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Determination of toxic metals in leather by wavelength dispersive X-ray fluorescence (WDXRF) and inductively coupled plasma optical emission spectrometry (ICP OES) with emphasis on chromium

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Abstract The tanning industry is one of the largest environmental polluters due to high generation waste in all production processes, but the tanning is particularly worrisome due to the use of significant amounts of chromium. Cr is an element potentially toxic to both health and the environment, depending on the concentration and the oxidation state. Cr(VI) can come in contact with human skin when using leather goods, which can cause allergies and dermatitis, besides being carcinogenic. Considering that approximately 90% of the world production of leather is performed with Cr salts, the determination of this element in leather is necessary to avoid exposure to the risks that the element can provide. The main goal of this study was the development of an alternative analytical method for the determination of Cr in leathers (ovine and bovine leather tanned with Cr and vegetable tannin) using wavelength dispersive X-ray fluorescence (WDXRF) for direct solid analysis. Besides performing analysis of the chemical composition and determination of Al, As, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn in leather by inductively coupled plasma optical emission spectrometry (ICP OES). Principal component analysis (PCA) was also used in the evaluation of the WDXRF and ICP OES data sets. WDXRF calibration models for Cr presented satisfactory figures of merit and the analysis of the leathers revealed an alarming concentration of total Cr in the samples reaching 21,353 mg kg^{-1}.

Keywords Cr · Wavelength dispersive X-ray fluorescence (WDXRF) · Leather · Direct solid analysis · Inductively coupled plasma atomic emission spectrometry (ICP OES) · Laser-induced breakdown spectroscopy (LIBS)

Introduction

The tannery industry was one of the earliest industrial activities developed by man (George et al. 2014) and currently provides significant contributions to the world economy. The processing of skins in leathers occurs during the tanning step, where the raw material is converted to a permanent and stable product (Thanikaivelan et al. 2004). This stage is possible with the help of tanning agents, metal sulfates, such as Cr, Al, Ti, and vegetable tannins (Quebracho, Mimosa, Almond, Tara). In finishing operations, pigments, solvents, oils, colorants, and coating products are added to give shine and

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softness (esthetic value) to the leather (Scheffler and Pozebon 2015; Thanikaivelan et al. 2004).

During each stage of the tanning process, a large amount of waste water, solid wastes, volatile organic compounds, and hazardous chemicals are generated (Dixit et al. 2015). These residues are responsible for causing environmental impacts on soil, vegetation, rivers, and sediments, contributing strongly to total dissolved solids, chemical oxygen demand, sulfates, chlorides, gaseous emissions, and toxic metals in the environment (Dixit et al. 2015). In addition, in each step of tanning processes, toxic elements can be incorporated into the leather (Aslan 2009; Aslan and Üzüm 2015; Karavana et al. 2011; Okoh et al. 2013; Scheffler and Pozebon 2015).

The determination of the concentration of toxic elements in leathers is necessary for quality control of the finished product, mainly because each type of tanning provides higher concentration of toxic metals depending on the type of chemical products used. During the leather tanned with Cr, $Cr(OH)SO_4$ is the most common tanning agent. This type of tanning process is more worrisome because high concentration of Cr causes environmental and human health concerns such as dermatitis and allergies (Miksche and Lewalter 1997). In addition, Cr used in leather manufacturing can be oxidized (Fuck et al. 2011) from Cr(III), an essential element for physiological functions (Miksche and Lewalter 1997), to immunogenic, genotoxic and carcinogenic Cr(VI) (Jerschow et al. 2001).

The three most common metals to cause allergy in the general population are Ni, Co, and Cr, and it is estimated that up to 1–3% are allergic to Cr (Thyssen and Menné 2010), that derives mainly from two types of exposition, cement and leather goods. In the last years, Cr(VI) allergy is currently increasing among Danish dermatitis patients due to leather exposure (Hedberg et al. 2014). In a study performed by German Federal Institute for Risk Assessment, it was verified that leather goods like gloves, shoes, or watch straps which come into direct contact with the skin contain high levels of Cr(VI). The study were performed during 6 years and Cr(VI) was detected in more than half of the 850 samples analyzed, and in one sixth of them, the levels were found higher than 10 mg kg^{-1} (Bundesinstitut fur Risikobewertung 2007). At a level of 5 mg kg^{-1} of Cr(VI), half of the sensitized individuals evaluated presented allergic skin reactions (Bundesinstitut fur Risikobewertung 2007), these values exceeding the limit of 3 mg per kg according to recommendations of the ISO 17075 standard (Hedberg et al. 2014; ISO 17075 2007).

Leather industry is one of the major sources for contribution of Cr to the environment (Kumar et al. 2014). Studies dedicated to determination of Cr in leather industry wastewater (Venezia et al. 2008), polluted soil (Gondal et al. 2009), soils near leather industry (Gitet et al. 2013), soil and plant from leather tanning area (Kumar et al. 2014), blood and urine of 44 workers of leather industries (Mignini et al. 2004), and leather (Hedberg et al. 2014; Zhao et al. 2015) have been performed and prove alarming concentrations of total Cr up to 34,246 mg kg^{-1} (Cr-tanned leather) (Zhao et al. 2015); thus, analytical procedures that are able to determine toxic metals with special emphasis on Cr in leather are necessary, mainly considering the environmental and toxicological effects of the element. Analytical methods for leather analysis normally employs mineralization with acids using inductively coupled plasma optical emission spectrometry (ICP OES) (Aslan 2009; Aslan and Üzüm 2015; Karavana et al. 2011) and microwave-induced plasma optical emission spectrometry (MIP OES) (Zhao et al. 2015). In addition, thermal analysis (Bañón et al. 2016; Budrugeac et al. 2011; Sebestyén et al. 2015) has also been employed.

Although the techniques previously mentioned are well consolidated in analytical laboratories, in all cases, sample destruction occurs before or during the analysis. Direct analysis techniques have been rarely quantitatively studied regarding their determination of analytes in leathers. Only a few studies have been published, such as the determination of As, Ca, Cr, Mg, Pb, and S by laser-induced breakdown spectroscopy (LIBS) in fully manufactured, wet blue, and natural leathers (Nasr et al. 2011) or the determination of SiO_2, Al_2O_3, Fe_2O_3, K_2O, CaO, TiO_2, ZnO, CO_2, SO_3, HgO, and P_2O_5 by X-ray fluorescence (XRF) in ancient leathers from the sixth century B.C. (Luo et al. 2011).

