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Applying multivariate analysis to characterize *waragi* spirits from Acoli, Uganda, by their metal contents

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Abstract

Quality control during spirits production and means of authenticating or verifying sources of spirits in the sub-Saharan region of Africa are limited due to lack of resources and the scientific acumen required to develop methodologies for characterizing spirits. However, the increasing needs to protect consumers from negligence, or willful contamination of spirits in this region underscores the urgency with which growth in this area must happen. In this paper, we describe a multivariate statistical framework upon which characterization, identification and authentication of spirits could be developed. The framework exploits the unique chemical fingerprints of spirits with the goal of accomplishing three functions simultaneously: the detection of class differences, the authentication of spirits and the verification of sources. In a test case using the metal contents of 17 Ugandan spirits, this framework shows (i) that a class of unrecorded spirits known locally as *Lira-Lira* can be singled out from other spirits by their Cu contents, (ii) that localities from where the *Lira-Lira* spirits were purchased can be resolved to within 8 km by cluster analysis and principal component analysis,

(iii) that cluster analysis loadings and scores, placed side-by-side, can pair spirits and their unique discriminating contaminants directly, (iv) that the most important metals for authenticating 13 spirits, source verification and production methods are Al, Sr, Ba, Mn, Zn and Cu (high concentration variability across samples is the qualifying factor), (v) that common sources of contamination can be detected by Pearson correlation analysis (this study finds that Sn/Cd, Pb/Cr, Tl/Cr, Pb/Ni or Cu/Ag as well as the triad Se/As/Ni in the 13 Ugandan spirits are from similar sources), and (vi) that inconsistency in spirits production can be detected with empirical data. Such rudimentary solutions to characterizing spirits have never been offered to the sub-Saharan countries.

Keywords: Food science, Food analysis

1. Introduction

The regulation of domestic alcohol production and consumption in the sub-Saharan region of Africa is in a crisis due to the lack of basic elements required to make informed decision. These basic elements of control, which usually include having a functioning alcohol production policy (to require the alcohol industry to assure quality and public health), an alcohol consumption policy (to educate the public about responsible use of alcoholic products), and a policy enforcement mechanism duly supported by scientific and technical capacities (to keep stakeholders in check) are mostly absent. In Uganda for example, the scientific and technical knowhow required for monitoring the quality of alcoholic beverage, or just for understanding the characteristics of beverages sold to the public are beyond the proverbial reach of the country (Ferreira-Borges et al., 2017). In this scenario, the necessary task of combatting negligence and, at times, outright fraud in the blending and packaging industry (let alone public education about the danger of alcohol consumption) therefore becomes a superficial exercise of saving face (Lachenmeier et al., 2007). Unfortunately, the brunt of this incapacity is mainly borne by a segment of the African society which can ill afford better quality spirits to service their alcohol addiction. Consequently, it is not surprising that incidence of alcohol poisoning is increasing in this population (Adelekan, 2008). For this reason, solutions in all forms (be it scientific, technical or political) are required to provide relief. In this paper, we propose a temporary scientific measure which simply requires harnessing the power of multivariate statistics to explore the levels of residual chemical spirits contamination for answers. We believe that such an approach could help in a larger context to document the characteristics of spirits which can be used to discriminate among the spirits, to authenticate sources of spirits as has been shown with Scotch whiskies (Adam et al., 2002) and to combat fraud in the food industry in general (Fiorino et al., 2018). This approach has also been used successfully to differentiate Spanish

red wines from one another and Spanish brandies similarly (Núñez et al., 2000; Cameán et al., 2001).

Over the last 20 years, metals have provided avenues for authenticating spirits because of their chemical stability in spirits (Núñez et al., 2000; Ibanez et al., 2008). Copper in Scotch whisky for example, has been used to discriminate between a malt whisky and a blended whisky (Adam et al., 2002). In multivariate space, metals have also been used to discriminate among beverages by their origin, quality, variety and type (Núñez et al., 2000; Kokkinofta et al., 2003). The metal content of *zivania* (a Cypriot unrecorded drink) for example, has been used to discriminate this locally treasured drink from related products (Kokkinofta et al., 2003).

In the current study, the potential of using multivariate statistics similarly is explored. We examine the metal contents of 17 spirits from Uganda in a multivariate space for similarities and differences with the hope of characterizing, discriminating among spirits brands and beginning the process of cataloging those differences. Uganda, a sub-Saharan country, provides a perfect example of a country with a population struggling to overcome a high mortality rate due to excessive consumption of contaminated alcohol, but without scientific support and acumen needed to address the problem. The country is awash with poorly regulated spirits of questionable qualities, most of which differ superficially from one another by the labels on their packaging containers only or by the amounts of impurities they contain. The manufacturing of these spirits are not only shrouded in secrecy, but has regulatory laxity built into their production and distribution as well. This lack of a strong regulatory authority, when combined with lack of technical knowhow to back alcohol policies, has led to unrestrained consumption of spirits in the country. Indeed, not long ago, Uganda was recognized as the world leader in alcohol consumption – at over 19–24 L of consumption per capita over a 10-year period (WHO, 2004; WHO, 2014; disputed by Kabwama et al., 2016).

2. Materials and methods

The key components of the multivariate experimental design used in this study are shown in Fig. 1. The details of each component are provided below.

2.1. Sampling

Seventeen (17) Ugandan spirits purchased from the study area (Fig. 2) were examined in this study. Thirteen (13) of these samples were recorded spirits (i.e. factory distilled, blended and packaged), and four (4) were unrecorded *Lira-Lira* spirits. (In Uganda, *waragi* is the general term used to describe any distillation product of fermentation; regionally, *waragi* is also known as *Lira-Lira* or *arege* in northern Uganda and *Kasese-Kasese* in western Uganda). For logistical and safety reasons,

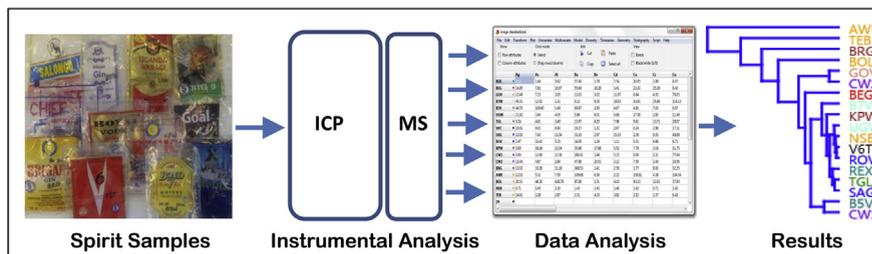


Fig. 1. A schematic diagram of the experimental design used to characterize the 17 *waragi* spirits from Acoli, Uganda.

sampling was done without attempting to pick true random samples but with as much diversity as possible on a first encounter basis. The 13 100-mL sachet brands were Big 5 Vodka (B5V), Beckham Spirit (BEG), Bond 7 Whisky (B7W), Brigade Spirit (BRG), Chief Waragi Spirit (CW1 and CW2, duplicates sampled one year



