



Comparative analyses of pesticide residues, elemental composition and mycotoxin levels in Spanish traditional and novel ciders

Pablo Alonso González^{a,*}, Eva Parga Dans^a, Iván de las Heras Tranche^b,
Andrea Carolina Acosta-Dacal^c, Ana Macías Montes^c, Manuel Zumbado Peña^{d,e}, Octavio Pérez
Luzardo^{d,e}

^a Institute of Natural Products and Agrobiology (IPNA-CSIC), Av. Astrofísico Francisco Sánchez, 3, 38206 San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain

^b Independent Researcher

^c Research Institute of Biomedical and Health Sciences (UIBS), University of Las Palmas de Gran Canaria, Paseo Blas Cabrera s/n, Las Palmas de Gran Canaria, 35016, Spain

^d Toxicology Unit, Research Institute of Biomedical and Health Sciences (UIBS), University of Las Palmas de Gran Canaria, Paseo Blas Cabrera s/n, Las Palmas de Gran Canaria, 35016, Spain

^e Spanish Biomedical Research Centre in Physiopathology of Obesity and Nutrition (CIBEROBn), Madrid, 28029, Spain

ARTICLE INFO

Keywords:

Cider
Chemical contaminants
Pesticide residues
Mycotoxin
Elemental composition
Trace elements

ABSTRACT

The apple cultivar, known for its adaptability and diverse varieties, has been extensively utilized for cider production, particularly in climatically suitable regions. Cider, an age-old alcoholic beverage derived from fermenting apple juice, is gaining popularity, especially among younger generations. Despite this trend, comprehensive knowledge regarding the toxicological profile of ciders remains limited, leaving room for potential chemical contaminants from raw ingredients or production methods. To address this gap, we conducted an unprecedented study analyzing sixty-eight cider samples from the Spanish market, encompassing both traditional ciders and newly developed apple-based flavored drinks referred to as “ciders.” Our investigation focused on pesticide residues, elemental profiles, and mycotoxin residues. In a groundbreaking approach, our study integrated the analysis of 225 pesticide residues, 50 Persistent Organic Pollutants (POPs), 11 mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, FB1, FB2, H-2, HT-2, OTA, PAT, and ZEN), and a total of 50 elements. Pesticide residues were identified using GCMSMS and LCMSMS, elemental composition determined via ICPMS, and mycotoxins analyzed using LCMSMS. The significance of our research lies in addressing the dearth of toxicological analyses of ciders, despite their burgeoning global consumption and production. For pesticide residues and elemental composition, our results underwent statistical processing, revealing distinct differences between the elemental profiles of traditional ciders and “ciders.” Additionally, disparities were observed between cider and other low-alcohol fermented beverages like wines and beers. Concentrations of most pesticide residues and elements in the cider samples were deemed non-toxic, falling below allowable limits established by international organizations for other beverages such as water or wine. However, certain elements, notably Br and Pb in traditional ciders, raised potential concerns. Our findings underscore the necessity of establishing regulatory limits for pesticide residues, potentially hazardous elements, and mycotoxins in cider, a regulatory framework currently lacking on a global scale.

1. Introduction

Apples (*Malus domestica*) and their derivatives, notably cider, wield a significant influence on global fruit cultivation (Fabien-Ouellet & Conner, 2018). Cider, an age-old alcoholic beverage fermented from apples, is experiencing a surge in consumption and production on a global scale

(Sousa et al., 2020). Rooted in Atlantic European tradition, cider boasts a history dating back to at least 900 BCE (Buglass, 2011). The United Kingdom leads as the primary global producer, followed by France and Spain, with the United States emerging as the leading non-European producer (Merwin et al., 2008). Major cider consumption zones include Western Europe (55.7%), Africa and North America (12% each),

* Corresponding author.

E-mail address: pablo.alonso.gonzalez@ipna.csic.es (P. Alonso González).

<https://doi.org/10.1016/j.foodcont.2024.110310>

Received 8 September 2023; Received in revised form 2 January 2024; Accepted 14 January 2024

Available online 23 January 2024

0956-7135/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Australia (8%), and Eastern Europe (6.4%) (Langley & Jenkin, 2019). Spain, despite its lower per capita consumption at 2.5 L per year compared to the UK’s 14 L, sees cider accounting for 3% of the alcoholic beverage market, exhibiting a 3.5% growth between 2014 and 2019, surpassing the 0.2% growth observed for beer during the same period (Büchele et al., 2022).

The definition of cider encompasses artisanal products resulting from the partial or complete fermentation of apple juice, including flavored variations incorporating other fruits. Cider can exhibit an alcohol content ranging from 1.2% to 8.5%, with or without additional sugar, water, or flavoring. It may also be tied to specific geographic regions and manufactured according to traditional methods. Traditional European ciders, such as Somerset, Bretagne, or Pays d’Auge, benefit from Protected Geographical Indications (PGI) and Protected Designations of Origin (PDO) within the European Union, safeguarding against fraud and ensuring consumers the authenticity of producers’ claims (Cayot, 2007). In Spain, only the traditional ciders of Asturias and the Basque Country hold official PDO recognition.

This study delves into the underexplored topic of cider in the Canary Islands, comparing natural ciders with a new category termed “cidere,” distinct from traditional craft ciders. The term “cidere” is adopted in Spain for apple-based drinks that may include additional ingredients, a departure from the original Spanish designation of *sidra* (Gobierno de España, 2017). Launched by major beer companies, these “cidere” have driven a substantial overall increase in cider and apple-based drink consumption in Spain.

As of 2021, natural cider has seen a decline in market share in Spain, constituting only 24% of sales compared to the dominance of carbonated ciders at 76% (Raffin, 2022). However, natural cider maintains its prevalence in traditional cider-producing regions such as the Basque Country (76% market share), Asturias, and Navarre (68%).

The primary innovation of this study lies in its comparative perspective, examining various regions and cider types, filling a gap in the limited literature on cider toxicology and composition. While existing research has well-established the chemical composition and sensory profile of cider, few studies provide comprehensive toxicological surveys covering pesticide residues, mycotoxins, and trace metals (Carballo et al., 2021). Notably, our study addresses this gap by evaluating 225 pesticide residues, 50 Persistent Organic Pollutants (POPs), 11 mycotoxins, and 50 elements.

Existing literature has primarily focused on the mycotoxin patulin, prompting investigations into its prevalence and strategies for degradation. Similarly, limited attention has been given to pesticide residues, with a recent survey covering 20 commercially available ciders in the Czech Republic (Zušfáková et al., 2023). Elemental composition studies have explored methods for verifying cider origin, authenticity, and correlation with raw materials.

Given the dearth of knowledge on the toxicological profile of ciders and other alcoholic apple-based drinks, our study aims to fill this gap by comprehensively examining the presence and levels of pesticide residues, POPs, mycotoxins, and elements. The information generated will lay a robust foundation for international comparisons and guide future improvements in toxicological monitoring strategies.

2. Methods and data collection

2.1. Cider samples

A total of sixty-eight cider samples were meticulously chosen for examination, as outlined in Table 1. This selection comprised forty-one traditional ciders and twenty-seven more recently introduced “cidere.” These beverages were procured from supermarkets and specialized shops specifically for this investigation, aiming to assemble a representative sample reflecting the most widely accessible and consumed ciders and “cidere” available in the Spanish market. Among the sampled beverages, six were identified as organic, with no organic counterparts

Table 1

Table 1 presents the sample list, including the product (cider vs. “cider”), the cider type, production method (organic vs. conventional) and region or country of origin.

