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Article

Miniaturized Microscale Solid-Phase Extraction-Based Module for Highly Efficient DNA Extraction and Purification from Grapevine Samples

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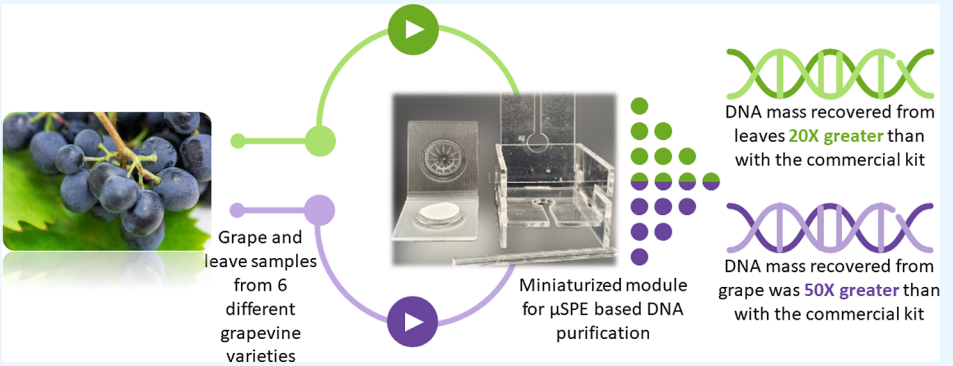

ABSTRACT: Several developments over the last few years are being directed toward improving DNA-based analysis to simplify, miniaturize, and reduce the time and cost of analysis, with the objective to allow its use in decentralized settings. One of the most interesting fields is DNA extraction and purification, a key step for ensuring good analytical performance. In this sense, microscale solid phase extraction (μSPE) offers paramount advantages for an improved DNA yield. In this work, we have developed a miniaturized module for DNA purification based on μSPE using a borosilicate glass microfiber filter as the solid phase. We also established a protocol for highly efficient DNA purification from vegetable samples, including leaves and grapes from four different varieties from the PDO Douro and two varieties from the Minho wine regions. The protocol demonstrated excellent performance when compared with a commercial kit with a DNA recovery yield of around 50%.

1. INTRODUCTION

Vitis vinifera is one of the oldest and most valuable plants domesticated by humankind, used for table grapes, raisins, juices, wines, and spirit production. There are currently between 6000 to 11,000 grapevine varieties cultivated worldwide, although only a reduced number is commercially significant.[1]

Food fraud, especially in the case of highly valued products, is an important concern. The European Parliament's 2013 report on the food crisis, fraud in the food chain, and the control thereof pointed out wine as one of the products most at risk of food fraud.[2] Particularly in the case of wine, reports on adulteration date back to the Roman Empire when correcting wine with grape juice concentrate was a popular fraudulent practice. As a result, the syrups, produced with unfermented grape juice in a leaden vessel over a slow fire, caused severe heavy metal poisoning among wine drinkers all over Europe.[3] Although with a different production method, grape syrup adulteration is still a widespread practice these days.[2] Moreover, in the autumn of 1794, imported port wine was reportedly sold in London for a fraction of its purchased price in Portugal, indicating a possible adulteration possibly by addition of white wine and/or sugar.[4]

Another more recent example refers to the fraudulent production of balsamic vinegar. In this particular case, the investigation led by the Italian police found out that the fraudsters allegedly sold common table grapes must to the producers instead of the one elaborated with prized "Sangiovese" and "Trebbiano" varieties, which are two of the

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seven grape varieties authorized for the production of traditional balsamic vinegar of Modena, one of the three protected balsamic vinegars. The rarest and finest balsamic vinegars have extremely high economic value.[5]

Fraudulent practices affect both the consumers and producers; therefore, analytical methods enabling the assessment of the authenticity and traceability of ingredients are of great interest.[6] Wines commercialized under certain EU quality schemes such as protected designation of origin (PDO) or protected geographical indication (PGI) need to be produced from specific grape varieties, exclusively for PDO and at least 85% for PGI. Such indications give the products a higher recognition by consumers and a higher economic value, which make these wines very appealing to fraudsters.[7]

Among other possible fraudulent practices, the substitution of certain grape varieties by others is a relatively common one. At the same time, in some cases, it is difficult for producers to guarantee their cultivars' genetic identity and purity. Conversely, a wide range of grapevine varieties resulted in conflicts in naming, considered synonyms (same grapevine with different names) and homonyms (different grapevines identified under the same name). The grapevine variety influences vine growth and grapes/wine quality.[8] Along with ampelographic methods, molecular techniques have allowed proper variety identification.[9,10] DNA-based methods can be of great value for variety identification in vineyards with unknown varieties.[1,3] On the other hand, it can provide nurseries a tool to assist in plant material authentication for vinegrowers.[11]

The development of DNA-based methods and bioinformatics tools has opened new and exciting venues for more efficient monitoring of wine and grapevine, since these methods are reproducible and reliable to trace among other *V. vinifera* varieties.[12] The main limitations of such analysis are related to the complexity of the vegetable sample, the loss of DNA integrity throughout food processing,[13] the risk of cross-contamination during various steps of DNA analysis,[14] and the presence of compounds that prevent DNA detection, such as PCR inhibitors.[15] Therefore, efficient DNA extraction and purification are crucial for the success of DNA-based methods.[15]

Likewise, there is increasing interest in fast and reliable methods with high sensitivity and multiplexing possibilities.[16] In this sense, micro-total analysis systems (μTASs), also known as lab-on-a-chip, aim to completely integrate multiple laboratory processes in a single miniaturized platform.[17] The main advantage of these DNA analysis systems is their portability, which allows decentralized analysis, and is cheaper and faster sample-to-result. In addition, these systems are usually more sensitive, requiring smaller volumes and fewer reagents, reducing the cost of analysis, and with possibilities for automatization, significantly reducing the risk of contamination.[18] These advantages make those devices promising to be used on-site by winemakers and enable better control of the whole production process and traceability and transparency. One of the most critical steps for miniaturization of analysis is sample preparation since this step should ensure efficient recovery of the target analyte and enable automatization and the connection with the next step.