Fig. 2. The locations of the study area in Uganda. Bolo Trading Center (BOL: 2° 44' 56"N and 32° 46' 45"E), Awere Trading Center (AWE: 2° 41' 17"N and 32° 47' 33"E), Teso Bar (TEB, a suburb of Lira Town: 2° 15' 24"N and 32° 54' 07"E) and Nsambya (NSB, a suburb of Kampala City: 0° 17' 39"N and 32° 35' 20"E).

apart), Goal Vodka (GOV), Kick Spirit Pineapple Waragi (KPW), Relax (REX), Royal Vodka (ROV), Salongo Spirit (SAG), Uganda Waragi (UGW), and V6 Tangawizi Vodka (V6T). For comparison, undocumented spirits (*Lira-Lira*) were also collected in duplicates in 50-mL Falcon screw-capped polypropylene conical centrifuge tubes (Fisher Scientific, Waltham, MA, USA) from three study areas Bolo (BOL), Awere (AWE), and Teso Bar in Lira (TEB), and from a Kampala suburb (NSB, Nsambya Police Barack).

2.2. Samples preparation

To prepare samples for ICP-MS analysis, 20 mL of each spirit was mixed with 0.4 mL of 50% nitric acid and 0.2 mL of 50% hydrochloric acid (TraceMetal Grade, Thermo Fisher Scientific, Waltham, MA, USA) in a digestion tube and refluxed on a heating block (Environmental Express, Charleston, SC, USA) at 93 °C for 2 h. The digested samples were cooled, filtered (No. 40 Whatman filter paper, Thermo Fisher Scientific, Waltham, MA, USA), and adjusted with distilled/deionized water back to 20 mL for analysis using a Thermo Scientific X-Series 2 ICP-MS (Thermo Electron North America LLC).

To ensure that Falcon tubes into which samples were transferred were not themselves introducing extractable metals into samples, two fresh tubes were filled with a 50/50 (v/v) mixture of 100% ethanol/distilled water and incubated for three months, the contents of which were analyzed as regular samples. A well characterized Scotch whisky (The Glenlivet (TGL); Adam et al., 2002) purchased in San Diego, California (USA) was also included as a ‘certified reference material.’ To ensure control over the reference conditions under which trace amounts of metals could be detected with 99% confidence in ethanol, a 50/50 (v/v) mixture of 100% ethanol/distilled water was used as a blank sample in each batched analysis, and as a platform for laboratory control spiked duplicate samples.

2.3. Instrumental analysis

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS), a robust analytical technique for addressing issues associated with contamination and adulteration of food and drinks (Nardi et al., 2009; Voica et al., 2009; Pedersen et al., 2016), was used similarly in this study. The ICP-MS was set to run on both the standard mode, and the Collision Cell Technology (CCT) and Kinetic Energy Discrimination (KED) mode (US EPA, 1994) to reduce interferences due to isobaric polyatomic ions. The ICP-MS operating conditions were as follows: Penning pressure, 8×10^{-8} mbar; Pirani pressure, 1 mbar; nebulizer gas pressure, 2.0 bar; nebulizer gas flow, 1.1 liter/min; spray chamber temperature, 2–4 °C range; plasma power, 1550 watt; and radio frequency power 1400 watts. The performance of the ICP-MS was optimized strictly according to US EPA Method 200.8 (US EPA, 1994)

with a solution containing 10 µg/L each of Li, Be, Co, In, Ba, Ce, Pb, Bi, Tl, Mg, Rh, Y, and U. For quantitation, a 5-point linear calibration range (5, 10, 20, 100 and 200 µg/L levels) was generated for each metal. In addition, (i) a 0.20 µg/L lowest point was added for Cd and Ag (levels of Ag is 1/8th of all the other elements because of its low solubility), (ii) a 0.5 µg/L lowest point was added for Sb, Be, Cr, Cu, Pb, and (iii) a 1 µg/L lowest point was added for As, Se, Tl, Ni and Zn. The final concentrations of metals in samples were calculated as recommended for US EPA Method 200.8 (US EPA, 1994). The method detection limits (MDL) and the reporting limits (RL) of the metals studied by the ICP-MS instrument are listed in Table 1.

All primary standards used to calibrate the ICP-MS were Certified Reference Materials obtained from SPEX Certiprep (Metuchen, NJ, USA) and Inorganic Ventures (Christiansburg, VA, USA). The accuracy of calibration was ascertained by analyzing Certified Reference Materials from an alternate source in the same batch in lieu of a Standard Reference Materials. Calibration was verified dynamically after

Table 1. The method detection limits (MDL^a) and the reporting limits (RL^b) of the ICP-MS used in this study. Concentrations are given in µg/L.

Metal		MDL	RL
Aluminium	²⁷ Al	0.61	5.0
Antimony	¹²³ Sb	0.01	0.5
Arsenic	⁷⁵ As	0.09	1.0
Barium	¹³⁷ Ba	0.02	5.0
Beryllium	⁹ Be	0.03	0.5
Cadmium	¹¹¹ Cd	0.02	0.2
Chromium	⁵² Cr	0.07	0.5
Cobalt	⁵⁹ Co	0.01	5.0
Copper	⁶³ Cu	0.03	0.5
Lead	²⁰⁷ Pb	0.01	0.5
Manganese	⁵⁵ Mn	0.14	5.0
Molybdenum	⁹⁸ Mo	0.03	5.0
Nickel	⁶⁰ Ni	0.14	1.0
Selenium	⁷⁸ Se	0.25	1.0
Vanadium	⁵¹ V	0.28	5.0
Silver	¹⁰⁷ Ag	0.004	0.2
Strontium	⁸⁶ Sr	0.001	-
Thallium	²⁰⁵ Tl	0.01	1.0
Tin	¹¹⁸ Sn	0.02	5.0
Zinc	⁶⁶ Zn	0.32	1.0

^a MDL = $t \times$ (standard deviation of seven replicates in distilled/dionized water); t is 3.14, the Student's value for 99% confidence level with n-1 degrees of freedom.

^b RL is the lowest concentration on the 5-point calibration graph (see Section 2.3.).

every 10 samples to ensure against instrumental drift or artefactual contamination. Precision was assured by maintaining less than 5% relative standard deviations of standards and samples analyzed in triplicates. Accuracy was assured by running quality control sample ICM-240 (ULTRA Scientific, North Kingstown, RI, USA) prepared at a concentration of 20 ppb (5 ppb for Ag) in the beginning (which must be within 90%–110% of stated value) and at the end of a batch run (which must be within 85%–115% of stated value).