Wavelength dispersive X-ray fluorescence (WDXRF) is an attractive technique that does not destroy samples, only a small amount of it is necessary, is multi-elementary, and performs fast analysis. XRF has been used in many leather analysis studies, but most had a qualitative approach (Chaplin et al. 2010; Gómez-morón et al. 2016; Mannina and Lombardo 2013; Nakano et al. 2011; Sano and Suzuki 2009; Vornicu et al. 2015); thus, quantitative methods for direct leather analysis need to be developed.

The main goal of this study was to evaluate and validate WDXRF as candidate technique for direct solid analysis for, mainly, the determination of Cr in leather, the calibration models were proposed taking into account the standard error of validation (SEV). In addition, concentration of essential and toxic hazards elements (Al, As, Ba, Ca, Cd, total Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn) was determined in leather samples tanned with Cr salts and with vegetable tannin using ICP OES and Cr(VI) was determined using UV–Vis spectroscopy. Leather also was discriminated and classified based on their chemical composition with a chemometric approach based in concentrations obtained by ICP OES and spectra obtained with direct analysis by WDXRF. Complementary experiments also were performed by LIBS (Costa et al. 2018) in a qualitative exploratory analysis.

Experimental

Samples

Sixty leather samples were analyzed in the experiments (24 from ovine and 36 from bovine). The leathers were tanned with Cr salts (50 samples) and with vegetable tannin (10 samples), including leather of 9 different colors. This sample variability provides more confidence in the generated models. These leathers were obtained from animals bred at Embrapa Pecuária Sudeste, and the experiments were approved by the Animal Research Ethics Committee of Embrapa Pecuária Sudeste (CEUA/CPPSE, Protocol No. 04/2011 and PRT 02/2015). This committee follows law number 11794 (October 08, 2008) that regulates incise VII of the first paragraph of article 225 from the Brazilian Federal Constitution. This law establishes the procedures for experiments with live subjects (http://www.mct.gov.br/upd_blob/0238/238343.pdf). A detailed description of the samples is shown in Table 1. Leather samples were cut in a hydraulic press (Metalúrgica Aço real, P-23) with cutting knives (3.0 cm × 4.0 cm) before LIBS and WDXRF analyses.

Determination of Al, As, Ba, Ca, Cd, total Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn in leather using ICP OES

Experiments were performed using an iCAP 6000 ICP OES spectrometer (Thermo Fisher, Madison, WI, USA) using axial and radial viewings and argon (99.996%, White Martins-Praxair, Sertãozinho, SP, Brazil) for elemental analysis. Table 2 presents the instrumental conditions and the emission lines used in the determinations using ICP OES.

The leather samples were cut into small pieces (around 5 by 5 mm) with a ceramic knife before analysis and were not ground to prevent contamination by metals from the mill blades. A digester block with PFA-closed vessels (perfluoroalkoxy, Savillex, MN, USA) of 50 mL and equipped with 24 vessels was used for sample mineralization. Samples were digested in triplicate according to the following procedure adapted from Scheffler and Pozebon (2015): 0.200 g of leather was weighed and transferred to the PFA vessel; 3.0 mL of 65% *w/w* HNO_3 (Synth, Diadema, SP, Brazil) was added and kept overnight for approximately 16 h; on the next day, 3.0 mL of H_2O_2 30% *w/w* (Synth, Diadema, SP, Brazil) was added, and the solution was heated at 100 °C for 180 min.

After digestion, the resulting extracts were filtered through quantitative filter paper with slow filtration for fine precipitates (Unifil, Germany), and a final volume of 30 mL was achieved using deionized water (resistivity > 18.2 MΩ.cm). Nitric acid was previously purified using a sub-boiling distillation system, Distillacid™ BSB-939-IR (Berghof, Eningen, Germany).

Aqueous multi-element standard solutions were prepared from stock standard solutions of 1000 mg L^{-1} (Fluka Analytical, Buchs SG, Qhemis, Switzerland) and used to construct calibration curves. All glassware and polypropylene flasks were washed and kept in 10% *v/v* HNO_3 for 24 h.

This procedure was used for the determination of Al, As, Ba, Ca, Cd, total Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn in the leather samples. Other digestion mixtures, such as aqua regia and 50% HNO_3, were also tested; however, insoluble residues remained in the resulting extract solution, indicating incomplete mineralization. The literature presents an official method (ISO 17072-2 2014) that employs a 3:1:1 acidic mixture of HNO_3, H_2SO_4, and $HClO_4$, respectively, for the decomposition of 1.00 g of leather. This method is not recommended for the determination of Cr in Cr-tanned leather. In this case, other official method can be employed using the same acidic mixture (ISO 5398-4 2007). However, H_2SO_4 makes it difficult to analyze using ICP OES due to of instrumental interference from the sample related to high viscosity when compared with other

Table 1 Description of the leather samples used in the experimental part

Sample number identification	Leather type	Tanning type	Color
1–4	Bovine	Chromium	Dark hazel
5–14	Bovine	Chromium	Green
15–18	Bovine	Chromium	Black
19–24	Bovine	Chromium	Light hazel
25, 26, 32, 42, 44, 45, 47, 48, 58	Ovine	Chromium	Light blue
27–31, 40, 41, 43, 46, 49	Ovine	Chromium	Dark blue
33, 35, 37, 57, 60	Ovine	Vegetable tannin	Yellow
34, 36, 38, 39, 59	Ovine	Vegetable tannin	Beige
50–56	Ovine	Chromium	Brown

acids. In addition the remaining SO_4^{2-} in solution can increase emission background from molecular species, such as SO, SO_2, and S_2 (Scheffler and Pozebon 2015). Furthermore, $HClO_4$ is potentially dangerous and should be avoided when possible. Therefore, the HNO_3/H_2O_2 mixture was employed for the digestion of the samples.

Due to the high concentration of Cr in the samples tanned with salts with this element, it was necessary to perform an additional 4-fold dilution; however, the leather samples tanned with vegetable tannin presented significantly lower concentrations of Cr, and no further dilution was required. To accommodate all the samples, a Cr calibration curve from 0.005 to 60 mg kg^{-1} was performed.