The present work describes the design, fabrication, optimization, and testing of a miniaturized module for highly efficient DNA extraction and purification from grapevine vegetable samples. The combined device and protocol provided a recovery of up to 50% of the DNA yield. The DNA mass recovered compared with commercial methods was 20 times higher for the leaf samples and 50 times for the grape samples of the 6 grapevine varieties tested.

2. MATERIALS AND METHODS

2.1. Materials. Six samples from 6 different well-identified grapevine varieties were kindly provided by Sogrape, Avintes, Portugal, and from one of the coauthors' farm. The grapevine varieties studied included four varieties from Douro PDO—"Tinta Barroca" (D1), "Tinta Francisca" (D2), "Tinta Roriz" (D3), and "Touriga Franca" (D4)—and two varieties from Minho region—"Arinto" (M1) and "Borraçal" (M2)—including both grapes and leaves from white and red grape varieties (Table S1).

For miniaturized devices, poly(methyl methacrylate) (PMMA) plates with 3 mm thickness were purchased from Plexicrill (Braga, Portugal), as well as polydimethylsiloxane (PDMS) (SYLGARD 184 Silicone elastomer kit, Sigma-Aldrich, St. Louis, MO, USA) and a borosilicate glass microfiber filter of 15 mm diameter, 0.42 mm thickness, and a pore size of 0.7 μm (GF/A, Whatman Inc., USA); inlet and outlet channels—1.5 mm × 2 mm × 15 mm (width × height × length). For DNA purification, λDNA (Invitrogen, USA) was used for the optimization of the DNA purification protocol, together with glycine, potassium chloride (KCl), guanidine thiocyanate (GuSCN), EDTA, Tris−HCl, Triton-X, ethanol (EtOH) (Sigma-Aldrich, St. Louis, Missouri USA), and the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) for comparison purposes. Total DNA quantification was performed using the Qubit 1× dsDNA High Sensitivity (HS) kit (Invitrogen, USA) for fluorimetry-based tests.

2.2. Miniaturized Device Prototype. The miniaturized device (Figure 1) is composed of a reusable PMMA support with a disposable PDMS cartridge placed inside. The PDMS chamber holds the borosilicate glass microfiber filter as described above.

The PMMA support and the molds were designed with an ArtCAM JewelSmith 2011. PMMA support was cut with a laser cutter (WildLaser LS1390plus, Portugal), and the mold's sketch was transferred to FlexiCAM software. The mold for PDMS was cut by a computer numerical control (CNC) cutting machine (Viper 606, FlexiCAM GmbH, Eibelstadt, Germany). The PDMS cartridge for the extraction was 30 × 35 mm in height and width and had a circle form in the center that matched the glass microfiber filter dimensions (Figure S1). There were one inlet and one outlet (8 mm in diameter) that connected with PVC tubes with 0.5 mm inner diameter. PDMS is a flexible material, and the tube connection does not need additional fixing with glue or other methods.

2.2.1. Miniaturized Device Assembly and Fluidics Performance. PDMS prepared in a 10:1 ratio (PDMS base/curing agent) was placed in the mold and vacuumed to ensure that possible bubbles did not interfere with the chamber design. PDMS was then cured in the oven (Thermo Scientific Heratherm OGS 60) at 65 °C for at least 2 h. A new PDMS cartridge was prepared for each assay and was discarded at the end of it. The miniaturized device was assembled as described below.

A hole was punched in the chamber's center of the PDMS cartridge to insert the inlet and outlet channels. The filter was centralized in the chamber, placed in the PMMA holder, and sealed. The inlet channel was connected to the injection pump (Harvard Apparatus PHD ULTRA, v. 3.0.2), and the outlet

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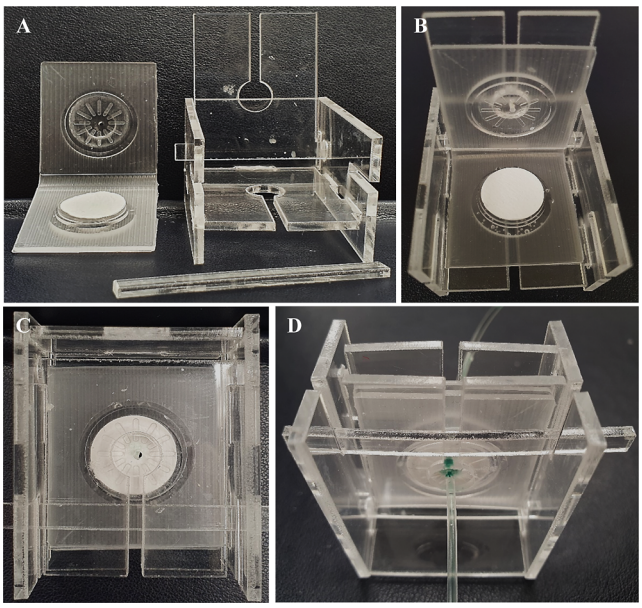

Figure 1. Miniaturized DNA-purification device module. (A) Prototype set: on the left, PMDS disposable cartridge and glass microfiber filter in the center; on the right, PMMA support. (B) Open view of the prototype. (C) Top view of the assembled prototype. (D) Diagonal view prototype with the fluid inlet and outlet channels.

channel was connected to the collecting tube. Solutions were injected at RT and at the flow rate and volume described in the protocol (Section 2.2.3) with the help of a syringe and an injection pump.