2.4. Data treatment

Standardized data and non-standardized concentration data were used in this study. Non-standardized concentrations were used to explore inter-brand relationship to retrieve the minimum number of metals needed for a general definition of Uganda *waragi*. But, in using non-standardized data, the *Relax* brand (REX) data and Cu levels had to be excluded to minimize distortion. REX had >10 times the levels of metals in other brands, and Cu was 100–1000 fold more in the unrecorded spirits and in the Scotch whisky reference than in other brands. For all other analyses described herein, the standardized form of the data was used. Data were standardized by subtracting the means of the duplicate measurements (μ) from variables (x) and dividing the results by respective standard deviations (α) (i.e., $[(x-\mu)/\alpha]$; i.e., rescaling data to have a mean of zero and a standard deviation of one). To test for production consistency, duplicate samples of a single brand were analyzed. To show how intra-company brand differences could be exploited to authenticated spirits, a randomly selected source offering a pair of differently branded products was used.

Randomizations of metals concentrations for brand similarity analysis (section 3.3.) were performed as follows. Random numbers between 1 and 5000 were first assigned by MS Excel 2010 software to each metal, and then the numbers were sorted from smallest to the largest before selecting the first seven for further analysis.

2.5. Statistical analysis

All data were imported into PAST software (Hammer et al., 2001) for principal component analysis (PCA) (Gergen and Harmanescu, 2012; Gewers et al., 2018), cluster analysis (CA) (Boutros and Okey, 2005) and for assessing the distribution of data by boxplots (Tukey, 1977; Benjamini, 1988). PCA was used to reduce data dimensionality, complement boxplot and CA results, and to estimate inter-brand relationship in a multivariate space. The Jolliffe cut-off values were used to informally determine the number of significant principal components to retain (Zwick and Velicer, 1982). CA, an unsupervised statistical procedure, was used to determine natural groupings among brands or contaminants from statistically derived numerical values describing how far apart the brands or the levels of metals are from each other. The application of CA and PCA in the food industry is well

established (Gergen and Harmanescu, 2012; Gewers et al., 2018; Boutros and Okey, 2005; Zwick and Velicer, 1982; Fotakis and Zervou, 2016); the same needs to take root in the alcohol industry. The Pearson product-moment correlation analysis (Pearson, 1901) was used to measure the strength of linear relationships among pairs of metal as an indirect means of determining potential common sources of metal contaminations.

3. Results and discussion

The mean levels of the 13 metals found in the 18 spirit samples studies are presented in Table 2 (Ag, Be, Cd, Co, Mo, Pb and Ti, detected below RLs, are not listed). The

Table 2. An 18×13 data matrix obtained in the sampled Uganda *waragi* gins. Ag, Be, Cd, Co, Mo, Pb and Tl were not included because they were detected below RLs in samples. Concentrations, given in µg/L, are means of duplicate measurements.

Brand ID	As	Ba	Cr	Cu	Al	Sr	Mn	Ni	Sb	Se	Sn	V	Zn	TOTAL ^f	% ^g
REX ^a	<u>9.74</u> ^e	42.62	4.74	10.22	157.9	98.45	48.76	5.44	0.64	35.79	0.38	27.59	350.35	792.5	29.5
B5V ^a	<u>1.68</u>	<u>19.79</u>	<u>0.18</u>	<u>7.48</u>	<u>97.86</u>	<u>62.96</u>	<u>6.14</u>	<u>0.52</u>	0.61	<u>1.54</u>	0.57	ND	<u>1.26</u>	<u>200.0</u>	<u>7.2</u>
B7W ^b	0.58	0.50	1.01	<u>31.48</u>	<u>13.13</u>	0.61	1.24	1.15	1.19	0.14	3.21	ND	9.81	62.4	1.2
UGW ^b	0.47	0.22	0.18	<u>1.60</u>	<u>6.67</u>	0.27	1.12	0.20	0.36	0.06	2.09	ND	2.68	15.1	0.5
SAG ^c	0.45	21.99	0.26	0.82	<u>16.89</u>	<u>82.06</u>	<u>12.34</u>	0.82	0.33	0.25	0.47	ND	1.92	137.8	5.2
ROV ^c	0.44	22.42	0.42	2.23	<u>38.08</u>	<u>66.91</u>	<u>4.78</u>	1.68	0.50	0.28	1.87	ND	24.19	162.9	6.0
CW1 ^d	0.59	<u>1.58</u>	0.21	1.96	<u>20.54</u>	<u>4.86</u>	<u>3.58</u>	0.32	0.45	1.75	0.79	ND	6.53	42.3	1.5
CW2 ^d	0.50	<u>12.86</u>	0.15	1.22	<u>10.51</u>	<u>60.38</u>	<u>10.87</u>	0.51	0.37	1.54	0.44	ND	14.10	112.6	4.2
KPW ^c	0.68	4.76	0.45	2.89	69.88	69.15	7.34	0.66	0.53	2.09	0.35	ND	7.46	165.4	6.1
BRG ^c	0.60	2.87	0.17	1.05	16.69	57.00	35.70	0.62	0.35	1.94	0.17	ND	5.94	122.4	4.6
BEG	0.56	49.36	0.63	3.82	2.61	80.57	1.08	1.67	1.07	0.18	0.39	1.07	5.00	148.0	5.4
GOV	0.63	15.74	0.13	1.45	33.27	55.71	11.73	0.54	0.89	0.06	1.07	ND	1.82	122.4	4.5
V6T	0.20	15.89	0.32	1.17	62.94	58.44	3.70	0.59	0.52	0.18	0.78	ND	3.51	147.2	5.5
TGL	0.67	1.41	6.39	406.9	87.72	4.13	16.06	1.73	0.77	0.22	1.90	ND	8.84	536.3	4.9
AWE	1.27	13.19	0.24	4213.5	42.86	7.01	13.38	0.72	0.88	2.43	0.18	ND	45.93	4341	4.8
BOL	1.98	8.81	0.20	1229.5	40.02	8.51	27.22	0.59	1.08	2.07	0.13	ND	19.20	1339	4.1
NSB	0.31	1.69	0.13	1396.8	30.91	2.16	19.38	1.07	0.78	1.06	0.96	ND	22.02	1477	3.1
TEB	1.22	1.92	0.37	12100.0	20.51	3.38	4.63	1.61	1.62	0.60	0.11	0.07	6.80	12143	1.6
TOTAL	22.54	237.59	16.17	19414.0	768.90	722.52	229.04	20.44	12.94	52.19	15.87	17.44	537.35		
%	0.1	1.1	0.1	88.0	3.5	3.3	1.0	0.1	0.1	0.2	0.1	0.1	2.4		

ND: Not detected.

^{a,b,c,d}Each letter represents a pair of brands from the same manufacturers stacked side by side. The underlined concentrations represent the intra-company differences and hence identifying the associated metals as discriminators for the pairs of company products studied.

^eUnique brand discriminators are in **bold**.

^f% Total concentrations of all 20 metals detected in each sample.