Standard solutions were added to 30 samples before digestion to evaluate the recovery of the adopted analytical procedure; thus, were added 1.0 mg kg^{-1} of Al, Fe, Mg, Sb; 0.5 mg kg^{-1} of Ba, Pb, Zn, As, Cd, Ni, Sr, Ti, Cu; 5.0 mg kg^{-1} of Ca to samples. In the case of Cr, 70 mg kg^{-1} was added to the samples tanned with this element and 0.5 mg kg^{-1} of Cr to the samples tanned with vegetable tannin. This procedure was elected due to the fact that no leather certified material is commercially available. In order to avoid acid dilution, the analytes added were divided in several samples.

In addition, determination of Cr(VI) in the leathers was performed using a spectrophotometer (Thermo Fisher, Shanghai, China), in 540 nm wavelength and a quartz cuvette with 1 cm of

Table 2 Operational parameters for ICP OES determinations

Instrument parameter	Operational conditions
Radio frequency applied power (kW)	1.15
Integration time for low emission line (s)	15
Integration time for high emission line (s)	5
Sample introduction flow rate (mL min^{-1})	2.1
Pump stabilization time (s)	5
Argon auxiliary flow rate (L min^{-1})	0.5
Argon plasma flow rate (L min^{-1})	12
Argon nebulizer flow rate (L min^{-1})	0.7
Replicates	3
Elements and wavelengths (nm); view modes: axial and radial^{a}, only axial^{b}, only radial^{c}	Al 308.215^{a} (I), As 193.759^{b} (I), Ba 233.527^{c} (II), C 193.091^{a} (I), Ca 317.933^{a} (II), Cd 226.502^{a} (II), Cr 267.716^{a} (II), Cu 327.396^{b} (I), Fe 238.204^{a} (II), Mg 279.559^{a} (II), Ni 231.604^{b} (II), Pb 220.353^{a} (II), Sb 206.833^{b} (I), Sr 228.200^{c} (II), Ti 336.121^{c} (II), Zn 202.548^{a} (II).

I, atomic lines; *II*, ionic lines

optical path. The adopted procedure was previously proposed by Oliveira et al. (2013).

WDXRF analysis and calibration

All samples were directly analyzed using a Perform-X ARL wavelength dispersive X-ray fluorescence instrument (Thermo Fischer, Madison, WI, USA). The samples were irradiated with X-ray emission provided by a Rh tube with a maximum of 4200 W. Moreover, the X-rays emitted from the electronic transitions of the elements present in the samples are recorded by the optical section of the equipment, passing first through collimators, and then they are reflected by specific crystals. Finally, the detectors register the counts per second of the X-ray emission lines that correspond to each crystal used. There are five crystals that can be used in the wavelength dispersion, and the choice is based on which crystal can disperse the characteristic wavelength from the element studied. The instrument is also equipped with four different collimators (0.15, 0.40, 1.00, and 2.60 mm) and two detectors: a flow proportional counter (FPC) and a scintillation counter (SC). For all elements evaluated in this study, only the electronic transition, Kα, was considered.

In order to build univariate calibration models, Microsoft Excel was used for data organization and calculations. MATLAB version 2017b (The Mathworks, Natick, MA, USA) was used to calculated signal-to-background ratio (SBR), signal area, and height for WDXRF spectra obtained for Al, Ca, Cr, Fe, Ti, and Zn. These elements were chosen based in the results of concentrations obtained previously by ICP OES and considering the sensitivity of WDXRF.

Calibration models were calculated using signal area and height for each six mentioned analytes. The predictive capabilities of WDXRF were based on SEV for all tested elements and the lowest values were used to select the best regression models (Augusto et al. 2017). The dataset composed by 60 samples was split in 40 samples for calculation of calibration models and 20 samples for validation. These samples were chosen at random.

The SEV values were calculated according to Eq. 1:

$$\mathrm{SEV} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n-1}} \qquad (1)$$

where y_i is the reference concentration determined by ICP OES, $\hat{y}_i$ is the predicted analyte concentration for dataset validation, and n is the number of samples used in the validation data set (20 in this case) (Augusto et al. 2017).

Limit of detection (LOD) and limit of quantification (LOQ) for WDXRF were calculated according to IUPAC recommendations (IUPAC 2017). The three sigma IUPAC method was used to express LOD values, and LOQ was estimated as 3.3-fold the LOD values. LOD and LOQ were calculated using the standard deviation (SD) of square root of the sum of the variance of the noise signal near each analyte signal. This process was repeated for each analyte. Analytes concentrations obtained after sample digestion and ICP OES determinations were used as reference values to calculate the WDXRF calibration models.

Principal component analysis

Exploratory analysis using PCA was calculated to evaluate the three techniques used in this study (ICP OES, LIBS, and WDXRF) considering its abilities to differentiate the samples according to the type of leather (ovine and bovine), type of tanning (Cr or vegetable tannin), and color of the leather.

The first PCA was obtained using a data matrix of 60 samples (rows) and the average concentration of each analyte, except Cd because was below LOD (14 columns), as determined using ICP OES. As discrete variables (concentrations) were employed, the data set was autoscaled. In this case, the average and standard deviation of each analyte were calculated. The original values were subtracted and divided by the average and standard deviation, respectively. The variables in the autoscaled matrix have average and standard deviation 0 and 1, respectively.

A complementary analysis was performed using the LIBS technique to evaluate the spectral profile of the studied leathers with a qualitative approach. LIBS experiments were conducted using a J200 (Applied Spectra, Fremont, CA, USA) Q-switched Nd:YAG laser at 1064 nm and 100 spectra were recorded for each sample in raster mode. The instrumental conditions used were a laser pulse energy, spot size, and delay time of 90 mJ, 50 μm, and 1.9 μs, respectively (Neiva et al. 2016; Neiva and Pereira-Filho 2018). The second PCA was calculated using a data matrix composed by 60 samples (rows) and 12,288 variables (columns) obtained from the LIBS spectra (emission lines from 186 to 1042 nm). The dataset was normalized by individual area and mean-centered.

The third PCA was calculated using a data matrix of 60 samples (rows) and 450 variables (columns) obtained via WDXRF spectra, and the dataset was only mean-centered. Pirouette version 4.5 (Infometrix, Bothell, WA, USA) was used for all PCA calculations.