2.2.2. Fluidics Performance of the Miniaturized Device. The fluidics performance of the device was tested with colored aqueous solutions at different flow rates to a maximum flow rate of 100 μL min^{-1}, to disregard leaks. The system volume capacity was measured by calculating the volume of liquid needed to fill the chamber completely.

2.2.3. Design and Optimization of the Miniaturized Device Protocol. For the optimization of the DNA purification protocol, a standard solution with a concentration of 10 ng μL^{-1} of λDNA was used and confirmed by fluorimetry quantification. Four protocols (P1, P2, P2.1, and P3), based on our previous study,[19] were tested (Tables S2 and S3).

Before starting the extraction protocol, the device's chamber and filter were conditioned with 300 μL of the binding solution (B1 or B2) used at a flow rate of 100 μL min^{-1}.

Protocol 1 (P1) uses a buffer without chaotropic salts for binding and washing: binding buffer 1 (BB1) composed of 0.25 M glycine and 400 mM KCl, pH 5. For the binding step, 10 ng μL^{-1} of λDNA in 1000 μL of BB1 was pumped into the system at a flow rate of 10 μL min^{-1}. Washing was performed with 500 μL of BB1 at 20 μL min^{-1}. Elution was done with 500 μL of TE buffer (10 mM Tris−HCl and 10 mM EDTA, pH 8) at 10 μL min^{-1}.

Protocol 2 (P2) uses a chaotropic buffer for binding: binding buffer 2 (BB2), composed of 6 M GuSCN, 20 mM EDTA pH 8, 10 mM Tris−HCl pH 6.4, and 4% (v/v) TritonX-100, pH 7.3. For the binding step, 10 ng μL^{-1} of λDNA on 1000 μL of BB2 was pumped into the system at a flow rate of 10 μL min^{-1}. Washing I was done with 500 μL of washing solution 1 (W1), EtOH 85% (v/v), at 20 μL min^{-1}. A second washing (WII) was introduced for EtOH removal with 300 μL of BB1 at 20 μL min^{-1}. Elution was done with 500 μL of TE buffer (10 mM Tris−HCl and 10 mM EDTA, pH 8) at 10 μL min^{-1}.

Protocol 2.1 (P2.1) is a modification of P2, and as such all steps except for WII were performed as described for P2. For WII, instead of 300 μL, 100 μL of BB1 was pumped into the system at a flow rate of 20 μL min^{-1}.

Protocol 3 (P3) is similar to P2 and P2.1, but air was used for W1 removal. All steps, with the exception of WII, were performed as described for P2. WII was replaced with a drying step. For it, the air was pumped through the system at a flow rate of 100 μL min^{-1} until the membrane was completely dried.

Aliquots along all steps (one of 300 μL at conditioning, five of 200 μL each at binding, five of 100 μL each at washing I, zero (P1, P3), one (P2.1), or three (P2) of 100 μL each for washing II, and five of 100 μL each for elution) were collected in triplicate to monitor DNA losses and the evolution of DNA recovery (Table S3). The aliquots were quantified by fluorimetry.

2.3. DNA Extraction and Purification from Grapevine Leaf and Grape Samples. For the DNA extraction and purification from *V. vinifera*, four leaf and grape samples from Douro grape varieties (D1−D4) supplied by Sogrape (Avintes, Portugal) and two from Minho (M1 and M2) provided by local producers were used, as described in Section 2.1.

2.3.1. Extraction and Purification with the Commercial Kit. For comparison purposes, the DNeasy PowerSoil Pro Kit commercial kit was chosen after a literature review[20−23] since it was previously reported to provide efficient DNA extraction and purification from both vegetable and wine samples.

Leaf and grape samples were previously ground to powder with liquid nitrogen. DNA purification extraction and purification were done following the protocol as described in the kit manual, in triplicate https://www.qiagen.com/us/resources/resourcedetail?id=9bb59b74-e493-4aeb-b6c1-f660852e8d97&lang=en. Total DNA was quantified by UV/vis spectrophotometry and stored at −21 °C.

2.3.2. Extraction and Purification with the Miniaturized Device. DNA purification from leaf and grape samples was carried out following protocol P2.1. Samples were previously ground to a fine powder with liquid nitrogen, and lysis was performed according to the DNeasy PowerSoil Pro Kit handbook.

The procedure started with a conditioning step with 300 μL of BB2 solution at 100 μL min^{-1}. For binding, 1000 μL of a mixed solution of lysate with BB2 in a 1:1 volume ratio was pumped through the system at a 10 μL min^{-1} flow rate. The filter was washed with 500 μL of 85% (v/v) EtOH (WI) at 20 μL min^{-1}. To remove EtOH, a second wash (WII) was performed with 100 μL of BB1 solution at 20 μL min^{-1}. DNA recovery was done with 500 μL of TE and divided into five aliquots of 100 μL (E1−E5) at 10 μL min^{-1}.

The aliquots collected at elution were quantified by UV/vis spectrophotometry, also measuring their purity, and stored at −21 °C.