| N | Product | Type | Organic/Conventional | Origin |
|----|---------|-------------------|----------------------|----------------|
| 1 | “Cider” | – | Conventional | Andalusia |
| 2 | “Cider” | – | Conventional | Andalusia |
| 3 | “Cider” | – | Conventional | Andalusia |
| 4 | “Cider” | – | Conventional | Madrid |
| 5 | “Cider” | – | Conventional | Navarre |
| 6 | “Cider” | – | Conventional | Navarre |
| 7 | “Cider” | – | Conventional | Asturias |
| 8 | “Cider” | – | Conventional | Asturias |
| 9 | “Cider” | – | Conventional | Valencia |
| 10 | “Cider” | – | Conventional | Valencia |
| 11 | “Cider” | – | Conventional | Valencia |
| 12 | “Cider” | – | Conventional | Valencia |
| 13 | Cider | Sparkling | Conventional | Asturias |
| 14 | Cider | Sparkling | Conventional | Asturias |
| 15 | Cider | Sparkling | Conventional | Asturias |
| 16 | Cider | Natural | Conventional | Asturias |
| 17 | Cider | Natural Sparkling | Conventional | Asturias |
| 18 | Cider | Natural | Conventional | Asturias |
| 19 | Cider | Natural Sparkling | Conventional | Asturias |
| 20 | Cider | Natural | Conventional | Basque Country |
| 21 | Cider | Natural | Conventional | Asturias |
| 22 | Cider | Natural | Conventional | Basque Country |
| 23 | Cider | Natural | Conventional | Asturias |
| 24 | Cider | Natural | Organic | Asturias |
| 25 | Cider | Sparkling | Conventional | Asturias |
| 26 | “Cider” | – | Conventional | Ireland |
| 27 | “Cider” | – | Conventional | Sweden |
| 28 | “Cider” | – | Conventional | Sweden |
| 29 | Cider | Sparkling | Organic | Galicia |
| 30 | Cider | Natural | Organic | Galicia |
| 31 | “Cider” | – | Conventional | Galicia |
| 32 | Cider | – | Conventional | Galicia |
| 33 | “Cider” | – | Conventional | Galicia |
| 34 | Cider | Natural | Organic | Galicia |
| 35 | “Cider” | – | Conventional | Galicia |
| 36 | “Cider” | – | Conventional | Galicia |
| 37 | Cider | Sparkling | Conventional | Asturias |
| 38 | Cider | Natural | Conventional | Basque Country |
| 39 | Cider | Natural | Conventional | Canary Islands |
| 40 | “Cider” | – | Conventional | Sweden |
| 41 | “Cider” | – | Conventional | Sweden |
| 42 | “Cider” | – | Conventional | Sweden |
| 43 | “Cider” | – | Conventional | Sweden |
| 44 | “Cider” | – | Conventional | Sweden |
| 45 | “Cider” | – | Conventional | Sweden |
| 46 | “Cider” | – | Conventional | Navarre |
| 47 | “Cider” | – | Conventional | Navarre |
| 48 | “Cider” | – | Conventional | Navarre |
| 49 | Cider | Natural | Conventional | Asturias |
| 50 | Cider | Natural Sparkling | Conventional | Asturias |
| 51 | Cider | Natural Sparkling | Conventional | Asturias |
| 52 | Cider | Natural | Conventional | Asturias |
| 53 | Cider | Natural | Organic | Asturias |
| 54 | Cider | Natural Sparkling | Conventional | Asturias |
| 55 | Cider | Natural | Conventional | Asturias |
| 56 | Cider | Natural | Conventional | Canary Islands |
| 57 | Cider | Natural Sparkling | Conventional | Canary Islands |
| 58 | Cider | Natural | Conventional | Canary Islands |
| 59 | Cider | Natural Sparkling | Conventional | Canary Islands |
| 60 | Cider | Natural | Organic | Canary Islands |
| 61 | Cider | Natural Sparkling | Conventional | Canary Islands |
| 62 | Cider | Natural Sparkling | Conventional | Basque Country |
| 63 | Cider | Natural Sparkling | Conventional | Basque Country |
| 64 | Cider | Natural Sparkling | Conventional | Basque Country |
| 65 | Cider | Natural | Conventional | Basque Country |
| 66 | Cider | Natural | Conventional | Basque Country |
| 67 | Cider | Natural | Conventional | Basque Country |

found among the available “cidere.” To mitigate potential biases, the samples were randomized and blinded.

The traditional cidere encompassed three styles: natural ($n = 24$), sparkling ($n = 6$), and natural sparkling ($n = 11$). The alcohol concentration reported on the labels ranged from 3.5% to 8.5%. All samples were contained in glass bottles to prevent interference in the subsequent analyses. Each sample received a unique code for identification in the analysis process. To prepare the samples, each bottle was opened to allow degassing for a minimum of 72 h. Subsequently, 100 mL of each sample was collected in plastic containers and stored at $-20\text{ }^{\circ}\text{C}$. Prior to each treatment, any residual gas was eliminated by subjecting the sample to ultrasonication for a duration of 30 min.

2.2. Reagents, chemicals, and standards

We utilized analytic-grade acetonitrile, methanol, acetone, and formic acid procured from Honeywell (USA), while ultrapure water was produced in-house. Mycotoxin standards, including aflatoxins, fumonisins, ochratoxin, T-2 and HT-2 toxins, deoxynivalenol, and zearalenone, were supplied by Trilogy (USA), with a zearalenone compound serving as an internal standard. Stock solutions were stored in methanol at $-20\text{ }^{\circ}\text{C}$, and working solutions were maintained at $4\text{ }^{\circ}\text{C}$.

Certified pesticide standards, aligning with the EU multi-annual plan, were obtained from CPA Chem (Bulgaria) and other suppliers. A working solution containing all pesticides at $0.833\text{ }\mu\text{g}/\text{mL}$ was prepared by combining commercial mixtures and in-house stock solutions. Certified Persistent Organic Pollutant (POP) standards were acquired in five mixes from CPA Chem., and procedural internal standards were sourced from multiple suppliers.

Analysis of fifty elements, encompassing essential nutrients, rare earth, and other minority elements, was conducted. Standards for all elements were acquired in acid solution from CPA Chem (Bulgaria). Four elements were employed as internal standards in a mixed solution at a stock concentration of $20\text{ mg}/\text{mL}$ each.

Sample preparation and instrumental analysis of contaminants were carried out using gas and liquid chromatography coupled with mass spectrometry (GC-MS/MS and LC-MS/MS) and inductively coupled plasma with mass spectrometry (ICP-MS). These methods were adapted from our prior investigation of beer samples (Alonso González et al., 2023). The subsequent subsections provide concise descriptions of the procedures.

2.3. Sample preparation

Mycotoxin Analysis: We prepared samples by diluting cidere with ultrapure water (1:1, v/v) and adding the synthetic mycotoxin analog zearalenone (ZAN) as internal standard. We created a 12-level calibration curve, spanning $500\text{--}0.02\text{ ng}/\text{mL}$, by adding working mixes to cidere samples that previously tested negative for a mycotoxin. This method was previously validated using in-house fortified samples.

Pesticide and POP residue analyses: We adopted a QuEChERS-based method to extract selected pesticides and POPs. Each cidere sample was vigorously shaken, followed by ultrasonic treatment. After addition of reagents and another round of shaking, the sample was centrifuged, filtered, and analyzed in GC-MS/MS or LC-MS/MS. Quality Control samples, blanks, and a ten-point calibration curve were prepared for a cidere free from the selected analytes, using the same methodology. All samples were added to a procedural internal standard mix solution and left in the dark prior to extraction.

Elemental analysis: We subjected cidere samples to agitation and sonication, then they were mixed with nitric acid and digested in a microwave oven. The internal standard solution was added to each vessel. The digested sample was transferred into an ICP-MS analysis tube, with an internal standard included for recovery control. We performed all determinations in triplicate. The method was earlier validated using in-house fortified cidere samples, yielding recoveries of

81–114% for toxic and essential elements and regression coefficients >0.998 for all elements.

2.4. Instrumental analysis

Analysis of contaminants, including pesticides, POPs, and mycotoxins in cidere was conducted using gas and liquid chromatography coupled with mass spectrometry (GC-MS/MS and LC-MS/MS). For the confirmation of compound identity, 2 MRM transitions were used: one for quantification and one for confirmation. In relation to the standards in the calibration curve, a maximum deviation of $\pm 30\%$ was tolerated for the ion ratio. In the same way, a maximum deviation of $\pm 0.1\text{ min}$ was established for the retention time. The detailed technical parameters, equipment, and materials are given in Tables S1 and S2. The LOD is provided in Table S3.

The GC-MS/MS utilized a GC System 7890B with a Triple Quad 7010 mass spectrometer (Agilent Technologies), back-flushed with helium (99.999% purity) as carrier gas. Chromatographic separations involved two fused silica ultra-inert capillary columns connected in series, with a controlled temperature ramp and specific gas flow settings for optimal results. Electron impact ionization was used in multiple reaction monitoring mode.

The LC-MS/MS was performed with a 1290 Infinity II LC System and Triple Quad 6460 mass spectrometer (Agilent Technologies). A Poroshell 120 EC-C18 column and a pre-column were used for separation. The Agilent Jet Stream Electrospray Ionization Source was used in both positive and negative ionization mode with dynamic multiple reaction monitoring. Different methods were used for pesticides and mycotoxins. Nitrogen was used as desolvation, drying, and collision gas. Data from both GC-MS/MS and LC-MS/MS were analyzed using Agilent’s MassHunter Quantitative Analysis software (for QQQ) version B.07.01.