^g% of total metal concentrations in each sample with Cu values excluded.

results show that each sample had a unique metal fingerprint. These fingerprints, the first of their kind for Ugandan spirits, can be used not only to assure quality in production, but to catalog products, or company differences and implicitly for detecting sources of spirits. These potential applications are illustrated in this study by the following examples.

3.1. Detecting empirical differences and production inconsistency

The rudimentary exercise of detecting the empirical differences among spirits as will be demonstrated below may appear a very basic one, but it is contextually a necessary first step towards cataloging spirits in Uganda by their chemical characteristics. To illustrate how empirical differences can be detected then by the nature and the extent of contamination in products, a pair of company spirits was chosen from three different companies. The first were a pair of spirits blended and packaged by King Albert Distilleries: *Relax* (REX, a 20% (v/v) ethanol/water) and *Big 5 Vodka* (B5V, a 40% (v/v) ethanol/water). These spirits were found to be constitutively dissimilar with the REX brand showing the most contamination (compare the underlined values for REX with values for B5V in [Table 2](#)). The observation means that these King Albert's products can be discriminated crudely by the number of metals and the level of contamination in spirits.

The second examples are a pair of East African Brewery products *Bond 7 Whisky* (B7W) and the *Uganda Waragi* (UGW) brands for which the basis of the within-company discrimination appears to be limited to the levels of Cu and Al in each spirit ([Table 2](#)). In the B7W spirit sample, both Cu and Al were present at higher levels than in UGW (Cu in $\mu\text{g/L}$: B7W 31.5 ± 0.3 and UGW 1.6 ± 0.1 , and Al in $\mu\text{g/L}$: B7W 13.1 ± 10.0 and UGW 6.7 ± 1.6). This means the focus of discriminating these spirits should be on the levels of Cu and Al.

The third examples are a pair of Parambot Distilleries products *Salongo Gin* (SAG) and *Royal Vodka* (ROV), the discrimination of which can be based on only three metals Sr, Mn and Al. In these samples, the levels of Sr and Mn were higher in SAG (82.1 ± 2.7 and 12.3 ± 2.5 $\mu\text{g/L}$, respectively) than in ROV (66.9 ± 4.6 and 4.8 ± 2.4 $\mu\text{g/L}$, respectively), but the level of Al was higher in ROV (38.1 ± 7.2 $\mu\text{g/L}$) lower in SAG (16.9 ± 1.5 $\mu\text{g/L}$).

A rather surprising discovery was the inconsistency observed in the levels of Ba, Al, Sr and Mn between the *Chief Waragi (Gin)* duplicates CW1 and CW2 collected one year apart ([Table 2](#)). We believe this to be a consequence of poor reproducibility in the production of spirit which, in this case, is detected without rigor. A few additional ancillary observations can also be made from [Table 2](#): (i) that the REX sample was the most contaminated overall comparatively (which offer a unique potential to identify this product on its own merit), (ii) that high levels of Ba are associated

exclusively with two brands: *Relax* (REX) and *Beckam Gin* (BEG), (iii) that non-standardized levels of Sb and Sn are not constitutively unique enough for brand discrimination and (iv) that Cu contamination alone can be used to discriminate the *Lira-Lira* spirits (BOL, AWE, TEB and NSB) as a class from all of the remaining 13 Ugandan spirits (resulting from the use of Cu tubes submerged in cold water to efficiently condense alcohol vapor during *Lira-Lira* production). The production of the Scottish whisky (TGL) likewise involves sustained contact with Cu (Adam et al., 2002). TGL though can be discriminated distinctively from the *Lira-Lira* spirits by the more than 1000-fold higher levels of Cu in the latter. Within the four *Lira-Lira* spirits, discrimination was only achievable by exploratory statistical analysis (section 3.4).

3.2. Determining the minimum discriminating factors for each spirit

To determine the minimum factors required to discriminate the 17 Ugandan spirits from each other, CA and PCA were both used to explore the internal structure of a 17×12 non-standardized concentration data matrix derived from Table 2 after excluding two sets of outlier data to minimize distortion: Cu because 88% of all metals detected by mass was Cu (see last row in Table 2) and REX brand because 30% of all metals detected beyond Cu were in this sample (see last column in Table 2).

Cluster analysis in the R-mode was used to determine natural groupings of contaminants across brands. The results, summarized in Fig. 3a, suggest that there are six recognizable groupings at a dissimilarity distance of 50. Five of these groupings are interesting in that each grouping contains only Al, Sr, Ba, Mn or Zn as a member. This complete segregation means Al, Sr, Ba, Mn and Zn each occupy a very distinct place in the multivariate space of this matrix. Additionally, the sixth grouping contains all the seven remaining metals (Cr, Se, Ni, Sn, V, As and Sb) at a dissimilarity distance close to zero (i.e., being very similar to each other statistically). These metals are therefore not important for discriminating the spirit samples studied. This elimination then leaves only Al, Sr, Ba, Mn and Zn (and Cu by default) as the discriminating factors for the 12 spirit brands included in the 17×12 matrix. To verify the preceding CA results, PCA, a statistical technique whose underlying principles are different from those of CA, was also applied to the same data matrix. The scatter plot of the loadings along principal components PC1 and PC2 (jointly explaining 92.6% of variance) from PCA are displayed in Fig. 3b. (PC3 was not considered here because its eigenvalue of 170.2 is barely above the Jolliffe cut-off value of 169.7 for the 17×12 data matrix when compared to 2995.3, the eigenvalue for PC1 and 821.7, the eigenvalue for PC2). The plot shows that Al, Sr, Ba, Mn and Zn data loaded *positively* along PC1 while the rest of the data loaded *negatively* on the same axis. This means these five metals (plus Cu) were largely responsible for