Results and discussion

Determination of Al, As, Ba, Ca, Cd, total Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn in leather by ICP OES

The concentration (mean ± standard deviation, $n = 3$) of elements determined in the samples after digestion is shown in Tables S1 and S2 in the Supplementary Material and their ranges of concentrations were in milligrams per kilogram: 94–1011 for Al; < LOD–13 for As; < LOD–40 for Ba; < LOD–3348 for Ca; < LOD for Cd; 10–23,407 for total Cr; < LOD–15 for Cu, 32–831 for Fe; < LOD–1126 for Mg, < LOD–5 for Ni, < LOD–25 for Pb, 31–318 for Sb; < LOD–9 for Sr, < LOD–6 for Ti, and < LOD–186 for Zn.

These concentrations are comparable to the results obtained by other studies dedicated to leather analysis that also used ICP OES (Aslan 2009; Aslan and Üzüm 2015; Karavana et al. 2011) with the exception of As and Cd because values were below those found in the literature (< LOD–0.74 for As and < LOD–1.76 mg kg^{-1} for Cd).

As expected, the Cr concentrations were extremely high in the leather samples tanned with salts that present this element, ranging from 8775 to 23,407 mg kg^{-1}; thus, special care must be taken with the disposal of this type of material in the environment. Strategies for greener environment towards sustainable development in leather industry have been applied (Kanagaraj et al. 2015), such as the immobilization of Cr ion present in the ash from the incineration of chromium-tanned leather shavings by means of vitrified ceramic bodies (Basegio et al. 2009). Production of basic Cr sulfate by using recovered Cr from ashes (Dettmer et al. 2010) or the incorporation of chromium-tanned leather residue to asphalt micro-surface layer (Krummenauer and de Oliveira Andrade 2009).

The Cr concentration range in the leather tanned with vegetable tannin (samples marked with an asterisk in Tables S1 and S2 in the Supplementary Material) was from 10 to 285 mg kg^{-1}; however, this concentration of Cr was higher than expected for this type of tanning and may be related to the contamination by machines and tools employed in the tanning process.

The Cr results are comparable with those obtained by (Scheffler and Pozebon 2015) which concentration obtained was in the range of 100 mg kg^{-1} (vegetable tanned) to 30,000 mg kg^{-1} (Cr tanned) using ICP OES. In another study (Zhao et al. 2015), Cr concentration ranged from 36 to 34,246 mg kg^{-1} for leather samples using MIP OES as analytical technique.

Tables S1 and S2 in the Supplementary Material also show that in leathers tanned with vegetable tannin, the Ti concentration was high, whereas the As concentration was lower when compared to samples tanned with Cr. The Sb concentration was high in all samples, indicating possible use of dyes containing this element; however, the concentrations of Cu, Ni, Pb, and Sr were low in most samples.

The concentrations of the elements differed between bovine and ovine leather in some cases; for example, the Al, Mg, Ca, Ti, and Cu concentrations were higher in ovine leathers (samples from 25–60) than in bovine (samples from 1–24), but Pb was only found in bovine leather samples. A comparison of each element in leather from different studies is not easy because concentrations vary significantly due to different products used in the process of tanning, finishing, and dyeing. In addition, several sources of these elements can be found in leather samples, as salts that were added for tanning, such as metal sulfate, dyes, pigments, water, pesticides, fungicides, animal feed (bioaccumulation), impurities of industrial chemicals, among others (Aslan 2009; Aslan and Üzüm 2015; Karavana et al. 2011; Okoh et al. 2013).

In addition, Tables S1 and S2 in the Supplementary Material show that high concentrations of potentially hazardous elements such as As, Cr, Cu, Ni, Pb, and Zn were determined on leathers. These elements are toxic contaminants to human health and the environment, so the handling, use, and disposal of leathers must be done in a conscious manner.

The recovery of the procedure for determining Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Ni, Pb, Sb, Sr, Ti, and Zn in leathers was verified, and the results are shown in Table S3 in the Supplementary Material. Recoveries were in the range of 74 to 100% for all elements in the samples. These results show that the reference method developed using ICP OES and decomposition in HNO_3/H_2O_2 in a digester block with PFA-closed vessels showed accurate results, as confirmed by the recovery tests.

PCA evaluation for ICP OES data

To obtain a global picture of all chemical elements in the samples and due to the fact the data sets obtained were related to three different techniques used in this study (ICP OES, LIBS, and WDXRF), three PCAs were separately calculated. In the case of ICP OES data set, elements concentrations were obtained and the variables were autoscaled. Figure 1 shows the scores (Fig. 1a–c) and loadings (Fig. 1d) plot for the ICP OES data for PC1 versus PC2, and both explain a total variance of 53%.

Figure 1a–c shows the scores with different classes highlighted. From Fig. 1a, a differentiation between the two leather groups (bovine, squares and ovine, circles) was observed, and PC1 discriminates ovine and bovine leathers with positive and negative scores, respectively. Figure 1b shows that PC1 discriminates leather tanned with vegetable tannin (triangles) with positive scores and leather tanned with Cr (squares) with negative scores.

Figure 1c shows the same results, but with each color of leather in evidence. A remarkable difference among the colors was not clearly perceivable, but it was possible to associate yellow (half tone diamonds) and beige (open squares) colors with Ti, Sr, Ni, and Fe (see loadings at Fig. 1d). Brown leathers (black circles) were related with As, Cr, and Sb elements. The samples with other colors are mixed or few were obtained for each one. Unfortunately, information about the dyes composition used in the leather preparation was not available, compromising an adequate evaluation between the color of the dye added to the leather and the metals contained in those dyes.

Figure 1d shows the loading plot of the first two PCs for the analytes. The difference between the leather type is due to ovine leather (Fig. 1a) has a correlation with Fe, Ni, Sr, Ti, Ba, Al, Ca, Cu, and Mg, whereas bovine leather has a correlation with Cr, Sb, As, Pb, and Zn. Leathers tanned with Cr (Fig. 1b) were associated to Cr (as expected), Sb, As, Pb, and Zn and leathers tanned with vegetable tannin were related to Fe, Ni, Sr, Ti, Ba, Al, Ca, Cu, and Mg.

Scores plot for PC1 versus PC3 (total variance of 51%) and PC2 versus PC3 (total variance of 22%) datasets are show in Fig. S1 in the Supplementary Material. Principal component 1 (PC1) versus PC3 presented a separation between bovine and ovine leather and samples tanned with Cr and vegetable tanning. On the other hand, PC2 versus PC3 did not presented any remarkable characteristic. This additional PC (PC3) did not bring further information about the color either.