Elemental analysis was conducted using an Agilent 7900 ICP-MS with standard nickel cones and a crossflow nebulizer. The procedure ensured avoidance of isobaric polyatomic interferences by using different isotopes and the Octopolar Reaction System (ORS4) in helium mode. A cleaning solution was used to avoid the memory effect associated with mercury. The ICP-MS data was processed with Agilent MassHunter data analysis software (version 4.2).

2.5. Data analysis

The mean and standard deviation for each pesticide, mycotoxin, and element under analysis is presented in Table 2. We employed software R (version 4.0.5) to perform a first t -test analysis for each group to check if the difference between groups was statistically significant. Then, we analyzed variance using Stata (version SE 17) (ANOVA) in all groups to explore differences in the concentrations between traditional cidere and “cidere”. Finally, the relationship between pesticides with statistically significant differences and elemental composition was assessed through an OLS regression, using robust standard errors that permit correcting for heteroscedasticity.

3. Results and discussion

3.1. Pesticide residues

Pesticides are routinely applied to apples for the management of various pests and diseases, particularly insecticides and fungicides (Ioriatti et al., 2011). In Spain, major apple orchards are situated in the humid northern regions of Galicia, Asturias, and the Basque Country, leading to heightened fungal pressure. Agrochemicals are employed to combat black-rot, cedar apple-rust, flyspeck, powdery mildew, apple-scab, and a range of insects such as maggots, moths, and various aphids (Ticha et al., 2008). While these treatments enhance productivity and profitability, residues may persist, especially in apple peel, and be transmitted to cidere during the pressing process (Zee et al., 1973).

Table 2
Summary of statistics for pesticides.

| Component | Total | Cider | “Cider” | (2)–(3) | MRL |
|-----------------------|----------------|-----------------|----------------|---------------------|--------------|
| | Mean (sd) | Mean (sd) | Mean (sd) | Diff. [p-value] | |
| | (1) | (2) | (3) | (4) | |
| Acetamiprid | 0.20 (0.64) | 0.235 (0.80) | 0.16 (0.30) | 0.077 [0.63] | 400 µg/kg |
| Range | 0.00–4.63 | 0.00–4.63 | 0.00–1.04 | | |
| Azoxystrobin | 0.01 (0.05) | 0.000 (0.00) | 0.02 (0.08) | –0.016 [0.24] | 10 µg/kg |
| Range | 0.00–0.44 | 0.00–0.00 | 0.00–0.44 | | |
| Boscalid | 0.01 (0.07) | 0.008 (0.05) | 0.02 (0.08) | –0.014 [0.39] | 2000 µg/kg |
| Range | 0.00–0.34 | 0.00–0.32 | 0.00–0.34 | | |
| Carbendazim | 0.09 (0.34) | 0.132 (0.43) | 0.04 (0.16) | 0.091 [0.29] | 200 mg/kg |
| Range | 0.00–1.81 | 0.00–1.81 | 0.00–0.78 | | |
| Chlorantranilprole | 0.02 (0.06) | 0.000 (0.00) | 0.04 (0.10) | –0.043*** [0.01] | 400 µg/kg |
| Range | 0.00–0.28 | 0.00–0.00 | 0.00–0.28 | | |
| Fluopyram | 0.09 (0.27) | 0.011 (0.07) | 0.19 (0.39) | –0.180*** [0.01] | 800 µg/kg |
| Range | 0.00–1.26 | 0.00–0.41 | 0.00–1.26 | | |
| Imazalil | 0.07 (0.23) | 0.024 (0.15) | 0.13 (0.29) | –0.109* [0.05] | 10 µg/kg |
| Range | 0.00–0.95 | 0.00–0.93 | 0.00–0.95 | | |
| Imidacloprid | 0.14 (0.46) | 0.000 (0.00) | 0.32 (0.67) | –0.324*** [0.00] | 10 µg/kg |
| Range | 0.00–2.42 | 0.00–0.00 | 0.00–2.42 | | |
| Mepiquat | 0.30 (1.01) | 0.046 (0.29) | 0.65 (1.47) | –0.609** [0.01] | 20 µg/kg |
| Range | 0.00–5.80 | 0.00–1.79 | 0.00–5.80 | | |
| Methoxyphenozide | 0.03 (0.10) | 0.022 (0.12) | 0.04 (0.06) | –0.020 [0.42] | 10 µg/kg |
| Range | 0.00–0.74 | 0.00–0.74 | 0.00–0.31 | | |
| N-dimethylformamidine | 0.09 (0.51) | 0.150 (0.67) | 0.00 (0.00) | 0.150 [0.24] | N.A. |
| Range | 0.00–3.47 | 0.00–3.47 | 0.00–0.00 | | |
| Pyrimicarb | 0.04 (0.09) | 0.038 (0.10) | 0.04 (0.06) | –0.000 [0.99] | 500 µg/kg |
| Range | 0.00–0.59 | 0.00–0.59 | 0.00–0.16 | | |
| Pyrimethanil | 0.21 (0.55) | 0.093 (0.24) | 0.37 (0.79) | –0.274** [0.04] | 15,000 µg/kg |
| Range | 0.00–3.70 | 0.00–1.05 | 0.00–3.70 | | |
| Tebufoenozide | 0.03 (0.08) | 0.012 (0.04) | 0.05 (0.11) | –0.041** [0.04] | 1000 µg/kg |
| Range | 0.00–0.41 | 0.00–0.19 | 0.00–0.41 | | |
| Thiabendazole | 0.09 (0.35) | 0.019 (0.09) | 0.19 (0.53) | –0.174** [0.05] | 4000 µg/kg |
| Range | 0.00–2.47 | 0.00–0.48 | 0.00–2.47 | | |
| Thiacloprid | 0.14 (0.43) | 0.021 (0.07) | 0.30 (0.63) | –0.277*** [0.01] | 300 µg/kg |
| Range | 0.00–3.07 | 0.00–0.31 | 0.00–3.07 | | |
| Thiophanate-methyl | 0.02 (0.10) | 0.008 (0.05) | 0.05 (0.14) | –0.038 [0.12] | 500 µg/kg |
| Range | 0.00–0.51 | 0.00–0.32 | 0.00–0.51 | | |
| (n) | 67 | 39 | 28 | | |

Summary of statistics (mean, standard deviation, and range - minimum-maximum) of the pesticides in µg/L. Column (1) shows the results for the whole sample, while columns (2) and (3) detail those data for the subsamples of traditional ciders (*sídras*) and novel “ciders”, respectively. Column (4) displays the difference between the subsamples and p-values computed using ANOVA. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

Although limited research has been conducted on commercial ciders, experimental analyses suggest a substantial reduction of pesticides from apples to cider, primarily through sorption to dry matter during fermentation (Banna & Kawar, 1982). Nevertheless, the remaining contaminants pose potential risks to consumers. Despite the necessity to monitor and control pesticide levels in cider, the European Union (EU) has not established a Maximum Residue Limit (MRL) specifically for cider. Consequently, we adhere to the limits set for apples in our study.

Out of the 275 compounds tested for presence, we detected 17 pesticide residues (see Table 2). Furthermore, only one of the 50 persistent organic pollutants (PCB 52) was found. Among the samples, 24 were free of residues, 14 exhibited one residue, and 29 showed more

than one. In total, 67 pesticide residues were identified, commonly associated with apple orchard treatments. The most prevalent was pyrimethanil (n = 27), followed by pyrimicarb (n = 19), methoxyphenozide (n = 17), thiacloprid, and acetamiprid (n = 13 each). The occurrence of other pesticides was below n = 10 (Fig. 1). Pyrimethanil, a broad-spectrum systemic fungicide, was notably high, linked to post-harvest treatments due to the extended period between harvest and cider-making, leading to increased patulin levels resulting from mold development (Bücheler et al., 2022). In comparing ciders and “ciders,” distinct differences in pesticide profiles were evident. Seventeen ciders were free of residues, while only seven “ciders” achieved this status. Additionally, 12 ciders had one residue, while 10 had more than one,

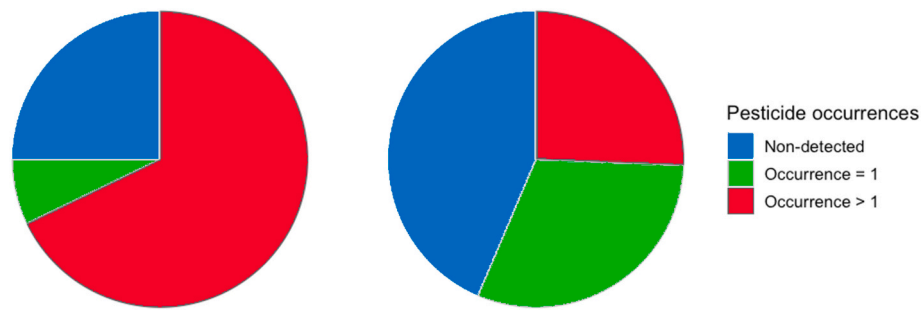


Fig. 1. Occurrence by type of product. Left: cider; Right: "cider".

whereas two "ciders" had one, and 19 had one or more residues (See Table 3). One "cider" exhibited 11 different residues, four with nine residues each, and the cider with the most residues contained only five.