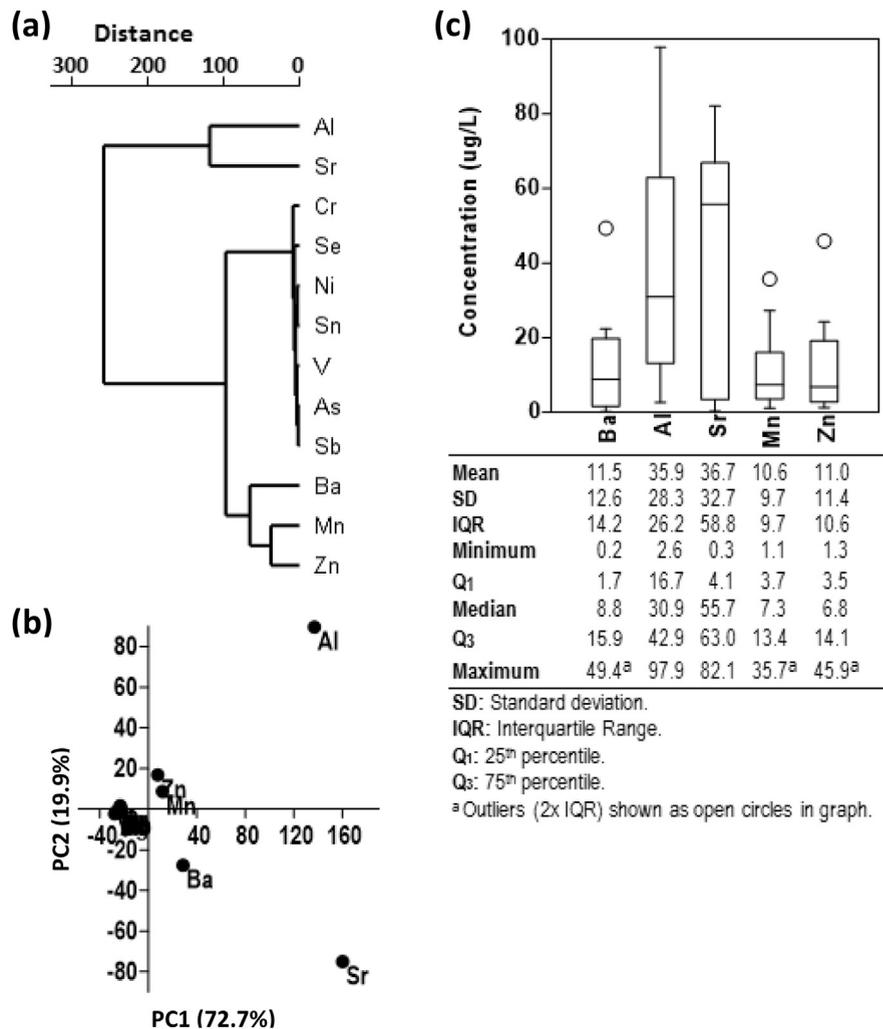


Fig. 3. Determining the minimum number of contaminants required to uniquely identify the Ugandan sachet-packaged spirits studied. Non-standardized data were used; Cu data, higher in unrecorded spirits, are excluded. (a) CA results (Ward's method; cophenetic correlation coefficients: 0.99) showing similarities in the nature of contamination by each metal in the 17 remaining spirit samples. (b) PCA results showing the separation of the five important metals (positive values) from the rest (negative values) along PC1 (PC1 explains 72.7% of variance). (c) The box and whisker plots displaying the distributions of the five important metal contaminants (and Cu, not shown) in the 17 alcoholic beverages sampled in Uganda. Each box encloses 50% of the measured values; the upper and the lower limits of the box are the 75% and the 25% percentiles, respectively. The horizontal lined within each box is the median. The relevant statistics are listed below each metal.

variations in the 17×12 raw concentration data matrix. Note that very distinct differences also exist between Al, Zn and Mn (positive values), and Sr and Ba (negative value) along PC2 even though PC2 explains only 19.9% of variance. We offer no interpretation of this distinction at this time.

The preceding CA and PCA results evidently set Al, Sr, Ba, Mn and Zn (and Cu by default) apart as the minimum discriminating factors for the spirits studied. The

distribution of these metals across the samples therefore holds information that may aid in understanding why the six metals so clearly separate out. This distribution is shown in Fig. 3c as boxplots. First, the distinctively difference shapes of the boxplots — all skewed — points to the enormous concentration variations from sample to sample. For example, the boxes for Al and Sr concentrations which have median lines that lie entirely outside the boxes of Ba, Mn and Zn means there are additional differences between Al and Sr on the one hand and Ba, Mn and Zn on the other hand. Al, Sr and Mn boxes additionally have longer upper whiskers implying large concentrations variability for these metals in highly polluted spirits. The boxplots for Al and Sr also are taller which means the concentration variations for these two metals are larger across all samples. Even the boxes for Ba, Mn and Zn have different shapes although they are comparatively short and with similar medians and heights. These large variations are the likely basis qualifying these metals as the best discriminating factors here.

3.3. Brand similarities

To test whether natural groupings exist among the 18 spirits we studied (17 from Uganda and a reference from San Diego, California, USA), an 18×12 non-standardized metals data matrix was explored by CA in Q-mode without the distorting effect of Cu (i.e., Table 2 without Cu). The integrity of the resulting cluster had to be tested since the raw REX data were included in the analysis. The integrity testing was done by comparing two dendrograms generated prior to removing seven randomly selected metals from the data matrix (Fig. 4) and after the removal (not shown). The two dendrograms were found to be identical with the same cophenetic correlation coefficient of 0.90 (cophenetic correlation is an overall measure of the similarities among brands for being included into the same clusters).

The CA results, color coded by manufacturers and displayed in Fig. 4, show three major brand groupings (Clusters 1, 2 and 3) at a dissimilarity distance of 150. The first grouping (Cluster 1) contains only REX, a distinctiveness earned by having rather higher levels of 12 metals comparatively (Table 2), and perhaps because V was detected only in this brand. The second grouping (Cluster 2) divides into two sub-clusters with each containing four members. One sub-cluster contains brands B7W and UGW (two East Africa Breweries products with the lowest metal contamination levels), CW1 (a recorded spirit duplicate showing real difference with CW2, the second duplicate) and TEB (one of the four unrecorded spirits). The second sub-cluster contains the three remaining unrecorded spirits (AWE, BOL and NSB) together with TGL, the reference spirit. This finding suggests that there are strong similarities between the *Lira-Lira* spirits and the Scottish whisky, Cu level notwithstanding. Cluster 2 additionally contains all unrecorded spirits alongside UGW. This means that the *Lira-Lira* spirits TEB, AWE, BOL and NSB without Cu

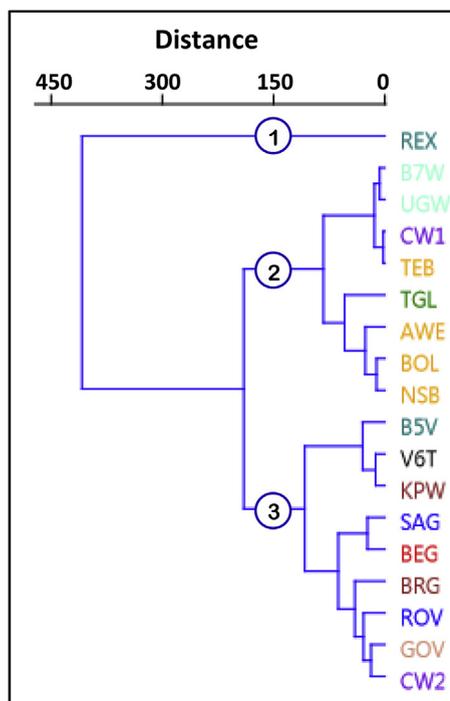


Fig. 4. Results of CA of non-standardized waragi data (Table 2). Silver, Be, Cd, Co, Mo, Pb and Tl were not included because of detections mostly below MDLs (Table 1). Cu was also excluded for reasons given in Fig. 3 caption. Products from the same manufacturer are color coded similarly.

contamination would have a background as ‘clean’ as that of UGW (*Uganda Waragi*, the ‘gold’ standard from Table 2). And given that these four unrecorded spirits are the mainstay for maintaining alcohol addiction in Acoli, the reduction in metal-related health risks associated with consuming *Lira-Lira* spirits would then lie heavily in reducing the amount of Cu in the *Lira-Lira* spirits. Interestingly, the composition of Cluster 2 also means that the residual metal contaminations left in the *Lira-Lira* spirits after Cu is removed are still enough to uniquely discriminate them as a class from the other brands of Uganda spirits we studied. This observation means that the *Lira-Lira* spirits are indeed a very unique class of spirits on a broader scale.