Direct analysis

A complementary analysis was performed and Fig. 2a shows representative LIBS spectra obtained for three leathers: bovine and ovine tanned with Cr and ovine tanned with vegetable tannin. The most intense signals and their corresponding emission lines are listed in Table 3. The three leathers presented Cr, C, Si, Mg, Al, Ca, Ti, Na, N, K, O and the spectra of these samples showed in Fig. 2a were similar, and basically, the only element that differed was Cr. However, for the samples tanned with Cr, there were 11 very intense emission lines for Cr (267.71 (II), 357.86 (I), 359.34 (I), 360.53 (I), 425.43 (I), 427.48 (I), 428.97 (I), 464.61 (I), 465.21 (I), 520.60 (I), 520.84 (I)). In the case of the sample tanned with vegetable tannin, only a small signal for Cr emission line 302.15 (I) nm was observed. In addition, with the help of Table 3, it is possible to see the capability of LIBS in order to presents dozens of emission lines in few minutes of analysis even for light elements as Al and C.

PCA calculated using the entire LIBS emission lines (Fig. S2 in the Supplementary Material) did not show significant separations between leather type, tanning type process, or among colors. In this case, a new PCA was calculated using only the 11 Cr emission lines described before, but the reliable information observed was an expected difference between the samples tanned with Cr and vegetable tanning (see Fig. S3 in the Supplementary Material). As preliminary conclusion, the use of LIBS permitted to perform an initial chemical inspection of the solid samples with detection of several chemical elements.

The 60 leather samples also were directly analyzed via WDXRF. The samples were positioned in specific sample holders with an aperture of a 29-mm diameter. For all elements evaluated, the electronic transition Kα 1 and Kα 2 were considered of the most sensitive lines. A collimator of 0.4 mm and a count time of 1 s were used in the experiments. The analytes required two different operational conditions: 30 kV of voltage and

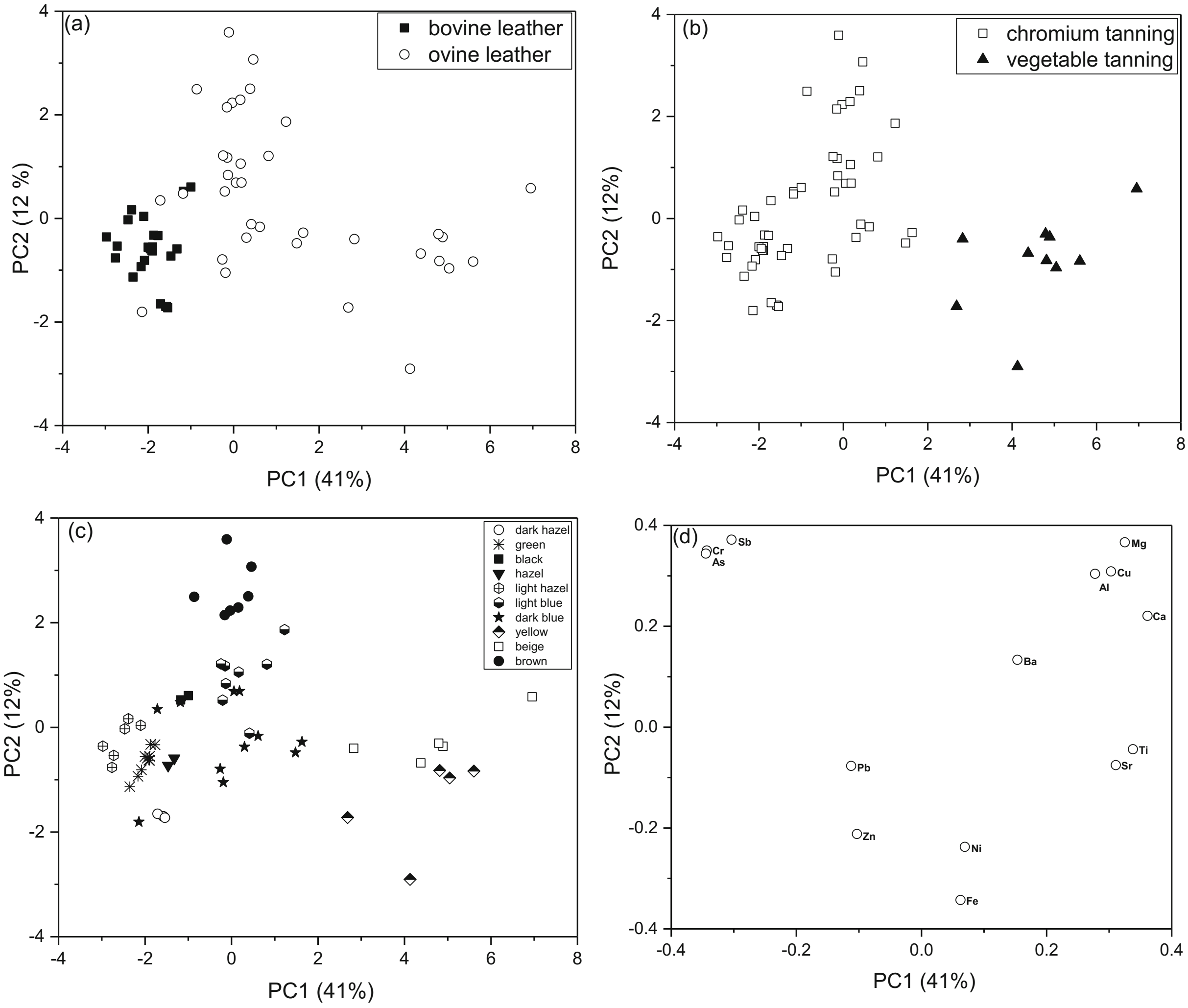

Fig. 1 Scores for leather type (**a**), tanning type (**b**), leather color (**c**), and loadings plot (**d**) for dataset obtained by mineralization and determinations via ICP OES

80 mA of current for Al, Ca, Mg, Na, S, and Si, and 50 kV and 55 mA for Cr, Fe, Ni, Zn, and Ti.