Among pesticides with high occurrences, "ciders" showed statistically significant higher levels of pyrimethanil (0.21 vs 0.093 $\mu\text{g/L}$; $P < 0.274$) and thiacloprid (0.30 vs 0.021 $\mu\text{g/L}$; $P < 0.277$) compared to ciders (see Table 4). The elevated pyrimethanil levels in "ciders" may be attributed to differences in production methods, with traditional cider production dependent on apple harvest periods, pressing, and fermentation, while "ciders" are often produced on demand, independent of such cycles. Additionally, "cider" production facilities are typically more distant from apple orchards compared to traditional cider production. The presence of thiacloprid in "ciders" is noteworthy as this insecticide targeting aphids and whiteflies was banned in the European Union in April 2020. Its presence in "ciders" suggests potential illegal use, reconstitution of stored apple juice predating 2020, or importation of apples/juice from countries where thiacloprid is still legal for "cider" production. "Ciders" also exhibited statistically significant levels of imidacloprid, tebufenozide, chlorantraniliprole (insecticides), thiabendazole, imazalil, fluopyram (fungicides), and the growth regulator mepiquat. Traditional ciders showed higher average levels of the insecticide acetamiprid, N-dimethylformamide (DMF), and the fungicide carbendazim, although the differences were not statistically significant (see Table S4). No MRL exceedances were observed, with all samples well below the threshold, establishing cider as a safer product compared to other fermented beverages such as wine or beer (Čuš et al., 2010; Pires et al., 2021).

Our results align with Zušáková et al. (2023), who found at least two residues of eighteen pesticides in twenty samples of Czech and foreign ciders. Notably, regional differences were observed among traditional ciders, with Canary Islands ciders showing the lowest occurrences and

Table 3
Number of pesticides in cider samples.

| Pesticide | Number |
|---------------------|--------|
| Acetamiprid | 13 |
| Azoxystrobin | 1 |
| Boscalid | 3 |
| Carbendazim | 6 |
| Chlorantraniliprole | 5 |
| Fluopyram | 7 |
| Imazalil | 6 |
| Imidacloprid | 7 |
| Mepiquat | 7 |
| Methoxyphenozide | 17 |
| N-dimethylformamide | 2 |
| Pyrimicarb | 19 |
| Pyrimethanil | 27 |
| Tebufenozide | 9 |
| Thiabendazole | 9 |
| Thiacloprid | 13 |
| Thiophanate-methyl | 4 |

Number of times each pesticide was detected (occurred) in the cider samples ($n = 67$).

Table 4
Pesticide detections in types of cider.

| Number | Total | Cider | "Cider" |
|--------|-------|-------|---------|
| 0 | 24 | 17 | 7 |
| 1 | 14 | 12 | 2 |
| >1 | 29 | 10 | 19 |

Number of times zero, one, or more than one pesticide were detected in the total sample ($N = 67$) and the subsamples according to type of product (cider or "cider").

residues, followed by Basque and Asturian ciders with the most occurrences and the widest residue spectrum. The inclusion of six ciders with organic certification revealed low pesticide residue levels, albeit their presence in the final product emphasizes the need for stricter monitoring to ensure pesticide-free products for consumers. This issue is particularly significant in Spain, one of the largest pesticide consumers globally (Alonso González et al., 2021).

Despite the presence of pesticide residues in Spanish ciders (both traditional and new "ciders"), it is crucial to note that these levels are significantly lower than those reported for commercially available beers and conventionally produced wines in Spain. Spanish ciders demonstrated a mean level of 1.33 $\mu\text{g/L}$, substantially lower than the 29.02 $\mu\text{g/L}$ found in both industrial and craft beers ($P < 0.005$) (Alonso González et al., 2023) and remarkably lower than the 109.81 $\mu\text{g/L}$ detected in conventional wines ($P < 0.0001$) (Alonso González et al., 2022). These findings are consistent with global literature on fermented beverages (Schusterova et al., 2021; Walsh et al., 2016, 2016uš et al., 2010).

Moreover, the average number of residues per sample highlights significant differences among these alcoholic beverages. Spanish ciders, with an average of 1.91 pesticide residues per analyzed sample, contrast starkly with the 5.26 residues found in the most consumed Spanish beers (Alonso González et al., 2023) and the 4.14 residues in wines (Alonso González et al., 2022). These results underscore substantial variations in pesticide residue levels among popular beverages, both in Spain and globally. However, further research is essential to understand the underlying reasons for these differences, with the potential role of additional ingredients and additives beyond apples in "ciders" requiring investigation. The lack of compulsory ingredient labeling in alcoholic beverages globally, including cider (Staples et al., 2023), currently hinders comparison and understanding of the toxicological profile.

3.2. Elemental composition

The analysis of the elemental composition encompassed essential elements (9) and trace elements with known toxicity or potential toxicity, including rare earths. These constituents play a pivotal role in human nutrition, while elements like mercury, lead, or cadmium can be highly toxic at elevated concentrations or upon repeated ingestion. Knowledge about the elemental composition of ciders is crucial not only for evaluating potential health risks associated with consumption but also for discerning the origin and authenticity of the product (Gajek

et al., 2021). However, only traditional ciders bearing an appellation of origin can be definitively linked to a specific region (only 6 of our samples in total). In contrast, “ciders” can be produced anywhere using apple juices from various origins. Given this disparity and the limited comparative data on ciders worldwide, elemental analysis proves most valuable in exploring potential health risks. However, as of now, neither the European Union nor other international agencies have established standards for maximum allowable concentration limits for hazardous substances in ciders. Consequently, we applied standards set for water by the World Health Organization (WHO, 2004) and for wine by the International Organization of Vine and Wine (OIV, 2018). The WHO has established standards for Al (0.9 mg/L), Ba (1.3 mg/L), Cd (0.003 mg/L), Cr (0.05 mg/L), Cu (2.00 mg/L), Ni (0.07 mg/L), and Pb (0.01 mg/L), while the OIV has set standards for As (0.2 mg/L), B (80 mg/L), Br (1 mg/L), Cd (0.01 mg/L), Cu (1 mg/L), Pb (0.15 mg/L), and Zn (5 mg/L).

Significant differences were observed in the presence and concentrations of macro- and trace-elements, including metals and metalloids, between traditional ciders and “ciders” (see Table 5 and Table 6). In summary, while both traditional ciders and “ciders” exhibit a comprehensive array of elements, the respective hierarchies and the detection of specific elements underscore their unique compositions. These variations in elemental profiles could serve as distinctive markers indicative of their distinct production methods, raw materials, and possibly, origin.

The average content of trace elements among the analyzed ciders varied significantly. Among traditional ciders, B, Br, Fe, Rb, and Si exceeded 1000 µg/L; followed by Al, Mn, Sr, and Zn between 100 and 1000; then Ba, Cr, Cu, Ni, and V between 10 and 100; As, Co, Cs, Ga, Mo, Pb, Sn, and Ti between 1 and 10; and finally, Au, Be, Cd, Ce, Dy, Er, Eu, Gd, Hg, Ho, La, Li, Lu, Nd, Os, Pd, Pr, Pt, Ru, Sb, Se, Sm, Ta, Tb, Th, Tm, U, Y, and Yb between 0 and 1 µg/L. For “ciders,” Fe and Si registered over 1000 µg/L; then Al, B, Br, Mn, and Rb between 100 and 1000; Ba, Cu, Sr, V, and Zn between 10 and 100; As, Ce, Co, Cr, Cs, Ga, Li, Mo, Ni, Sn, and Ti between 1 and 10; and lastly, Au, Be, Cd, Dy, Er, Eu, Gd, Hg, Ho, La, Lu, Nd, Os, Pb, Pd, Pr, Pt, Ru, Se, Sm, Ta, Tb, Th, Tm, U, Y, and Yb between 0 and 1 µg/L.

