 10 mm path length) to record the UV spectra between 200 and 700 nm. Separation was performed at 37 °C on a 100×4.6 mm^2, 1.8 μm Agilent Zorbax SB-C18 column protected with an Acquity ultra-performance liquid chromatography (UPLC) in-line filter (Waters, Milford, MA) and a 5.0 μm SB-C18 guard

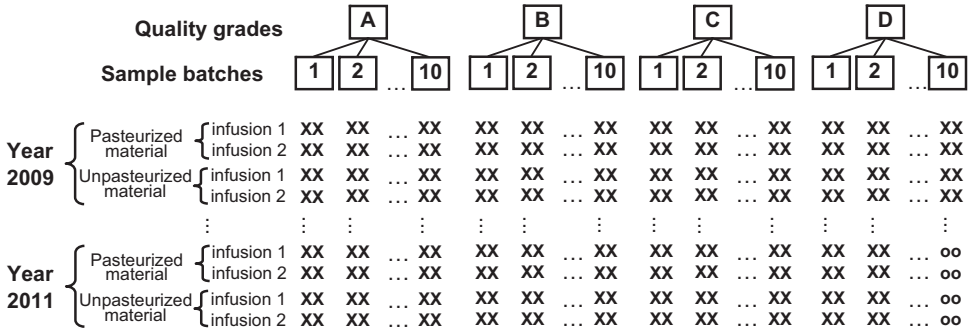

Fig. 1. Scheme of the experimental design with four factors, e.g. production season, steam pasteurization, quality grade (A, B, C and D) and sample batches (1, 2, …, 10) that are nested within the levels of the quality grade and production season factors. Each 'X' represents one HPLC-DAD measurement, while 'o' represents a missing measurement.