2.4. DNA Amplification. For evaluation of the amplificability of the obtained DNA extracts, a quantitative polymerase chain reaction (qPCR) test for amplification of fragments of the leucoanthocyanidin dioxygenase (LDOX) gene was selected. The LDOX is a precursor enzyme involved in proanthocyanin biosynthesis, and the selected primers LDOX_H2fwd and LDOX_H2rev3 amplify the 201bp fragment of this gene. For the amplification reaction, 10 μL of

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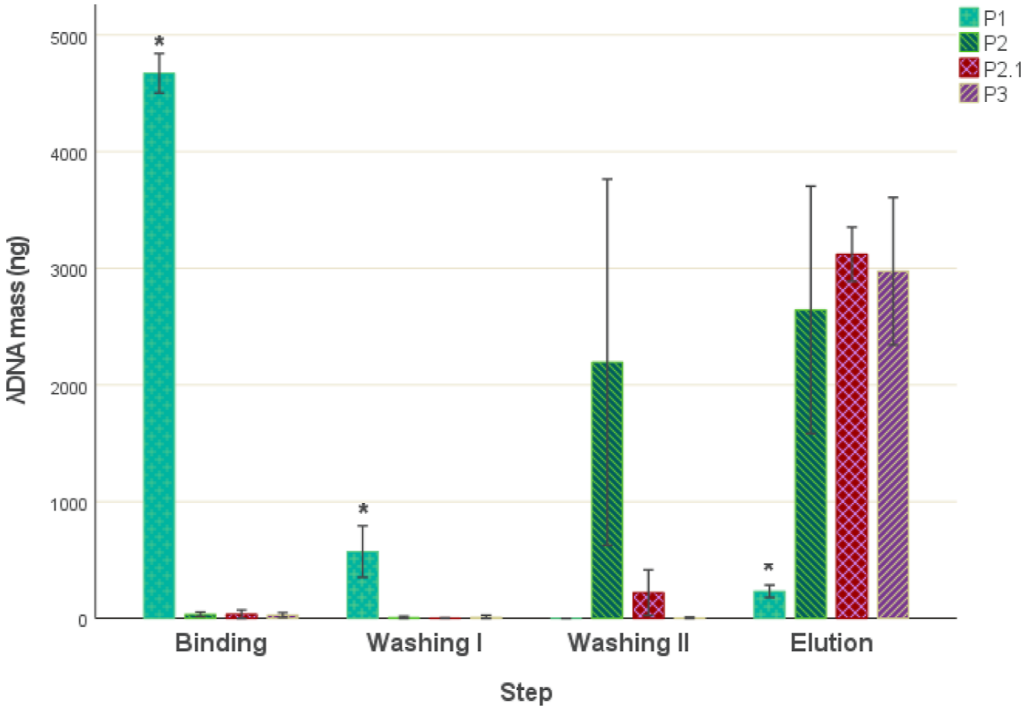

Figure 2. Protocols (P1, P2, P2.1, and P3). λDNA total mass, ng, was collected after each extraction step. Results are represented as mean ± 90% CI, n = 3, independent replicates. Results show a high DNA loss in binding for P1 and a high DNA recovery in elution for the remaining protocols.

PowerUp SYBR Green Master Mix (Applied Biosystems) was used together with 0.5 μM of each of the primers, 5 μL of DNA extract from leaves from the variety Tinta Francisca, and 2.6 μL of water in 20 μL of total volume. The qPCR reaction was performed on a StepOnePlus real-time PCR system (Applied Biosystems) with the following cycling conditions: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Tested samples included aliquots from the elution step, E1−E5 being subsequent aliquots in the elution step, E the mixture of all 5 aliquots, and E1D10 the E1 aliquot diluted 10 times in PCR water.

2.5. Statistics. During protocol optimization, the DNA yield was determined for each protocol tested for comparison between the protocols. In P1, the initial DNA mass was obtained from quantification of the BB1 + λDNA (1:1) solution used for binding. For the remaining protocols (P2, P2.1, and P3), the initial DNA amount was estimated from the P1 results since the binding solution used in these protocols—BB2—contains chaotropic salts that have previously shown to interfere with DNA quantification.[24] Results are presented as mean ± 90% confidence interval (CI), n = 3 independent replicates.

Statistical treatment was performed with IBM SPSS Statistics software (version 27.0.1.0). Comparison between protocols was made by one way ANOVA and Bonferroni post hoc test. Comparisons of the DNA extraction with the kit were done by one way ANOVA and Bonferroni post hoc test between varieties and by Levene's test and the t-test between samples (grapes/leaves). Comparison of the DNA extraction with the miniaturized device for vegetable samples was made by one way ANOVA and Bonferroni post hoc test, comparing DNA concentrations between aliquots of the same variety and between the same aliquot of various varieties. The p-value ≤ 0.05 was taken as a minimum for significance.

3. RESULTS AND DISCUSSION

3.1. Device Assembly and Fluidics Performance. Fluidics performance was evaluated with colored aqueous solutions to a maximum flow rate of 100 μL min^{-1}. Fluid leaks were corrected by modifications to the PDMS holder's design. The formation of sporadic air bubbles was observed. The system volume capacity is approximately 100 μL.

The miniaturized device described was based on a previous device developed by our group[19] and tested for DNA extraction and purification from olive oil samples. This new device is more user-friendly than the previous one due to its simple and quicker assembly, since our previous device included a set of 6 screws with their locknuts and rings and a PDMS O-ring which were substituted, in the current device, by a PDMS holder and a simplified closing system based on a PMMA simplified closure system as seen in Figure 1 In addition, in this prototype, the single-use cartridge is our main improvement with a smaller extraction chamber, which allows a reduction in the volumes of the buffer solutions and sample needed. Consequently, the present protocol is less time-consuming, more economic, and more straightforward than our previously built device,[19] and the reduction in the volume of buffers allows us to reduce both the cost and time of analysis, enabling us to recover the bound DNA in a lower volume, which provides a higher DNA concentration in the final solution for the subsequent steps of analysis.

3.2. Evaluation and Optimization of a Protocol for the Miniaturized Device. Four protocols were evaluated to be suitable for automation in a miniaturized setting. An optimum DNA purification profile should result in no or reduced loss of DNA during the initial steps (binding and washing) and high DNA recovery during elution.