In general, traditional ciders exhibited higher average concentrations of most elements, attributable to the absence of the clarification and filtration techniques employed in “cider” production, which reduce

overall content of organic and non-organic matter (see Table S5). Beyond differences in concentrations and hierarchy of elements, ANOVA revealed other significant distinctions between traditional ciders and “ciders.” Ciders contained statistically higher average levels of the macro-elements K, Mg, and P and trace-elements B, Br, Co, Cr, Cs, Pb, Rb, and Ru. In turn, “ciders” had statistically higher levels of Cu, Li, Si, Ta, and U. The elemental composition of both types of ciders differed significantly from beers and wine. For instance, after studying craft and mainstream beers in Spain, Alonso González et al. (2023) found that only Mn ranged between 100 and 1000 µg/L among the craft beers analyzed, followed by Zn, Sr, Ni, Fe, Cu, Ba, and Al ranging between 10 and 100 µg/L. In contrast, traditional ciders presented overall higher amounts of elements and a completely different hierarchical ranking with B, Br, Fe, Rb, and Si at quantities higher than 1000 µg/L, followed by Al, Mn, Sr, and Zn between 100 and 1000 µg/L.

Cider displays more similarities with wine as a fruit-derived drink, rather than with beer, fermented from cereals. Indeed, and in line with previous research (Čepo et al., 2018; Santana-Mayor et al., 2020), Alonso González et al. (2021) also reported different results for wines, using a similar methodological approach. In wine, the group with concentrations above 1000 µg/L was Fe and Al, followed by Zn, Sr, Cu, Ba, and Mn ranging between 100 and 1000 µg/L. The similarities between wine and cider are greater in both hierarchical order and average concentration levels than for beer, but they are still relatively different.

Regarding the exceedance of maximum concentration limits of metals, no infringements occurred for B, Ba, Cd, Cr, Cu, and Zn. Aluminum exceeded the WHO limits for water 17 times; there were 20 infringements for Br in both OIV and WHO limits (OIV, 2018; WHO, 2004); 3 for Ni concerning WHO limits; and 7 for Pb for both OIV and WHO limits. For Al, 9 “ciders” and 8 traditional ciders exceeded the limits; and for Ni, one “cider” and two ciders. The clearest difference appears in Br exceedances (18 traditional ciders and only 2 “ciders”); and Pb (7 traditional ciders). This clear trend in Br and Pb levels of traditional ciders probably reflects different cidermaking processes and machinery, but more research is needed in this area to clarify the issue.

Among the six organic ciders, two surpassed the limits for aluminum (Al), and five out of six exceeded the thresholds for bromine (Br). The rationale behind these exceedances extends beyond the scope of this paper, and the scarcity of research on cider toxicology underscores the

Table 5
Summary statistics of macro elements.

| Component | Total | Cider | “Cider” | (2)–(3) |
|-----------|--------------------|----------------------|--------------------|----------------------|
| | Mean (sd) | Mean (sd) | Mean (sd) | Difference [p-value] |
| | (1) | (2) | (3) | (4) |
| Ca | 52.17 (23.47) | 51.298 (22.06) | 53.40 (25.68) | –2.098 [0.72] |
| Range | 13.50–132.69 | 32.37–132.69 | 13.50–109.14 | |
| K | 939.43 (431.26) | 1143.778 (230.60) | 654.80 (485.25) | 488.975*** [0.00] |
| Range | 222.61–2434.81 | 738.03–1981.25 | 222.61–2434.81 | |
| Mg | 39.47 (19.12) | 46.930 (14.43) | 29.09 (20.22) | 17.844*** [0.00] |
| Range | 13.76–110.88 | 31.65–98.18 | 13.76–110.88 | |
| Na | 43.27 (59.93) | 49.298 (70.91) | 34.89 (39.74) | 14.412 [0.34] |
| Range | 6.18–304.51 | 6.18–304.51 | 12.10–187.38 | |
| P | 56.13 (40.83) | 65.763 (47.13) | 42.72 (25.08) | 23.044** [0.02] |
| Range | 16.85–340.74 | 34.64–340.74 | 16.85–115.53 | |
| S | 58.05 (45.07) | 56.005 (51.91) | 60.90 (34.05) | –4.893 [0.66] |
| Range | 0.45–188.13 | 0.45–185.37 | 0.79–188.13 | |
| (N) | 67 | 39 | 28 | |

This table presents the summary statistics (mean, standard deviation, and range –composed by the minimum and maximum value–) of the macro-elements in mg/L. Column (1) shows the results for the whole sample, while columns (2) and (3) do the same for the subsamples of the observations that contain sidra and cider, respectively. Column (4) displays the difference between subsamples and the p-value of this difference computed using an ANOVA. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

Table 6
Summary statistics of elemental composition.

| Component | Total | Cider | “Cider” | (2)–(3) |
|-----------|----------------------|-----------------------|----------------------|----------------------|
| | Mean (sd) | Mean (sd) | Mean (sd) | Difference [p-value] |
| | (1) | (2) | (3) | (4) |
| Al | 698.07 (612.11) | 664.940 (525.61) | 744.22 (723.37) | −79.284 [0.60] |
| Range | 121.46–3014.50 | 121.46–2192.59 | 127.38–3014.50 | |
| As | 1.31 (3.28) | 1.047 (1.81) | 1.68 (4.63) | −0.633 [0.44] |
| Range | 0.00–18.54 | 0.00–7.53 | 0.00–18.54 | |
| Au | 0.11 (0.19) | 0.132 (0.19) | 0.09 (0.18) | 0.044 [0.35] |
| Range | 0.00–0.87 | 0.00–0.87 | 0.00–0.74 | |
| B | 1152.78 (713.64) | 1422.662 (617.70) | 776.88 (675.41) | 645.785*** [0.00] |
| Range | 112.50–2831.05 | 258.29–2831.05 | 112.50–2624.21 | |
| Ba | 67.44 (59.91) | 76.263 (63.85) | 55.15 (52.61) | 21.114 [0.16] |
| Range | 16.75–255.16 | 18.53–219.72 | 16.75–255.16 | |
| Be | 0.33 (0.69) | 0.388 (0.67) | 0.26 (0.72) | 0.130 [0.45] |
| Range | 0.00–3.30 | 0.00–2.39 | 0.00–3.30 | |
| Bi | 0.00 (0.00) | 0.000 (0.00) | 0.00 (0.00) | 0.000 [0.00] |
| Range | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Br | 931.60 (596.27) | 1156.539 (602.78) | 618.29 (427.59) | 538.251*** [0.00] |
| Range | 211.68–2674.52 | 309.37–2674.52 | 211.68–2513.13 | |
| Cd | 0.04 (0.17) | 0.012 (0.04) | 0.07 (0.27) | −0.055 [0.21] |
| Range | 0.00–1.40 | 0.00–0.17 | 0.00–1.40 | |
| Ce | 0.98 (1.71) | 0.954 (1.92) | 1.01 (1.38) | −0.051 [0.91] |
| Range | 0.00–11.35 | 0.00–11.35 | 0.00–5.38 | |
| Co | 1.83 (1.65) | 2.164 (1.61) | 1.36 (1.62) | 0.804** [0.05] |
| Range | 0.00–8.36 | 0.34–7.18 | 0.00–8.36 | |
| Cr | 10.86 (9.06) | 13.780 (7.60) | 6.79 (9.47) | 6.989*** [0.00] |
| Range | 1.46–50.55 | 3.58–40.64 | 1.46–50.55 | |
| Cs | 4.53 (4.26) | 5.880 (3.42) | 2.64 (4.65) | 3.241*** [0.00] |
| Range | 0.00–20.93 | 0.94–16.19 | 0.00–20.93 | |
| Cu | 63.68 (123.92) | 39.340 (83.44) | 97.58 (160.29) | −58.239* [0.06] |
| Range | 0.00–486.11 | 0.00–486.11 | 0.00–469.66 | |
| Dy | 0.13 (0.18) | 0.133 (0.19) | 0.13 (0.15) | 0.007 [0.87] |
| Range | 0.00–1.08 | 0.00–1.08 | 0.00–0.65 | |
| Er | 0.09 (0.10) | 0.097 (0.11) | 0.07 (0.09) | 0.028 [0.27] |
| Range | 0.00–0.55 | 0.00–0.55 | 0.00–0.38 | |
| Eu | 0.03 (0.06) | 0.032 (0.07) | 0.03 (0.05) | −0.000 [0.98] |
| Range | 0.00–0.42 | 0.00–0.42 | 0.00–0.21 | |
| Fe | 1288.10 (1774.75) | 1372.850 (1898.01) | 1170.05 (1613.83) | 202.803 [0.65] |
| Range | 94.43–10,155.98 | 320.06–10,155.98 | 94.43–7620.22 | |
| Ga | 25.83 (22.70) | 29.090 (24.12) | 21.29 (20.11) | 7.802 [0.17] |
| Range | 6.38–96.77 | 7.33–83.79 | 6.38–96.77 | |
| Gd | 0.13 (0.19) | 0.128 (0.21) | 0.12 (0.16) | 0.006 [0.89] |
| Range | 0.00–1.21 | 0.00–1.21 | 0.00–0.67 | |
| Hg | 0.05 (0.10) | 0.047 (0.09) | 0.04 (0.12) | 0.004 [0.89] |
| Range | 0.00–0.61 | 0.00–0.39 | 0.00–0.61 | |
| Ho | 0.03 (0.04) | 0.031 (0.04) | 0.03 (0.03) | 0.001 [0.91] |
| Range | 0.00–0.21 | 0.00–0.21 | 0.00–0.15 | |
| In | 0.00 (0.00) | 0.000 (0.00) | 0.00 (0.00) | 0.000 [0.00] |
| Range | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| La | 0.47 (0.80) | 0.403 (0.86) | 0.57 (0.70) | −0.166 [0.41] |