Cluster 3 contains three sub-clusters with the following [membership] at a distance of 65: [B5V, V6T and KPW], [SAG and BEG] and [BRG, ROV, GOV and CW2]. The composition of these sub-clusters reveals a potential to separate spirits from the same companies (color coded similarly) as well as from different producers. For example, B5V is very different from REX (Cluster 1). Likewise, KPW and BRG, or SAG and ROV are in separate sub-clusters of Cluster 3. Notice how far CW2 is from CW in Fig. 4.

To validate the CA results, PCA scores derived from the complete data matrix (Table 2) were used to generate the two scatter plots shown in Fig. 5. In Fig. 5a (in which PC1 and PC2 together explain 91.6% of variance), four brand groupings are

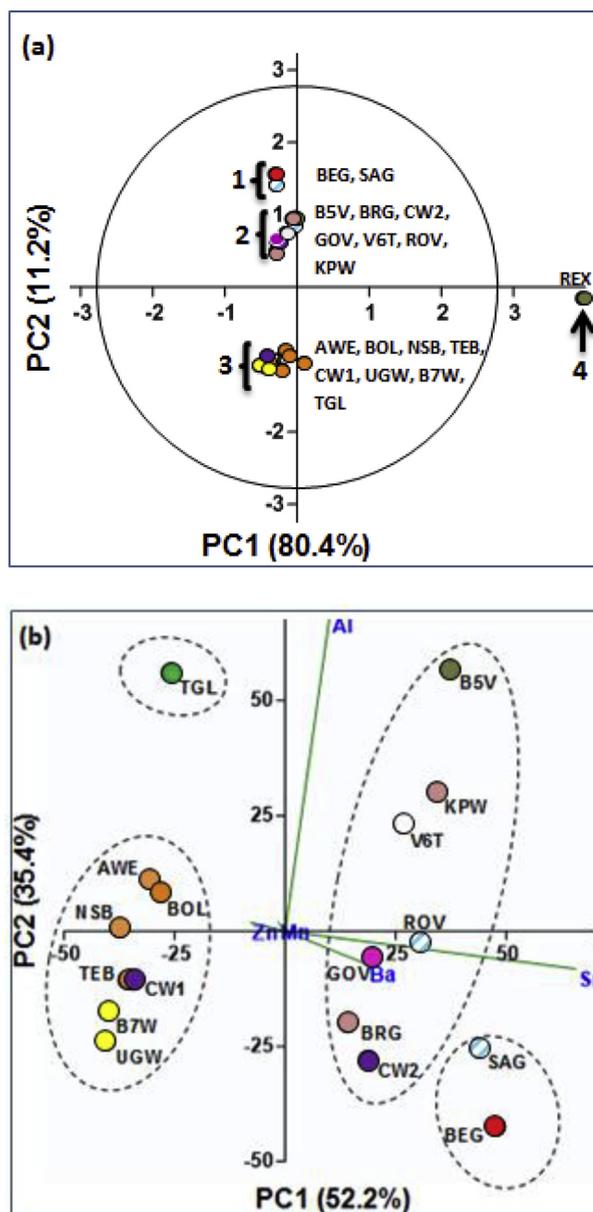


Fig. 5. PCA plots using metals levels in $\mu\text{g/L}$ (Table 2) in Eigenvalue scale (a) with the 95% ellipses shown; copper levels excluded to minimize distortion). Four clustering of brands are revealed. Cluster 1 contains BEG, SAG; Cluster 2: B5V, BRG, CW2, GOV, V6T, ROV, KPW; Cluster 3: AWE, BOL, NSB, TEB, CW1, UGW, B7W, TGL; and Cluster 4 contains only REX. (b) Detail PCA scores without copper and REX to amplify the nature of clustering.

revealed. Group 1 contains BEG and SAG as found by CA. Group 2 contains B5V, BRG, CW2, GOV, V6T, ROV, KPW. These brands, together with BEG and SAG belong to the same CA cluster (Cluster 3). Group 3 has all the unrecorded spirits AWE, BOL, NSB, and TEB, the reference Scottish whisky TGL, one of the lesser contaminated *Chief Waragi (Gin)* duplicate CW1 and both the least contaminated spirits UGW and B7W from the East African Breweries. Group 4 contains only

REX. The memberships of both Groups 3 and 4 match the groupings observed in CA results. These PCA results therefore validate the CA results.

To bring out details overshadowed by the inclusion of REX and Cu data in the matrix, PCA was performed again on the same data set after removing REX and Cu data from Table 2. The results, displayed in Fig. 5b (in which PC1 and PC2 together explain 87.6% of variance), establishes and confirms the distinctiveness of the *Lira-Lira* spirits (AWE, BOL, TEB and NSB) as a separate class of Uganda spirits (even without Cu) along PC1. Note that CW1, UGW and B7W still falls in this PCA grouping without the REX and Cu data (emphasizing the fact that *Lira-Lira* spirits would be 'clean' without Cu). Also note that the sample duplicates CW1 and CW2 are still separated.

In general, these observations together highlight the potential of discriminating at an empirical level all brands of spirits sold in Ugandan.

3.4. Resolving sources of spirits

3.4.1. *Lira-Lira* spirits

Having established that unrecorded *Lira-Lira* spirits could be clearly delineated as a class by using non-standardized variables, the challenge was to find the discordance within the class. This was found by analyzing the standardized form of the whole 18×20 data matrix by PCA (Fig. 6a). Two members of the class AWE and TEB can be seen to have been separated completely from each other. While AWE is strongly and positively correlated with PC1 (explaining 47.1% of variation) and driven by a vector associated with Sb, TEB is correlated likewise but with PC2 (explaining 21.6% of variation) and driven by a Se vector. This level of resolution is remarkable given that the two samples were collected from related localities 75 km (47 mi) apart. Even more compelling is the resolution of the AWE and the BOL samples data along PC2 in which AWE assume a positive value while BOL assumes a negative value. Given that AWE and BOL were collected from culturally identical localities which are also only 8 km (5 mi) apart is a clear testament to the power of PCA to discriminate between similarly brewed, distilled and stored spirits.