Protocol 1 (P1) uses a buffer solution without chaotropic salts (BB1), which is also used for the washing step. Results (Figure 2) present DNA losses in the binding, suggesting that

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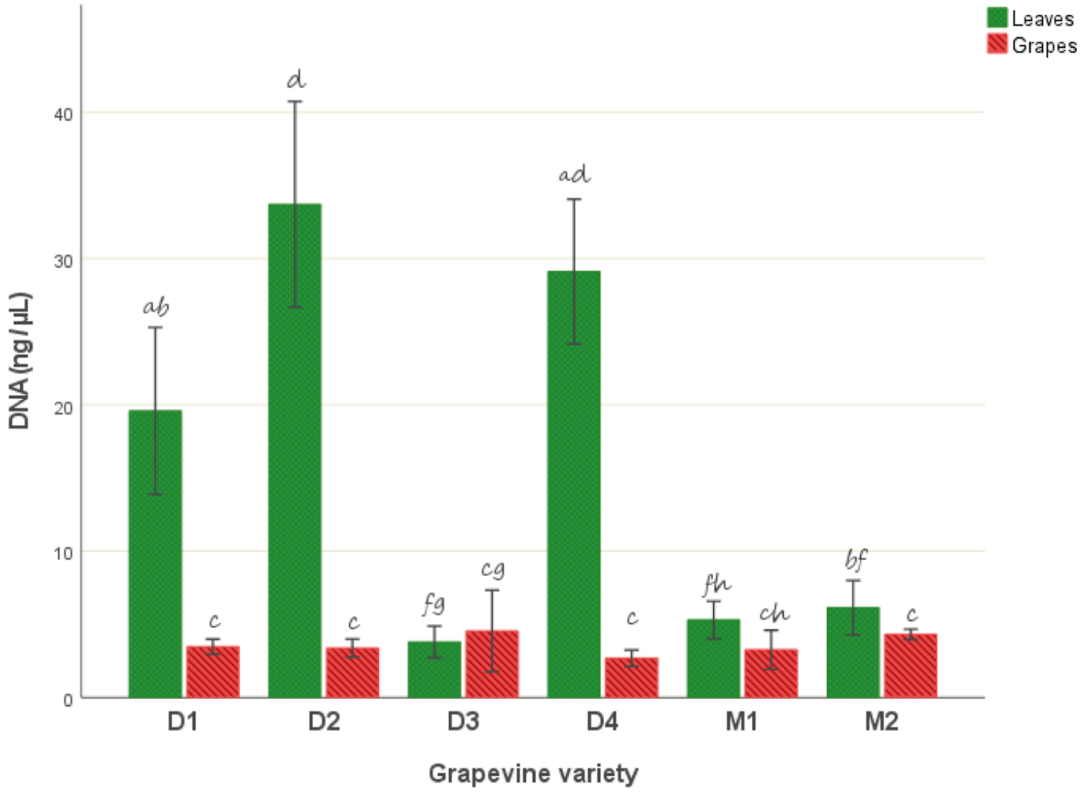

Figure 3. DNA extraction and purification from leaves and grapes with the DNeasy PowerSoil Pro Kit (Qiagen). Recovered DNA concentration, in ng μL^{-1}, for each grapevine variety is represented. Results are shown as mean ± 90% CI, $n = 9$, independent and dependent replicates. Results show a significant difference in the concentration of DNA extracted between leaves and grapes in most varieties.

the solid phase did not adsorb the DNA optimally. Some DNA was lost during the washing step, and the DNA recovery yield at elution was 3.8% ± 0.3. Thus, this protocol showed to be ineffective, which is contrary to Carvalho et al. 2018[19] results, where this one was the most efficient. The different filters in use could explain the conflicting results, since the one from this work has a smaller diameter, pore, and is thicker than the one from Carvalho et al., 2018.

Protocol 2 (P2) uses a chaotropic buffer (BB2) for binding, ethanol for the first wash, and BB1 for ethanol removal. As shown in Figure 2, there was no loss of DNA in the binding and first wash (WI). During the second wash (WII), DNA loss was observed, mainly in the second aliquot collected. DNA recovery yield was 33.2% ± 1.1.

Protocol 2.1 (P2.1) is a modification of P2, intending to reduce the DNA losses seen in WII by using a smaller volume of BB1 for ethanol removal. A reduction in DNA losses during WII was observed (Figure 2). DNA recovery yield was 51.2% ± 1.3.

Protocol 3 (P3) is similar to P2 and P2.1, but the filter was dried with air instead of BB1 for ethanol removal. As such, no DNA losses were observed (Figure 2) in the first steps (binding, washing, and drying). DNA recovery yield was 48.8% ± 3.6.

It is notable that the DNA losses observed in the binding step for the protocols that use BB2 for it may not correspond to the real losses at this step since this buffer has chaotropic salts that interfere with the quantification,[24] and as such accurate DNA quantification to track DNA losses in the binding step was not possible for P2, P2.1, and P3.

The highest DNA recovery yield outcomes from the tested protocols showed that P2.1 is the most optimized protocol. Thus, it was selected to proceed to the next phase of the present work, DNA extraction and purification from grapevine leaves and grapes.

3.3. DNA Extraction and Purification from Leaf and Grape Samples. *3.3.1. Extraction and Purification with the Commercial Kit.* DNA extraction and purification were performed according to the DNeasy PowerSoil Pro-Kit (Qiagen) kit manual. As shown in Figure 3, DNA concentration extracted from grapevine leaves is significantly higher than that extracted from grapes, except for D3 and M1. Additionally, in leaves, the extracted DNA concentration was significantly different between varieties. There was no significant difference between varieties in grape extraction.