(continued on next page)

Table 6 (continued)

| Component | Total | Cider | “Cider” | (2)–(3) |
|-----------|----------------------|-----------------------|----------------------|----------------------|
| | Mean (sd) | Mean (sd) | Mean (sd) | Difference [p-value] |
| | (1) | (2) | (3) | (4) |
| Range | 0.00–5.07 | 0.00–5.07 | 0.00–2.62 | |
| Li | 1.55 (2.54) | 0.817 (1.92) | 2.56 (2.95) | –1.746*** [0.00] |
| Range | 0.00–10.40 | 0.00–8.68 | 0.00–10.40 | |
| Lu | 0.02 (0.02) | 0.020 (0.02) | 0.01 (0.02) | 0.006 [0.19] |
| Range | 0.00–0.08 | 0.00–0.08 | 0.00–0.07 | |
| Mn | 378.37 (674.54) | 326.425 (125.57) | 450.72 (1039.57) | –124.294 [0.46] |
| Range | 54.23–4485.11 | 110.10–740.92 | 54.23–4485.11 | |
| Mo | 5.15 (12.53) | 4.920 (9.38) | 5.47 (16.11) | –0.549 [0.86] |
| Range | 0.00–68.67 | 0.00–40.38 | 0.00–68.67 | |
| Nb | 0.00 (0.00) | 0.000 (0.00) | 0.00 (0.00) | 0.000 [0.00] |
| Range | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Nd | 0.59 (0.94) | 0.572 (1.06) | 0.62 (0.77) | –0.050 [0.83] |
| Range | 0.00–6.25 | 0.00–6.25 | 0.00–3.16 | |
| Ni | 12.02 (20.63) | 14.591 (21.93) | 8.43 (18.46) | 6.161 [0.23] |
| Range | 0.00–115.21 | 2.17–115.21 | 0.00–83.75 | |
| Os | 0.01 (0.02) | 0.009 (0.03) | 0.00 (0.00) | 0.008 [0.21] |
| Range | 0.00–0.16 | 0.00–0.16 | 0.00–0.03 | |
| Pb | 5.03 (12.21) | 8.001 (15.33) | 0.90 (1.84) | 7.104** [0.02] |
| Range | 0.00–68.74 | 0.00–68.74 | 0.00–7.00 | |
| Pd | 0.01 (0.02) | 0.012 (0.02) | 0.00 (0.01) | 0.008 [0.12] |
| Range | 0.00–0.11 | 0.00–0.11 | 0.00–0.07 | |
| Pr | 0.14 (0.22) | 0.127 (0.25) | 0.15 (0.18) | –0.019 [0.73] |
| Range | 0.00–1.48 | 0.00–1.48 | 0.00–0.71 | |
| Pt | 0.01 (0.03) | 0.012 (0.04) | 0.00 (0.00) | 0.011 [0.14] |
| Range | 0.00–0.22 | 0.00–0.22 | 0.00–0.00 | |
| Rb | 909.12 (729.96) | 1146.352 (580.79) | 578.68 (795.75) | 567.669*** [0.00] |
| Range | 77.67–3389.78 | 525.32–2501.91 | 77.67–3389.78 | |
| Ru | 0.00 (0.00) | 0.001 (0.00) | 0.00 (0.00) | 0.000** [0.02] |
| Range | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Sb | 0.04 (0.32) | 0.067 (0.42) | 0.00 (0.00) | 0.067 [0.40] |
| Range | 0.00–2.62 | 0.00–2.62 | 0.00–0.00 | |
| Se | 0.59 (1.60) | 0.582 (1.00) | 0.61 (2.20) | –0.026 [0.95] |
| Range | 0.00–11.56 | 0.00–3.63 | 0.00–11.56 | |
| Si | 5732.63 (9350.02) | 3539.248 (9561.73) | 8787.69 (8270.27) | 5248.45** [0.02] |
| Range | 59.01–58,567.13 | 77.86–58,567.13 | 59.01–31,721.88 | |
| Sm | 0.12 (0.19) | 0.123 (0.22) | 0.12 (0.15) | 0.004 [0.93] |
| Range | 0.00–1.30 | 0.00–1.30 | 0.00–0.60 | |
| Sn | 3.41 (4.92) | 3.760 (5.35) | 2.92 (4.28) | 0.845 [0.49] |
| Range | 0.00–24.81 | 0.01–24.81 | 0.00–17.47 | |
| Sr | 149.12 (138.37) | 136.548 (159.90) | 166.64 (101.37) | –30.090 [0.38] |
| Range | 20.31–642.01 | 20.31–642.01 | 61.70–477.58 | |
| Ta | 0.04 (0.07) | 0.019 (0.04) | 0.06 (0.09) | –0.046*** [0.01] |
| Range | 0.00–0.36 | 0.00–0.19 | 0.00–0.36 | |
| Tb | 0.02 (0.03) | 0.022 (0.03) | 0.02 (0.03) | 0.003 [0.72] |
| Range | 0.00–0.19 | 0.00–0.19 | 0.00–0.12 | |
| Th | 0.10 (0.19) | 0.097 (0.24) | 0.09 (0.10) | 0.002 [0.96] |
| Range | 0.00–1.51 | 0.00–1.51 | 0.00–0.37 | |
| Ti | 17.37 (25.13) | 14.882 (19.47) | 20.85 (31.45) | –5.965 [0.34] |

(continued on next page)

Table 6 (continued)

| Component | Total | Cider | “Cider” | (2)–(3) |
|-----------|-------------------|---------------------|-------------------|----------------------|
| | Mean (sd) | Mean (sd) | Mean (sd) | Difference [p-value] |
| | (1) | (2) | (3) | (4) |
| Range | 0.93–143.75 | 0.93–89.30 | 4.13–143.75 | |
| Tl | 0.00 (0.00) | 0.000 (0.00) | 0.00 (0.00) | 0.000 [0.00] |
| Range | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Tm | 0.01 (0.02) | 0.017 (0.02) | 0.01 (0.02) | 0.006 [0.18] |
| Range | 0.00–0.08 | 0.00–0.08 | 0.00–0.07 | |
| U | 0.54 (0.74) | 0.325 (0.46) | 0.83 (0.93) | –0.505*** [0.00] |
| Range | 0.00–2.72 | 0.00–1.46 | 0.00–2.72 | |
| V | 42.82 (113.91) | 25.879 (66.99) | 66.42 (156.23) | –40.540 [0.15] |
| Range | 0.07–627.23 | 0.07–353.59 | 0.12–627.23 | |
| Y | 0.81 (0.98) | 0.824 (1.03) | 0.79 (0.91) | 0.031 [0.90] |
| Range | 0.00–5.77 | 0.00–5.77 | 0.08–4.01 | |
| Yb | 0.10 (0.11) | 0.107 (0.11) | 0.09 (0.11) | 0.022 [0.44] |
| Range | 0.00–0.51 | 0.00–0.51 | 0.00–0.51 | |
| Zn | 99.75 (149.45) | 117.082 (163.23) | 75.62 (126.71) | 41.466 [0.27] |
| Range | 0.00–854.83 | 9.39–854.83 | 0.00–380.55 | |
| (n) | 67 | 39 | 28 | |

This table presents the summary statistics (mean, standard deviation, and range—composed by the minimum and maximum value—) of the elements in µg/L. Column (1) shows the results for the whole sample. Columns (2) and (3) show the subsamples of the observations that contain cider and “cider”, respectively. Column (4) presents the difference between subsamples and the p-value of this difference computed using an ANOVA. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

urgency of providing an explanation. In general, the elemental composition of a food product is intricately linked to soil characteristics and plant uptake processes, as different elements can be selectively absorbed through the roots of plants. Anthropogenic factors also play a pivotal role in influencing elemental composition, notably the use of fertilizers and pesticides in orchards, air pollution, additives utilized in the cellar, machinery employed during cider production (Tariba, 2011), and vessels/recipients such as metal cans (beyond our sampled range) and unlined bottle-tops.