Figure 2b shows X-ray emission wavelengths from ten elements present in the three spectra of leathers evaluated: Zn, Fe, Cr, Ti, Ca, S, Si, Al, Mg, and Na. As expected, the XRF spectra present less signals than LIBS. Since there are two tanning processes, one with Cr and another with vegetable tannin, the signal intensities for Cr are different in each case (third signal from left to right in Fig. 2b). Additionally, the tanning process with vegetable tannin is free of Cr; thus, a small signal intensity for Cr was observed for this process. The emission of the other assessed elements varied from sample to sample due to their composition.

A PCA for WDXRF data was calculated and Fig. 3 shows the score plots from PC1 versus PC2, separated by bovine and ovine leather (a), Cr and vegetable tanning process (b) and different colors of leathers studied (c), and the loads from PC1 and PC2 (d and e). As shown in the Fig. 3a, bovine and ovine leathers have different elemental compositions and are mainly grouped by the presence of S and Si in the samples (see Fig. 3e), PC2 discriminate bovine leather with negative scores. In Fig. 3b, the grouping is due to the tanning process, and Cr in positive values in PC1 is the element responsible for the grouping. Finally, in Fig. 3c, the samples were separated by their color. Beige leathers (open squares) and yellow (half tone diamonds) were associated to Zn, Fe, Ca, Si, Al, and Na (negative values

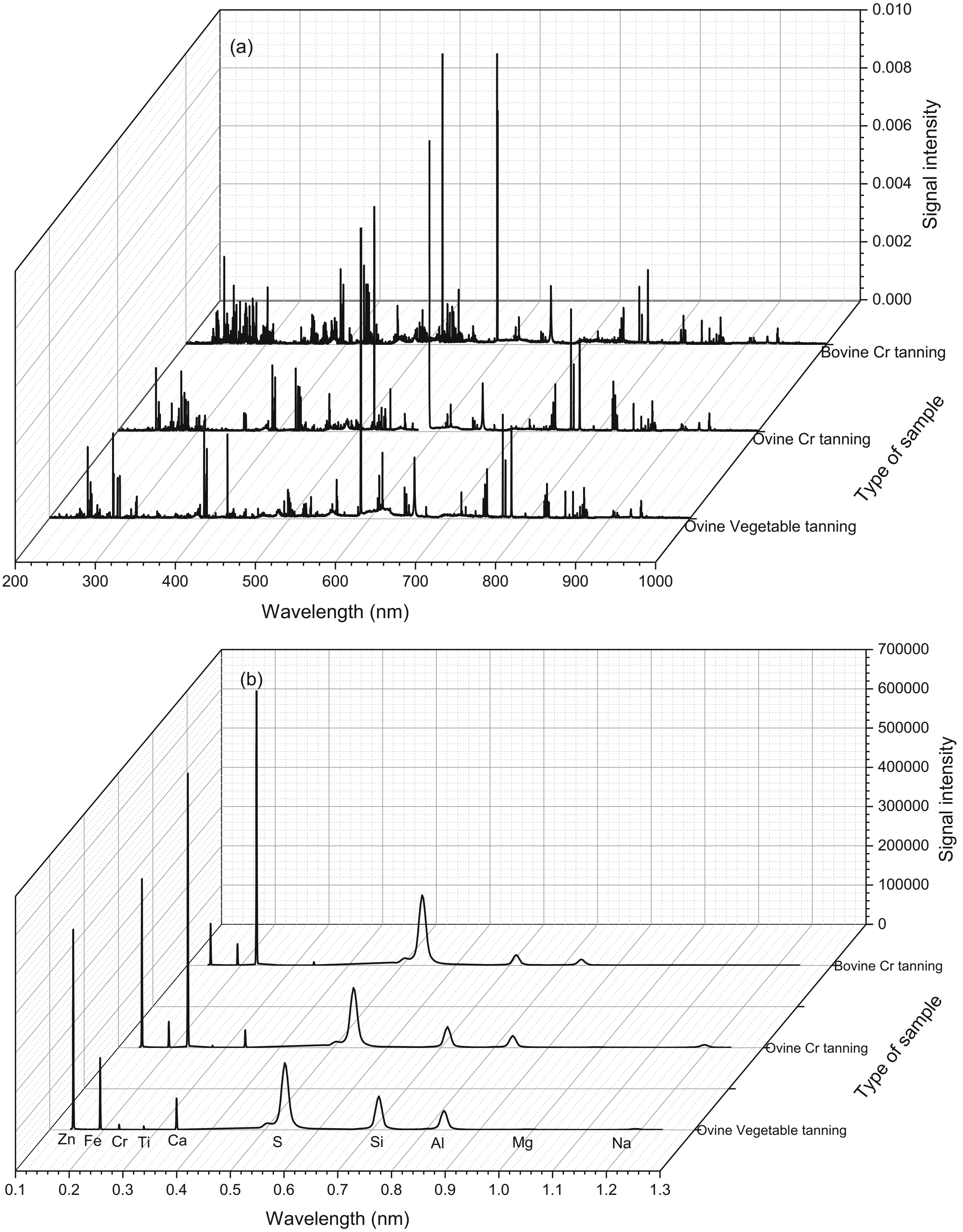

Fig. 2 Typical spectra obtained via LIBS (**a**) and WDXRF (**b**) for three leather samples (bovine and ovine tanned with chromium and ovine tanned with vegetable tannin)

Table 3 Emission lines present in the spectra of all leather samples analyzed

Elements	Emission lines (nm)
Cr	267.716 (II), 357.869 (I), 359.349 (I), 360.533 (I), 425.435 (I), 427.480 (I), 428.972 (I), 464.617 (I), 465.216 (I), 520.604 (I), 520.844 (I)
C	193.091 (I), 247.856 (I)
Si	250.690 (I), 251.920(I), 252.851 (I)
Mg	279.553 (II), 280.270(II), 285.213 (I)
Al	308.215 (I), 309.271 (I)
Ca	393.366 (II), 396.847 (II), 422.673 (I), 558.197 (I), 558.876 (I), 559.447 (I), 559.850 (I), 610.272 (I), 612.216 (I), 616.216 (I), 643.907 (I), 854.209 (II)
Ti	498.173 (I), 499.107 (I), 499.952 (I)
Na	588.995 (I), 589.593 (I), 819.483 (I)
N	742.368 (I), 744.230 (I), 746.831 (I), 818.483 (I), 818.802 (I), 821.630 (I), 822.312 (I), 824.235 (I)
K	766.490 (I), 769.896 (I)
O	777.421 (I), 844.639 (I)

I, atomic; *II*, ionic lines

in PC1). Brown leathers (black circles) were related to Cr (positive values in PC1). The other colors are mixed and it is not possible to observe a clear pattern.