Subsequently, the A260/A280 purity ratio values were recorded to be between 1.2 to 1.6 for leaves and between 1.1 and 1.6 for grapes. These results suggest that some contaminants, such as proteins, and reagents, such as phenol, are present.

3.3.2. DNA Extraction and Purification with the Miniaturized Device. DNA extraction and purification from leaf and grape samples were carried out following P2.1. DNA was recovered in five aliquots (E1−E5) and quantified.

3.3.2.1. Grapevine Leaf Samples. The robustness of our prototype for DNA extraction is depicted in Figure 4, showing the DNA concentration (ng μL^{-1}) of the aliquots (E1−E5) collected in the elution for each variety (D1, D2, D3, D4, M1, and M2). Results positively suggest that most DNA is recovered in E1, with its concentration varying between varieties. E2 is the one that contains the second-largest DNA

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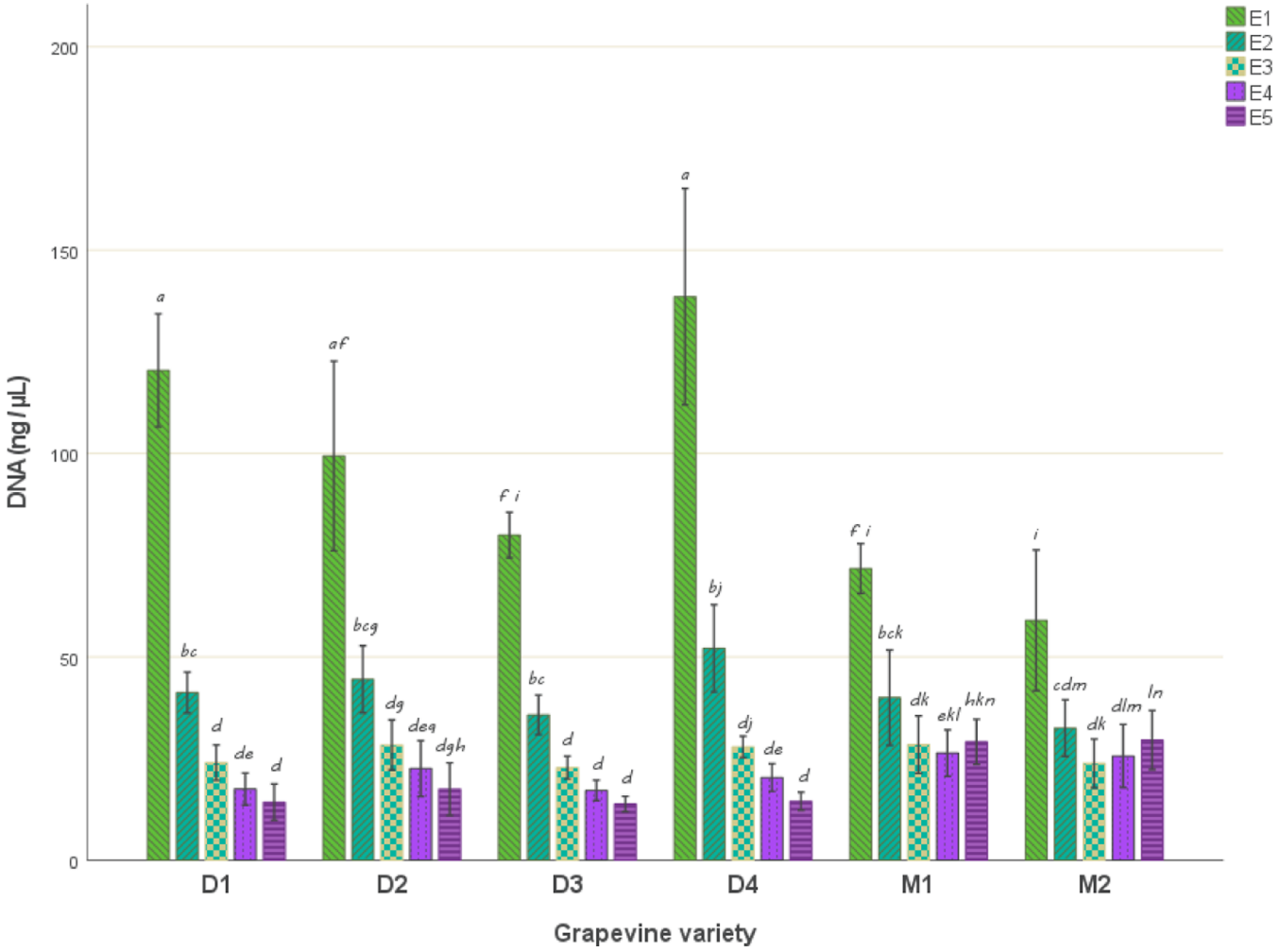

Figure 4. DNA extraction and purification from leaves with the miniaturized device and P2.1. Graphic shows recovered DNA concentration, ng μL^{-1}, for each variety and evolution of DNA recovery along the elution (E1−E5). Results are represented as mean ± 90% CI, n = 9, independent and dependent replicates.

amount, with some exceptions in its significance compared with the following aliquots. As expected, E3, E4, and E5 have no significant differences and resulted in the lowest DNA concentration.

The A260/A280 purity ratio values for each elution aliquot (E1−E5) demonstrate that E1 has the lowest purity ratio, and an increase of the purity ratio was observed in the following aliquots saturated after E4. This trend is plausible since impurities might be reduced in the later elution collection as a consequence of the lower DNA yield in comparison with the first elution collection (Figure S2).