Elevated concentrations of aluminum in wines have been associated with the use of bentonite clay for fining and contact with surfaces of this reactive metal (Tariba, 2011). Concentrations exceeding 10 mg/L have been demonstrated to result in aluminum clouding, a phenomenon analogous to cupric and ferric cloudiness in enology (Tariba, 2011). The use of bentonite is permitted under Spanish cider legislation (Ministerio de la Presidencia y para las Administraciones Territoriales, 2017). Bromide levels, although generally problematic in drinking water, have not been identified as a risk through food consumption (World Health Organization, 2009). However, the OIV has established limits for bromine in wines. The presence of bromine compounds in wine has been linked to environmental contamination in cellars, corks, and barrels that may transfer to the wine, prompting proposed remediation strategies (Palacios et al., 2010).

Contamination with both nickel (Ni) and lead (Pb) can often be attributed to the use of cellar machinery, such as stainless-steel deposits, pipes, filters, etc., similar to the situation in wine production (Wyrzykowska et al., 2001). Additionally, many stainless-steel deposits are made with an alloy that includes some zinc (Zn) to prevent corrosion. Contamination with lead often results from the continued use of old machinery or plumbing, including metal-wooden apple presses and press-housing. Lead levels have also been linked to bentonite and colloidal clay use (Gajek et al., 2021). The absence of other metals such as copper (Cu), employed in various plant protection formulations, indicates the low levels of pesticides used in cider production compared to wine or even beer. In conclusion, despite the various exceedances highlighted in this study, the absence of standards for ciders and other low-alcohol beverages poses a challenge in determining whether the

analyzed samples are safe for consumers or not.

3.3. Mycotoxins

Mycotoxins pose a significant concern for various fermented beverages, with beer being particularly scrutinized. Patulin, the primary mycotoxin affecting apples and apple-derived products, including cider, has been the central focus of numerous surveys and toxicological investigations on ciders (Harris et al., 2009; Jackson et al., 2003; Murillo-Arbizu et al., 2009). The mold responsible for patulin production is *Penicillium expansum*, particularly prevalent when fallen or decaying apples are collected, subsequently leading to contamination during storage and transportation (Wang et al., 2022). Interestingly, patulin residues, along with those of most analyzed mycotoxins such as aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), deoxynivalenol (DON), fumonisin B2 (FB2), T-2 and HT-2 toxins, ochratoxin A (OTA), and zearalenone (ZEN), were not detected in our study.

However, residues of fumonisin B1 (FB1) were identified in seven samples, ranging from 3.57 to 3.99 µg/L. Three of these samples were categorized as “ciders,” and four as traditional ciders. Fumonisin typically pose concerns in cereal and cereal-derived products, particularly in corn; their presence in ciders had not been reported before (Azam et al., 2021). Fumonisin B1 is known to be present at elevated levels in fermented beverages like beer (Piacentini et al., 2015). Classified as possibly carcinogenic to humans by the IARC, especially concerning esophageal cancer (Franceschi et al., 1990), its detection in cider is a notable finding. Additionally, T-2 toxin was found in ten samples, with concentrations ranging from 0.76 to 4.44 µg/L, affecting four “ciders” and six traditional ciders. T-2 toxin is primarily produced by various *Fusarium* species and has been identified in cereals, cereal drinks, and plant-based milks but not in cider until now (Azam et al., 2021). Associated with hematotoxicity, myelotoxicity, and growth retardation, its presence in cider raises potential health concerns, especially in cereals and cereal-derived products (European Commission, 2006).

However, it is essential to note that the levels of both mycotoxins detected were well below the Maximum Residue Limits (MRLs) set for most food products in the European Union (European Commission,

2006). In conclusion, the cider samples analyzed in this study exhibit considerably lower occurrences and average levels of mycotoxins, particularly when compared to beers. For instance, Rodríguez-Carrasco et al. (2015) found higher levels of T2 ranging from 24.2 to 38.2 µg/L in 14 out of 154 beers, all classified as wheat beer style. Moreover, the high prevalence of fumonisins in mainstream beers from Italy and Spain, reported at levels of 30 and 85 µg/L, respectively (Bertuzzi et al., 2011; Torres et al., 1998), was largely attributed to the use of rice and corn as adjunct cereals. These levels are several orders of magnitude higher than those observed in our cider samples.

4. Conclusion

The global market for apple-based fermented products, including traditional cider and newer variations labeled as “cidere,” is expanding. Examining their safety profiles, especially for the latter, is crucial due to potential long-term health impacts from trace toxic compounds. The findings reveal generally low concentrations of analyzed substances, such as pesticide residues, problematic chemical elements, and mycotoxins, establishing cider as a safe product for consumers. Notably, the absence of patulin in Spanish cidere is a positive outcome, with only low residues of Fumonisin B1 and T-2 toxin found. Differences between traditional cidere and newer apple-based drinks are evident, necessitating further research to elucidate their distinct characteristics, including elemental profiles and the presence of Br and Pb in traditional cidere, as well as differing pesticide residue levels. Pesticide levels were overall low in both cider types, with only 17 pesticides being detected and one organic pollutant. Pyrimethanil was the most commonly found pesticide. “Cidere” showed higher levels of pesticides in terms of both diversity of pesticide types and number of pesticides found per sample. One sample contained 11 different pesticide residues, which is a matter of concern. Some infringements in elemental toxic compounds among organic cidere also require further exploration and explanation. This study, unique in its exploration, focuses on Spain with some worldwide cidere included for comparison, aiming to prompt producers, policy-makers, and technology stakeholders to enhance production practices for improved food safety. The study underscores the current absence of regulations stipulating maximum residue and toxic element limits in cider.

CRedit authorship contribution statement

Pablo Alonso González: Writing – original draft, Writing – review & editing, Investigation, Conceptualization. **Eva Parga Dans:** Writing – review & editing, Funding acquisition, Conceptualization. **Iván de las Heras Tranche:** Formal analysis, Data curation. **Andrea Carolina Acosta-Dacal:** Validation, Methodology, Formal analysis, Data curation. **Ana Macías Montes:** Formal analysis, Data curation. **Manuel Zumbado Peña:** Methodology, Formal analysis, Data curation. **Octavio Pérez Luzardo:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by the Catalina Ruiz research staff training aid program of the Regional Ministry of Economy, Knowledge and Employment of the Canary Islands Government, granted to the University of Las Palmas de Gran Canaria through the European Social

Fund, via a post-doctoral grant to the author Andrea Acosta-Dacal (APCR2022010003). This work was supported by the Spanish Plan for Innovation, Technical and Scientific Research 2017–Ramón y Cajal RYC 2018-024025-I and the ACIISI project ProID2021010071 for Eva Parga-Dans.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2024.110310>.