3.4.2. *Recorded* spirits

To increase resolution of the recorded spirits, a smaller 14×11 standardized data matrix was subjected to a separate PCA. The smaller matrix resulted from the exclusion from the data matrix Se and Sb which are clearly associated with the *Lira-Lira* spirits AWE and BOL, respectively (Fig. 6a) and all the *Lira-Lira* spirits. The PCA results, displayed in Fig. 6b, show that BRG and BEG data score strongly and positively on PC1 and PC2, respectively, and that Sr and Ni loading are the unique discriminating factors on PC1 (69.4%) and PC2 (10.5%), respectively. These observation suggests

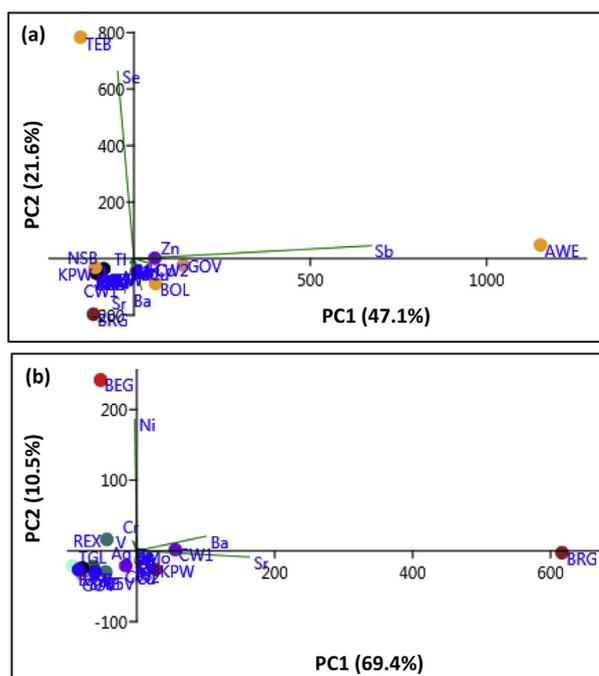


Fig. 6. PCA plots using an 18×20 variance-covariance matrix with all metals in all samples (a), and (b) and using an 13×18 variance-covariance matrix after Se, Sn and all metal data from the *Lira-Lira* spirits (AWE, BOL, NSB, and TEB) were excluded for finer resolution. Algorithm: Ward's method; cophenetic correlation coefficient: 0.90.

that BRG and BEG discrimination from each other are closely associated with the levels of Sr and Ni, respectively. By increasing the sensitivity of the scatter plot given in Fig. 6b, other unique brand/metal associations such as REX with V (confirming speculation, section 3.3.), KPW with Mo/Sn, CW1 with Ba, and CW2 with As are also observable around the intersections of the PC1 and PC2.

3.5. Novel application of cluster analysis

A novel application of CA with standardized data is shown in Fig. 7. In this application, the CA loading and score results (on the same scales) are placed side-by-side to reveal a direct one-to-one correspondence between spirits and their major discriminating factors. In this study, we are able to not only singled out correctly three of the four unrecorded spirits individually (color coded orange in Fig. 7a), but matched each to a factor that most characterizes the spirits (Fig. 7b). Specifically, AWE, TEB, and BOL are each matched and separated by levels of Sb, Se, and Al, respectively. It can also be seen that NSB is uniquely discriminated from the rest of the *Lira-Lira* spirits.

Similarly discriminated were recorded spirits BRG and BEG on the basis of Sr and Ni, respectively. The duplicates CW1 and CW2 are again shown to be very different from each other with CW1 closely relating to B5V and CW2 still relating to GOV

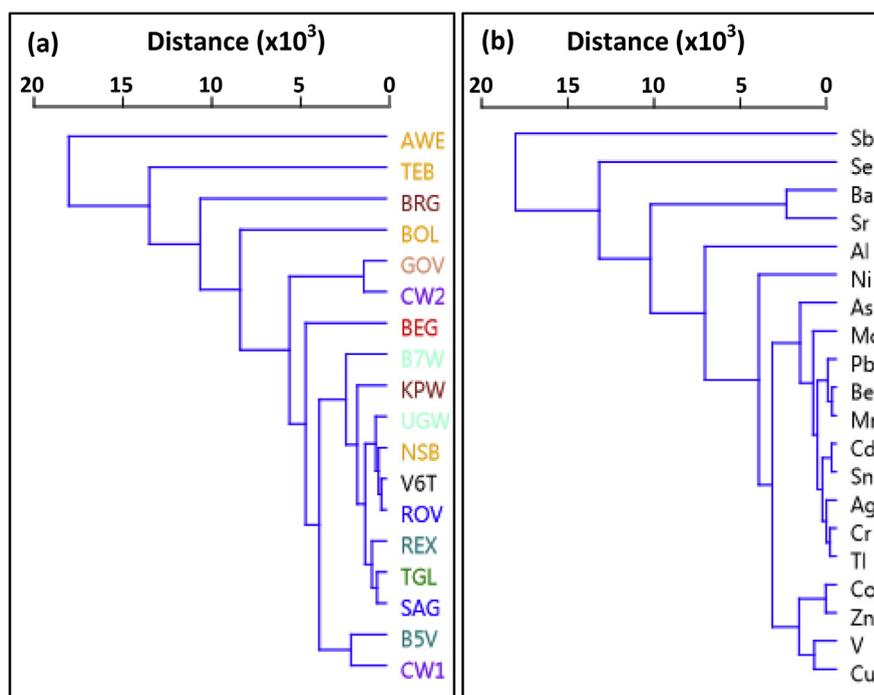


Fig. 7. Comparative CA using standardized scores (a) and loadings (b) side-by-side to show metals/brands relationships. Brands packaged by the same company are color coded similarly. Algorithm: Ward's method; Similarity index: Euclidean; cophenetic correlation coefficients: 0.98 (a) and 0.97 (b).

(as observed using non-standardized data in Fig. 4). Note that the attempted discrimination of the East African Breweries product samples B7W and UGW which failed using non-standardized data (Fig. 4) is now accomplished (Fig. 7).

3.6. Estimating common sources of contamination

The Pearson bivariate correlation analysis was used to estimate the common sources of metals in the spirits studied. Table 3 provides data supplemental to Table 2 which were used in correlation analysis. The results of these pairwise associations (Table 4) show a strong correlation between Se and As levels ($r = 0.98$), between Ni and As ($r = 0.88$) and between Se and Ni ($r = 0.89$) in all samples. The correlation between the levels of Se and As has been observed in canned food (Al-Rajhi, 2014) and that between Ni and As is known to occur naturally (Brown and Blin-Stoyle, 1959). But, the correlation between Se and Ni is unknown to us. Additionally, note the existence of a 'triad' correlation involving Se, As, and Ni suggesting perhaps a single contaminating source for all three metals in the spirits studied.

Also, strongly and positively correlated were levels of Ni and Be ($r = 0.81$) and those of Sn and Cd ($r = 0.96$). The strong correlation between Ni and Be are understandable since these two metals are commonly used together as beryllium-nickel alloys in some high-temperature applications (WHO, 1990). The correlation

Table 3. An 18×5 additional data matrix obtained in the sampled Uganda *waragi* gins used in correlations analysis (Table 4). Concentrations, given in µg/L, are means of duplicate measurements.