3.3.2.2. Grape Samples. Figure 5 shows the DNA concentration (ng μL^{-1}) of the aliquots (E1−E5) collected for each variety. With the same tendency as the leaf sample results, most DNA from grape extraction is recovered in E1. However, in terms of variability, in contrast to the results from grapevine leaves, there are no significant differences in the DNA concentration between varieties in grape extraction. In addition, E2 is also noticed as the second-largest DNA amount for D3, M1, and M2 varieties, with no difference in the remaining ones. Not only that, following similar trends resulting from leaves' extraction, E3, E4, and E5 from grape samples produced no significant difference between them and have the lowest DNA concentration. There was no significant difference in the DNA concentration between varieties, except for E4 of D1 or D4. These results indicate that our constructed prototype produced excellent reproducibility of DNA extraction and purification results from various raw samples.

Still aligning well with our findings in leaves' purity, the results from the A260/A280 ratio values for each elution aliquot (E1−E5) from the grape sample denote that E1 generates the lowest purity ratio, and an increase of the purity ratio was observed in the following aliquots. The purity keeps increasing, and the stability of purity from grape extraction with our prototype is reached at E3 with values around 1.0 to 1.3 (Figure S3).

3.3.3. Performance of the Proposed Prototype vs Commercial Kit. DNA was extracted with the developed microfluidics device and with a Qiagen DNeasy PowerSoil Pro-Kit for performance comparison. Table 1 summarizes the substantial features of our prototype that regardless of the considerably higher elution volume, our proposed system provides approximately 20 times greater for the leaves and 50 times greater in the grape samples than the commercial kit ones (Qiagen DNeasy PowerSoil Pro-Kit).

Furthermore, DNA purity results (Figures S4 and S5) represent that the commercial kit and device ratios are remarkably comparable, although all the values produced from

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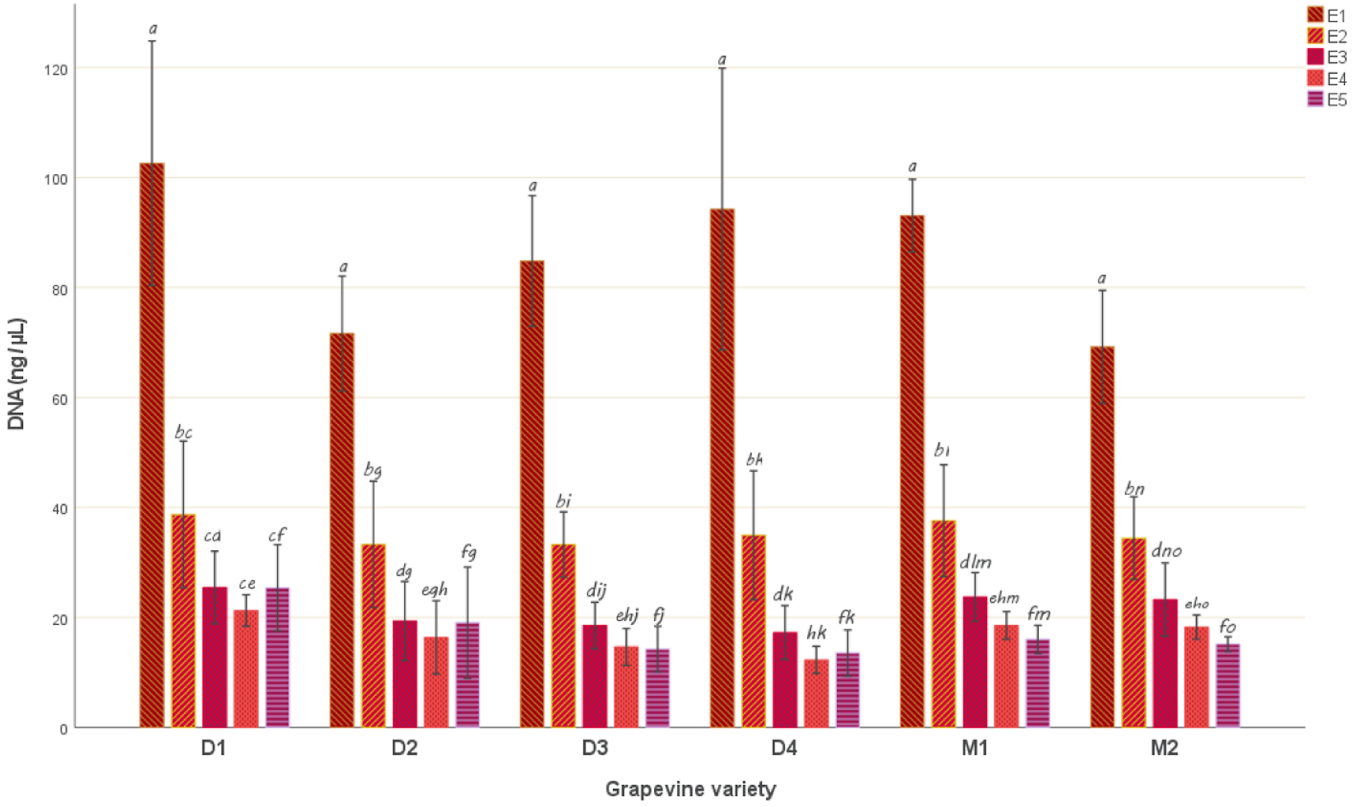

Figure 5. DNA extraction and purification from leaves with the miniaturized device and P2.1. Graphic shows recovered DNA concentration, ng μL^{-1}, for each variety and the evolution of DNA recovery along the elution (E1−E5). Results are represented as mean ± 90% CI, n = 9, independent and dependent replicates.