References

- Alonso González, P., Parga Dans, E., Acosta Dacal, A. C., Zumbado Peña, M., & Pérez Luzardo, O. (2022). Differences in the levels of sulphites and pesticide residues in soils and wines and under organic and conventional production methods. *Journal of Food Composition and Analysis*, 112, Article 104714.
- Alonso González, P., Parga Dans, E., de las Heras Tranche, I., Acosta-Dacal, A. C., Hernández, Á. R., Montes, A. M., Pena, M. Z., & Luzardo, O. P. (2023). Comparative analysis of mycotoxin, pesticide, and elemental content of Canarian craft and Spanish mainstream beers. *Toxicology Reports*, 10, 389–399.
- Alonso González, P., Parga-Dans, E., & Pérez Luzardo, O. (2021). Big sales, no carrots: Assessment of pesticide policy in Spain. *Crop Protection*, 141, Article 105428.
- Azam, M. S., Ahmed, S., Islam, M. N., Maitra, P., Islam, M. M., & Yu, D. (2021). Critical assessment of mycotoxins in beverages and their control measures. *Toxins*, 13(5), 323.
- Banna, A. A., & Kwar, N. S. (1982). Behavior of parathion in apple juice processed into cider and vinegar. *Journal of Environmental Science & Health Part B*, 17(5), 505–514.
- Bertuzzi, T., Rastelli, S., Mulazzi, A., Donadini, G., & Pietri, A. (2011). Mycotoxin occurrence in beer produced in several European countries. *Food Control*, 22(12), 2059–2064.
- Büchle, F., Neuwald, D. A., Scheer, C., Wood, R. M., Vögele, R. T., & Wünsche, J. N. (2022). Assessment of a postharvest treatment with pyrimethanil via thermonebulization in controlling storage rots of apples. *Agronomy*, 12(1), 34.
- Buglass, A. (2011). Cider and perry. *Handbook of alcoholic beverages: Technical, Analytical and Nutritional Aspects, tome 1*, 231–265.
- Carballo, D., Fernández-Franzón, M., Ferrer, E., Pallarés, N., & Berrada, H. (2021). Dietary exposure to mycotoxins through alcoholic and non-alcoholic beverages in Valencia, Spain. *Toxins*, 13(7). <https://doi.org/10.3390/toxins13070438>
- Cayot, N. (2007). Sensory quality of traditional foods. *Food Chemistry*, 101(1), 154–162.
- Čepo, D. V., Pelajić, M., Vrček, I. V., Krivohlavek, A., Žuntar, I., & Karoglan, M. (2018). Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. *Food Chemistry*, 246, 394–403.
- Čuš, F., Česnik, H. B., Bolta, Š. V., & Gregorič, A. (2010). Pesticide residues and microbiological quality of bottled wines. *Food Control*, 21(2), 150–154.
- European Commission. (2006). Setting of maximum levels for certain contaminants in foodstuffs. *Regulation*, 5–24, 1881.
- Fabien-Ouellet, N., & Conner, D. (2018). The identity crisis of hard cider. *Journal of Food Research*, 7(2), 54–68.
- Franceschi, S., Bidoli, E., Barón, A. E., & La Vecchia, C. (1990). Maize and risk of cancers of the oral cavity, pharynx, and esophagus in northeastern Italy. *Journal of the National Cancer Institute*, 82(17), 1407–1411. <https://doi.org/10.1093/jnci/82.17.1407>
- Gajek, M., Pawlaczyk, A., Wysocki, P., & Szykowska-Jozwik, M. I. (2021). Elemental characterization of cidere and other low-percentage alcoholic beverages available on the Polish market. *Molecules*, 26(8), 2186.
- Gobierno de España. (2017). *Ministerio de la Presidencia y para las Administraciones Territoriales. Real Decreto 72/2017, de 10 de febrero por el que se aprueba la norma de calidad de las diferentes categorías de la sidra natural y de la sidra*, (pp. 11416–11423).
- Harris, K. L., Bobe, G., & Bourquin, L. D. (2009). Patulin surveillance in apple cider and juice marketed in Michigan. *Journal of Food Protection*, 72(6), 1255–1261.
- Ioriatti, C., Agnello, A. M., Martini, F., & Kovach, J. (2011). Evaluation of the environmental impact of apple pest control strategies using pesticide risk indicators. *Integrated Environmental Assessment and Management*, 7(4), 542–549.
- Jackson, L. S., Beacham-Bowden, T., Keller, S. E., Adhikari, C., Taylor, K. T., Chirtel, S. J., & Merker, R. I. (2003). Apple quality, storage, and washing treatments affect patulin levels in apple cider. *Journal of Food Protection*, 66(4), 618–624.
- Langley, M., & Jenkin, E. (2019). *Westons cider report*.
- Merwin, I. A., Valois, S., & Padilla-Zakour, O. I. (2008). Cider apples and cider-making techniques in Europe and North America. *Horticultural Reviews*, 34, 365.
- Murillo-Arbizu, M., Amézqueta, S., González-Peñas, E., & de Cerain, A. L. (2009). Occurrence of patulin and its dietary intake through apple juice consumption by the Spanish population. *Food Chemistry*, 113(2), 420–423.
- OIV. (2018). International code of oenological practices, annex: Maximum acceptable limits. OIV code sheet—issue 2018/01. *Organisation Internationale de la Vigne et du Vin*.
- Palacios, A., Carrillo, D., Borinaga, I., Coulomb, S., Berducci, M. E., & Chatonnet, P. (2010). Contaminación medioambiental de anisoles clorados y bromados en bodegas elaboradoras de vino: Ejemplo de un caso práctico real. Trabajos presentados con motivo del VII Foro Mundial del Vino: [recurso electrónico]. *Logroño, La Rioja, España*, 12, 13, y 14 de mayo de 2010.

- Piacentini, K. C., Savi, G. D., Olivo, G., & Scussel, V. M. (2015). Quality and occurrence of deoxynivalenol and fumonisins in craft beer. *Food Control*, *50*, 925–929.
- Pires, N. A., Goncalves De Oliveira, M. L., Goncalves, J. A., & Faria, A. F. (2021). Multiclass analytical method for pesticide and mycotoxin analysis in malt, brewers' spent grain, and beer: Development, validation, and application. *Journal of Agricultural and Food Chemistry*, *69*(15), 4533–4541.
- Raffin, C. (2022). La otra apuesta del patrimonio de la sidra: Su expansión por España pasa por las burbujas. *El confidencial*. https://www.elconfidencial.com/empresas/2022-01-22/sidra-patrimonio-espana-cider-mercado_3362133/.
- Rodríguez-Carrasco, Y., Fattore, M., Albrizio, S., Berrada, H., & Mañes, J. (2015). Occurrence of Fusarium mycotoxins and their dietary intake through beer consumption by the European population. *Food Chemistry*, *178*, 149–155. <https://doi.org/10.1016/j.foodchem.2015.01.092>
- Santana-Mayor, Á., Rodríguez-Ramos, R., Socas-Rodríguez, B., Díaz-Romero, C., & Rodríguez-Delgado, M.Á. (2020). Comparison of Pesticide Residue Levels in Red Wines from Canary Islands, Iberian Peninsula, and Cape Verde. *Foods*, *9*(11), 1555.
- Schusterova, D., Hajslova, J., Kocourek, V., & Pulkrabova, J. (2021). Pesticide residues and their metabolites in grapes and wines from conventional and organic farming System. *Foods*, *10*(2), 307.
- Sousa, A., Vareda, J., Pereira, R., Silva, C., Câmara, J. S., & Perestrelo, R. (2020). Geographical differentiation of apple ciders based on volatile fingerprint. *Food Research International*, *137*, Article 109550.
- Staples, A. J., Howard, P. H., Conner, D. S., Serrine, J. R., Ostrom, M. R., & Miller, M. (2023). Apples to advocacy: Evaluating consumer preferences for hard cider policies. *Journal of Wine Economics*, 1–16.
- Tariba, B. (2011). Metals in wine—impact on wine quality and health outcomes. *Biological Trace Element Research*, *144*, 143–156.
- Ticha, J., Hajslova, J., Jech, M., Honzicek, J., Lacina, O., Kohoutkova, J., Kocourek, V., Lansky, M., Kloutvorova, J., & Falta, V. (2008). Changes of pesticide residues in apples during cold storage. *Food Control*, *19*(3), 247–256.
- Torres, M. R., Sanchis, V., & Ramos, A. J. (1998). Occurrence of fumonisins in Spanish beers analyzed by an enzyme-linked immunosorbent assay method. *International Journal of Food Microbiology*, *39*(1–2), 139–143. [https://doi.org/10.1016/s0168-1605\(97\)00113-x](https://doi.org/10.1016/s0168-1605(97)00113-x)
- Walsh, D. B., O'Neal, S. D., George, A. E., Groenendale, D. P., Henderson, R. E., Groenendale, G. M., & Hengel, M. J. (2016). Evaluation of pesticide residues from conventional, organic, and nontreated hops on conventionally hopped, late-hopped, and wet-hopped beers. *Journal of the American Society of Brewing Chemists*, *74*(1), 53–56.
- Wang, Z., Wang, L., Ming, Q., Yue, T., Ge, Q., Yuan, Y., Gao, Z., & Cai, R. (2022). Reduction the contamination of patulin during the brewing of apple cider and its characteristics. *Food Additives & Contaminants: Part A*, 1–14.
- WHO. (2004). *Guidelines for drinking-water quality* (Vol. 1). World Health Organization.
- World Health Organization. (2009). *Bromide in drinking-water: Background document for development of WHO guidelines for drinking-water quality*. World Health Organization.
- Wyrzykowska, B., Szymczyk, K., Ichichashi, H., Falandysz, J., Skwarzec, B., & Yamasaki, S.-i. (2001). Application of ICP sector field MS and principal component analysis for studying interdependences among 23 trace elements in Polish beers. *Journal of Agricultural and Food Chemistry*, *49*(7), 3425–3431.
- Zee, J. A., Simard, R. E., Chhem, C., Gosselin, C., & Martin, G. B. (1973). Organochlorinated Pesticides in Ciders. *American Journal of Enology and Viticulture*, *24*(3), 120–124.
- Zuřáková, V., Dušek, M., Jandovská, V., & Olšovská, J. (2023). Screening and quantification of pesticide residues in ciders by liquid chromatography-high resolution mass spectrometry. *Czech Journal of Food Sciences*, *41*(1), 29–35.