Determination of total chromium in the leathers using WDXRF: univariate calibration models

Calibration curves for Al, Ca, Cr, Fe, Ti, and Zn using WDXRF were obtained for 60 samples (40 for calibration and 20 for validation). In order to calculate the calibration models, SBR, signals areas, and heights for each analyte were generated and associated with the reference values obtained with ICP OES. Usually quantitative methods using XRF require a reference technique or consolidated method, such as ICP OES (Augusto et al. 2017; Guerra et al. 2014; Marguí et al. 2010; Silveste et al. 2016), inductively coupled plasma mass spectrometry (ICP-MS) (Marguí et al. 2010), or flame atomic absorption spectrometry (FAAS) (Bilge et al. 2016), to develop the most appropriate calibration method that can be univariate (single signal) or multivariate (several signals).

To evaluate the quality of the proposed models, SEV, LOQ, and LOD values were calculated for univariate calibrations for each mentioned analyte. However, the calibration that resulted in reliable predictive capacities, such as correlation coefficients above 0.9 and low SEV values, was only those for Cr.

Thus, some attempts to improve the calibration models for other analytes (Al, Ca, Fe, Ti, and Zn) were performed, such as follows:

(a) Separating the samples into four groups by type of leather (bovine and ovine) and by type of tanning (Cr and vegetable tannins) and calculate a calibration model for each group;
(b) Calculating the partial least squares (PLS) by software Pirouette using the area of all analytes together as a data matrix and the reference concentrations of the analytes obtained by ICP OES as a dependent variable (y); the same was performed using the signal heights; and
(c) Normalizing the area and height signals of each analyte by the area and height signals of Cr, Si, Zn, Fe, and S (analytes found at high concentrations) to minimize interference.

However, none of these attempts resulted in positive results in order to build a calibration model for the analytes described previously. Thus, the proposed method proved to be successful only for determination of Cr in the leather samples using WDXRF by direct analysis.

The figures of merit for the best results (lower value of SEV) for the calibration model obtained for Cr are shown in Table 4 that include in the first row the equation for the calibration curve (univariate linear model) when reference Cr concentrations obtained by ICP OES and signal height obtained by WDXRF were compared,

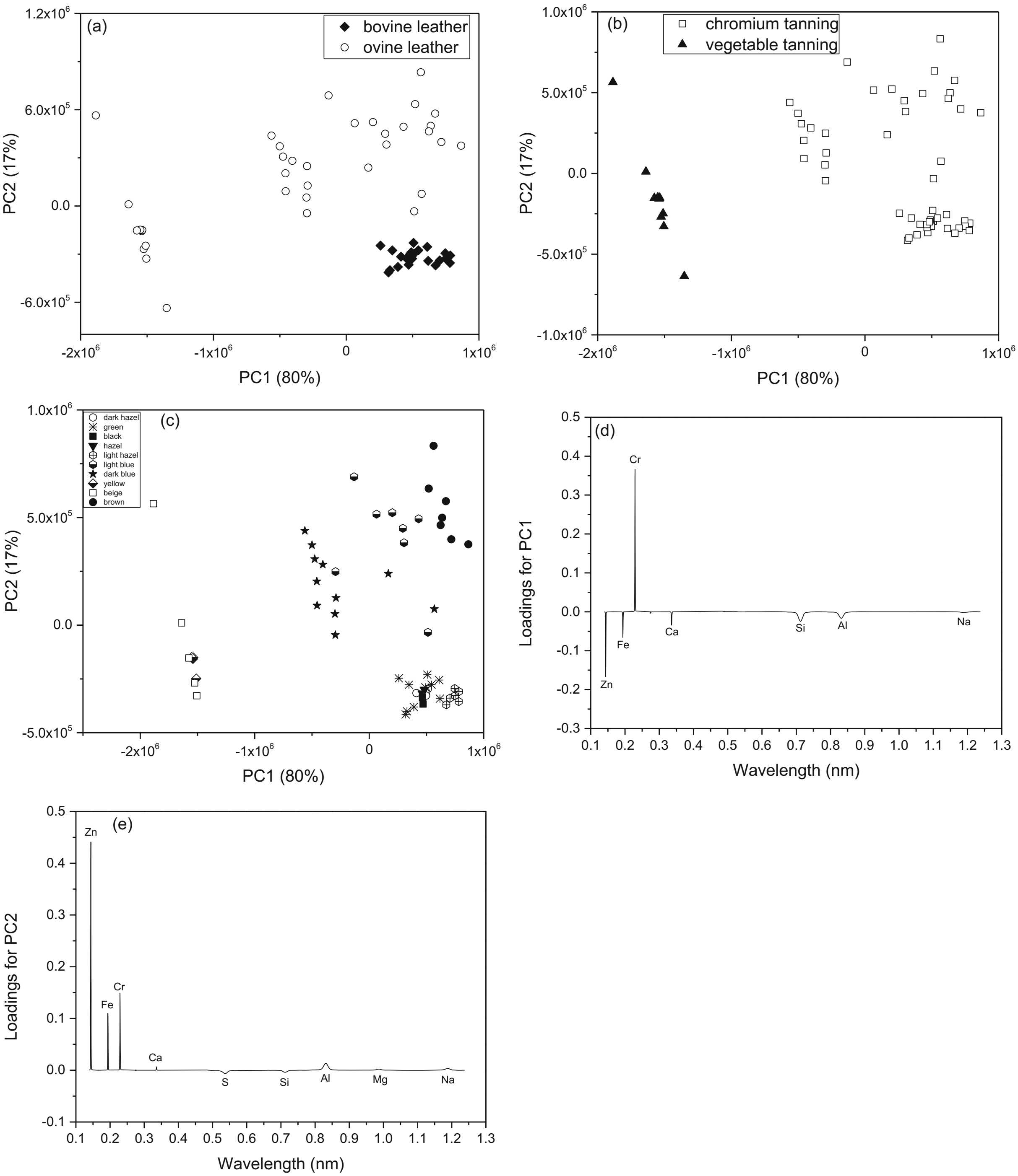

Fig. 3 Scores for leather type (**a**), tanning type (**b**), leather color (**c**), and loadings for PC1 (**d**) and PC2 (**e**) plots for datasets obtained from direct analysis via WDXRF

followed by the correlation coefficient from the linear calibration model, which was of 0.9901, standard error of calibration (SEC) of 1279 mg kg^{-1}, and SEV of 628 mg kg^{-1}.