Table 1. Comparison between Recovered DNA Amount with the Commercial Kit and the Miniaturized Device from Leaf and Grape Samples[a]

	Qiagen DNeasy PowerSoil Pro Kit [DNA (μg) ± EP (i), elution volume: 100 μL]		miniaturized device [DNA (μg) ± EP (j), elution volume: 500 μL]		recovered DNA rate of the device versus the commercial kit (j/i)	
	leaves	grapes	leaves	grapes	leaves	grapes
D1	2.0 ± 0.2	0.4 ± 0.0	21.8 ± 3.1	21.4 ± 2.7	11.1	61.6
D2	3.4 ± 0.3	0.3 ± 0.0	21.2 ± 2.6	16.0 ± 1.9	6.3	47.4
D3	0.4 ± 0.0	0.5 ± 0.1	17.0 ± 1.9	17.0 ± 2.0	44.8	37.4
D4	2.9 ± 0.2	0.3 ± 0.0	25.4 ± 3.7	17.2 ± 2.7	8.7	64.4
M1	0.5 ± 0.1	0.3 ± 0.1	19.6 ± 1.5	18.9 ± 2.2	37.0	58.1
M2	0.6 ± 0.1	0.4 ± 0.0	17.1 ± 1.5	16.0 ± 1.6	27.8	37.2
mean	**1.6±0.2**	**0.4±0.0**	**20.3±2.4**	**17.7±2.2**	**22.6**	**51.0**

[a]It represents the elution volume, mean of DNA mass with standard error (DNA ± EP), μg, and recovered DNA mass ratio between the device and the commercial kit (j/i).

both fall below 1.8 as some contaminants, such as proteins, may be present.[25] In leaf samples, for the A260/A280 value, our proposed device resulted in merely a difference from those resulting from the commercial kit, while for the grape sample a slight difference is also recorded. Interestingly, for A260/A230, our prototype produced almost a negligible difference compared to the commercial kit, as shown by the overlapping standard deviation. These findings attest to the effectiveness of the proposed system as an alternative to the widely used commercial DNA extraction kits for a vast range of samples.

The integration of the proposed prototype and established protocols in this work offers several advantages over other existing methods. First, its size allows portability and reduces sample and reagent volumes as well as bulky equipment needs, which can be scaled up toward decentralized and on-site sample analyses. Consequently, it reduces the cost and duration of the DNA extraction and purification procedure compared to other methods, such as solid-phase extraction (SPE), which uses larger volumes and equipment. The microscale SPE (μSPE) manifested in our miniaturized prototype also allows DNA concentration when it is scarce in matrices, such as wine, if using a larger volume during binding and a smaller volume for elution.

Other advantages are the simplicity of the device assembly and fabrication, its relative automation that reduces the number of interferences by the operator, the risk of contamination, and systematic or gross errors due to human limitations. Finally, this prototype can be integrated into a complete and fully automated device for DNA analysis, involving steps such as the amplification and detection of

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fragments of interest. Pertaining to disposability, the proposed device can also be tested and validated for its application in other biological matrices, for instance, in clinical applications that typically require short-term or rapid single-point measurements.

3.4. Amplificability of the DNA Extracts. As seen in Table 2, the C_q values resulting from amplification of the 5 elution aliquots obtained from Tinta Francisca leaves with the developed device and protocol showed that in the first elution aliquot, despite a higher DNA concentration, the presence of some inhibitor, most likely from the washing step, prevented the amplification; this was confirmed by the positive amplification of the same aliquot diluted 10 times in PCR water. The best results were obtained with this diluted aliquot, and with the combination of all obtained aliquots confirming this, since the presence of inhibitors might be diluted when all the aliquots combined and overtime during the elution step. The selection of the combined aliquots from the elution step for further analysis has shown to provide amplificable DNA. Furthermore, a further optimization of the washing step might provide a good alternative to samples with low DNA content particularly.

Table 2. C_q Values Obtained after Amplification of Different Elution Aliquots

	C_q values
E1	n.d.
E2	35.43 ± 1.2
E3	35.05 ± 1.7
E4	33.59 ± 0.45
E5	33.67 ± 3.65
E1D10	31.01 ± 0.68
E	32.91 ± 0.71

4. CONCLUSIONS

The miniaturized device demonstrated an efficient DNA extraction from grapevine leaves and grapes compared to the commercial kit DNeasy PowerSoil Pro-Kit (Qiagen). It presents several advantages, such as its portability, disposability, and integration possibility in μTAS. This device can be the basis for effective traceability and authenticity testing to guarantee the products' origins, particularly wine authenticity. The miniaturized device and the optimized protocol for it (P2.1) which uses a chaotropic buffer for binding, ethanol for the first wash, a nonchaotropic buffer for the second wash, and buffer TE for elution showed a DNA recovery yield of 50%. The DNA mass recovered with the miniaturized device was 20 times more significant for the leaf samples and 50 times greater with the grape samples when compared with the commercial kit. The purity ratio A260/A280 for both the commercial kit and the miniaturized device was outstandingly comparable with values below 1.8. Overall findings suggest that our semiautomatized miniaturized device is effective and reproducible for DNA extraction and purification of various types of raw samples and paves the way toward alternatives for the pricey and laborious commercial DNA extraction and purification kits. The future roadmap of this study is envisaged toward constructing DNA μTAS to be used in decentralized settings, enabling better control of different points of the wine value chain.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02717.

Details of grapevine samples used in this study, design and simulation of the cutting of the mold for the fabrication of the PDMS cartridge, composition of the buffers used, detailed description of the DNA purification protocols tested, purity ratios of different types of samples analyzed after DNA purification on the developed miniaturized device, and comparison of purity ratios of different samples after DNA purification with the commercial kit and with the newly developed miniaturized device and optimized protocol (PDF)

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Notes

The authors declare no competing financial interest.

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