Brand	⁹ Be	¹¹¹ Cd	⁵⁹ Co	⁹⁸ Mo	²⁰⁷ Pb
REX	0.13 ± 0.07	0.03 ± 0.00	0.48 ± 0.02	0.29 ± 0.22	1.06 ± 0.08
B5V	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.01	0.10 ± 0.01	0.09 ± 0.07
B7W	0.03 ± 0.00	0.10 ± 0.01	0.00 ± 0.00	0.06 ± 0.00	0.52 ± 0.03
UGW	0.02 ± 0.00	0.07 ± 0.10	0.00 ± 0.00	0.03 ± 0.03	0.07 ± 0.01
SAG	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.19 ± 0.00	0.11 ± 0.02
ROV	0.01 ± 0.01	0.06 ± 0.06	0.01 ± 0.03	0.12 ± 0.01	0.09 ± 0.01
CW1	0.01 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.08 ± 0.00
CW2	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.10 ± 0.00	0.07 ± 0.02
KPW	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.10 ± 0.00
BRG	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.12 ± 0.00	0.05 ± 0.00
BEG	0.10 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.14 ± 0.02	0.12 ± 0.01
GOV	0.02 ± 0.01	0.03 ± 0.00	0.00 ± 0.00	0.08 ± 0.03	0.13 ± 0.05
V6T	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.06 ± 0.01	0.17 ± 0.01
TGL	0.07 ± 0.01	0.08 ± 0.01	0.30 ± 0.03	0.10 ± 0.02	0.72 ± 0.04
AWE	0.06 ± 0.01	0.02 ± 0.01	0.11 ± 0.00	0.14 ± 0.01	0.32 ± 0.02
BOL	0.03 ± 0.01	0.01 ± 0.00	0.09 ± 0.00	0.09 ± 0.01	0.09 ± 0.02
NSB	0.04 ± 0.03	0.05 ± 0.03	1.80 ± 1.27	0.08 ± 0.01	0.18 ± 0.11
TEB	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.30 ± 0.11	0.11 ± 0.01

Values below MDLs (Table 1) are in **bold**.

between Sn and Cd may also be understood similarly because they are sometimes used together as corrosion resistant alloy (MCB, 1978). Strong and positive correlations were also observed between levels of Pb and Cr ($r = 0.87$), Pb and Ni ($r = 0.81$), and Tl and Cr ($r = 0.80$) suggesting common sources for each pair.

3.7. Study limitation

This study has contextual limitations worth considering. First, the picture presented here may change if a different batch of the spirit brands were to be used. This is demonstrated in this study by the lack of consistency in the production of CW1 and CW2 duplicates of the same spirit brand collected one year apart. Second, a defined metal fingerprints library of Ugandan spirits does not exist. This would have allowed a comparative analysis of quality. Third, the lack of the following information minimizes any strict interpretation of our results: (i) the materials that came into contact with spirits during production, (ii) the raw materials used in production, (iii) the soil in which the fermented plant products were grown, and (iv) the quality of water used during the brewing process. The picture presented here should

Table 4. Correlations among the levels of metals in the 18 *waragi* samples.

	As	Al	Ba	Be ^a	Cd ^a	Co ^a	Cr	Cu	Mn	Mo ^a	Ni	Pb ^a	Sb	Se	Sn	Sr
As	1.00															
Al	0.76	1.00														
Ba	0.50	0.32	1.00													
Be	0.70	0.46	0.56	1.00												
Cd	-0.09	0.00	-0.31	0.02	1.00											
Co	0.14	0.16	-0.10	0.25	0.18	1.00										
Cr	0.53	0.65	0.16	0.65	0.40	0.15	1.00									
Cu	-0.01	-0.14	-0.23	0.18	-0.21	0.01	-0.10	1.00								
Mn	0.72	0.53	0.20	0.46	-0.21	0.33	0.43	-0.10	1.00							
Mo	0.61	0.32	0.45	0.60	-0.32	0.02	0.30	0.58	0.43	1.00						
Ni	0.88	0.67	0.57	0.81	0.11	0.24	0.68	0.06	0.59	0.70	1.00					
Pb	0.74	0.70	0.25	0.74	0.43	0.23	0.87	-0.07	0.52	0.39	0.81	1.00				
Sb	0.04	-0.09	-0.02	0.40	0.09	0.06	0.05	0.69	-0.13	0.37	0.17	0.15	1.00			
Se	0.98	0.73	0.49	0.67	-0.08	0.19	0.53	-0.08	0.72	0.57	0.89	0.74	-0.07	1.00		
Sn	-0.23	-0.14	-0.30	-0.16	0.96	0.01	0.22	-0.30	-0.38	-0.43	-0.05	0.24	0.02	-0.21	1.00	
Sr	0.36	0.35	0.76	0.13	-0.43	-0.22	0.04	-0.40	0.25	0.33	0.38	0.06	-0.40	0.41	-0.36	1.00

Ag, V and Tl not included because of non-detect in many samples.

^aConcentrations data are in Table 3.

therefore be considered (i) a snapshot of the state of spirit contamination in Uganda and (ii) an illustration of capability to fingerprint spirits from Uganda. However, in cases where spirit production requires sustained contacts with metals, the concentrations of the metals in question should be the starting point for characterizing the spirit. This is demonstrated in this study by the *Lira-Lira* spirits and the Scottish whisky where production involved sustained contact with Cu. In these spirits, the consistently very high levels of Cu (when compared to the other metals) removes the uncertainty of arriving at a different conclusion due to variability in Cu levels among batches.

4. Conclusion

In this study, we fingerprinted 17 spirits from Uganda on the basis of their metals content and showed that the fingerprints can be manipulated by rudimentary exploratory statistics to identify factors important for characterizing and discriminating the spirits. A proof of concept was able to identify Al, Sr, Ba, Mn, Zn and Cu as the important metals for characterizing and discriminating the 17 spirits studied; the underlying qualification for listing appears to be a large concentration variation across the spirit brands. Fingerprinting is also shown here to have a potential for detecting production inconsistency. Duplicate samples of a spirit brand were

shown to be actually different by fingerprinting. Additionally, the common sources of the metal contaminations in the 17 spirits were also estimated by the Pearson correlation analysis. The Se, As and Ni contamination in the spirits for example were shown to have come from similar sources. Likewise, Sn and Cd, Pb and Cr, Tl and Cr, Pb and Ni, or Cu and Ag pairs probably came from similar sources as well. The contributions of soil, grains and water to contamination were not accounted for in this study. More studies are required to produce a complete picture of Ugandan spirits.

Declarations

Author contribution statement

Eric Oloya Otim: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

I Ru Chen: Analyzed and interpreted the data.

Ochan Otim: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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