Table 4 also shows that the Cr concentration range for 60 leather samples (fifth row of the Table 4) varied from not detected to 21,638 mg kg^{-1}. In this case, not detected means values below the SEV. The Cr

Table 4 Figures of merit for the WDXRF calibration model calculated for Cr

Calibration curve (coefficients ± standard deviation)	[Cr] = (43.9 ± 1.0) x + (19,583 ± 14,600)
R	0.9901
Standard error of calibration (SEC) (mg kg^{-1})	1279
Standard error of validation (SEV) (mg kg^{-1})	628
Cr concentration range (mg kg^{-1})	Not detected to 21,638
Wavelength (nm)	0.229
LOD (mg kg^{-1})	30
LOQ (mg kg^{-1})	100
Signal type	Height

concentrations in the leather samples tanned with vegetable tannins were below the SEV value of 628 mg kg^{-1} (10–285 mg kg^{-1} measured by ICP OES). Thus, it was not possible to predict the concentrations of these samples with acceptable accuracy impairing its calibration model; therefore, the proposed method can be used to successfully determine Cr in Cr-tanned leather, which normally contains Cr concentrations above SEV. Although SEV value is high, the concentrations of Cr in the leather samples are often higher, in the order of percentage, and do not compromise the procedure developed.

In addition, the height was the signal type that obtained best results for calibration models when compared with signal area. Table 4 present also values of LOD (30 mg kg^{-1}) and LOQ (100 mg kg^{-1}) for WDXRF measurements. In general, the method developed presented appropriate figures of merit for the univariate calibration models calculated for Cr.

The predictive capabilities of the calibration model based on SEV were appropriate for the determination of Cr via WDXRF and can be evaluated by Fig. 4, which show satisfactory correlation for samples used in calibration (open circle) and validation (black circle). This figure shows good concordance between the Cr concentration reference obtained via ICP OES versus the Cr concentration predicted via WDXRF.

In addition, Table S4 in the Supplementary Material shows the concentrations of Cr determined using ICP OES (reference) and WDXRF (proposed method) for ovine and bovine leathers. The comparative results with ICP show that good predictions were obtained using proposed method for determination of Cr, in the 50 leathers Cr-tanned analyzed that ranged from 9690 to

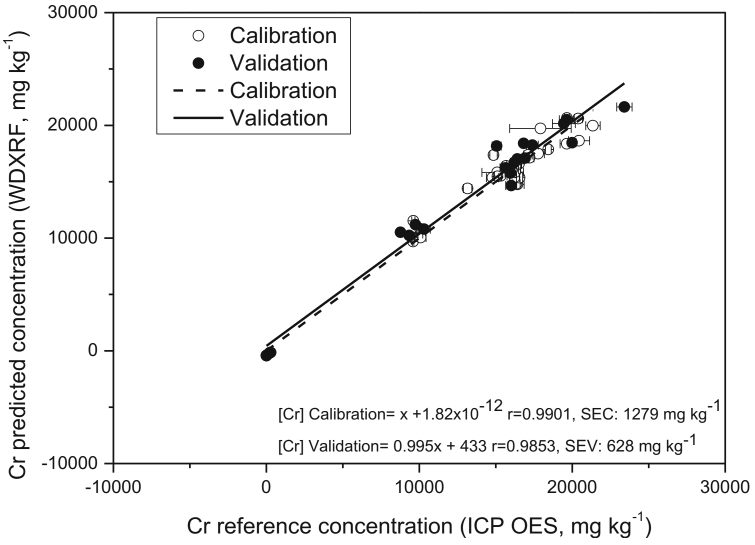

Fig. 4 Comparison between reference concentrations (ICP OES, mean ± standard deviation, *n* = 3) and predicted concentrations obtained from WDXRF (mean) for Cr in mg kg^{-1} for the calibration and validation samples

21,638 mg kg^{-1} (see Table S4 in the Supplementary Material).

In addition, determination of Cr(VI) in leather using UV–Vis spectroscopy was performed and the concentrations of Cr(VI) determined in the bovine and ovine leather samples tanned with Cr salts presented high values, varying from 740 to 2250 mg kg^{-1}. These values are extraordinarily high and worrying since exceeding the limits set of European Union that established maximum concentration of Cr(VI) (3 mg kg^{-1}) (ISO 17075 2007; Van Beik et al. 2017). Leather samples tanned with vegetable tannins were also analyzed and Cr(VI) concentrations were not detected because they were below the LOQ of the proposed method.

Conclusions

The reference method developed using ICP OES and decomposition in HNO_3/H_2O_2 in a digester block with PFA-closed vessels showed accurate results, as confirmed by the recovery tests. The concentrations of the elements in the leather vary according to the type of leather (ovine or bovine), the type of tanning product used (Cr or vegetable tannin), and the dye added to color the leather. Potentially hazardous elements such as As, Cr, Cu, Ni, Pb, and Zn were determined in high concentrations and these differences in concentrations were visualized clearly using PCA based on data obtained via ICP OES and WDXRF. Most of these elements are dangerous to humans and the environment due to high toxicity and can cause allergies, dermatitis, and cancer.

There are few studies on the quantitative determination of Cr in leathers via direct analysis techniques. The proposed method proved to be fast and effective for the determination of Cr in leathers tanned with salts containing this element. However, further studies need to be performed to determine other analytes, such as Al, Ca, Cr, Fe, Ti, and Zn via WDXRF. Additionally, it is important to develop mechanisms to reduce the SEV for the determination of Cr in leather tanned with vegetable tannins at a low concentration of this element.

Total Cr concentration obtained by WDXRF was compared with a reference method using ICP OES and the results showed that the proposed method present satisfactory figures of merit and the maximum concentration of total Cr was a high value of 21,353 mg kg^{-1}. WDXRF is an attractive technique because it is nondestructive, presents high analytical frequency, and performs multi-element analysis with no chemical residue generation.

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