



Evaluation of Covalent Organic Frameworks for the low-cost, rapid detection of Shiga Toxin-producing *Escherichia coli* in ready-to-eat salads

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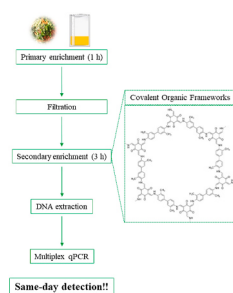
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HIGHLIGHTS

- Covalent Organic Frameworks have been applied for the first time for DNA extraction and purification.
- Short pre-enrichment method allowed to detect STEC in only 5 h.
- A simple filtration-based sample treatment allowed to efficiently remove vegetable tissues qPCR inhibiting compounds.
- The multiplex qPCR method targeted *stx1*, *stx2*, *eae*, *rfbE* and a NC-IAC.
- The LOD₅₀ of the method was 8.7 CFU/25 g.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Prof. N.W. Barnett

Keywords:

Shiga Toxin-producing *Escherichia coli*
Multiplex qPCR
Ready-to-eat salad
Covalent Organic Frameworks
Rapid methods
Same-day detection

ABSTRACT

Background: Ready-to-eat products, such as leafy greens, must be carefully controlled as they are directly consumed without any treatment to reduce the presence of potential pathogens. Food industries, especially those that process products with short shelf-life, demand rapid detection of foodborne pathogens such as Shiga Toxin-producing *Escherichia coli* (STEC). In this sense, molecular methods can fulfill both requirements of turnaround time and consumer safety. The most popular rapid methods are those based on real-time PCR (qPCR) however, vegetables contain inhibitory compounds that may inhibit the amplification reaction thus, there is a need for novel sample preparation protocols.

Results: In the current study, a low-cost sample treatment based on sequential filtration steps was developed. This protocol was combined with covalent organic frameworks (COFs), and compared against a chelating resin, to evaluate their performance by multiplex qPCR targeting the major virulence genes of STEC, namely *stx1*, *stx2*, and *eae*, along with the *rfbE* for the specific identification of serogroup O157 due to its particularly high incidence, and an Internal Amplification Control to assess reaction inhibition. The optimized sample treatment

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effectively removed vegetable qPCR inhibitory compounds, and it was possible to detect STEC in spiked ready-to-eat salad samples in one working day, roughly 5 h, with an LOD₅₀ of 8.7 CFU/25 g with high diagnostic sensitivity and specificity. The method was also assessed in samples with cold-stressed bacteria with good results, further demonstrating its applicability.

Significance: It was demonstrated for the first time that COFs are suitable for DNA extraction and purification. In addition to this, due to the tunable nature of these materials, it is envisioned that future modifications in terms of pore size or combination with magnetic materials, will allow to further improve their performance. In addition to this, the rapid and low-cost sample treatment protocol developed demonstrated suitable for the rapid screening of STEC vegetable samples.

1. Introduction

Foodborne diseases remain a major public health issue worldwide. Among them, infections associated to Shiga Toxin-producing *E. coli* (STEC) are some of the most relevant. They are characterized by bloody diarrhea and bacterial toxins may enter the bloodstream and lead to the development of hemolytic uremic syndrome (HUS), kidney damage, and long-term sequelae [1]. In 2020, STEC infections were the fourth most commonly reported zoonosis in the European Union (EU) with 4446 cases [2]. In the United States, in 2017, 6034 cases were reported (<https://www.cdc.gov/ecoli/surv2017/index.html>) although the actual number is estimated to be much higher [3]. Ready-to-eat (RTE) vegetables, such as leafy greens, have been commonly implicated in STEC infections, highlighting the importance of the detection of these pathogens in this particular food commodity [4,5].

Culture-based methods for pathogen detection are lengthy and not suitable for the current intensive food production systems. Official methods accepted in the EU already implement molecular-based methods, namely immunomagnetic separation and qPCR, for the specific detection of the most relevant STEC serotypes [6,7], but these methods need extensive enrichment steps. In addition to this, food inhibitors can influence qPCR performance [8]. More specifically, compounds found in vegetables, such as chlorophylls and polysaccharides, are known to be highly inhibitory [9–11]. Nanoporous materials like Covalent Organic Frameworks (COFs) have emerged as promising adsorbents for many different types of organic contaminants. It was previously demonstrated that COFs can adsorb different compounds from complex mixtures, as demonstrated in water treatment [12] and food contaminant analysis applications [13]. For this reason, we envisioned that these materials could allow for the enhancement of the DNA extraction procedure by capturing qPCR inhibitory compounds [13,14]. Additionally, their tunable pore size and functionality provide an added value to these materials, as they can be tailored towards the capture of specific compounds [15]. To this end, the aim of the present study was to evaluate the suitability of COFs as a novel material for DNA extraction, and to provide rapid, low-cost method for the processing of RTE-vegetable samples in order to detect STEC in one single day, avoiding qPCR reaction inhibition.

2. Materials & methods

2.1. Strains and culture media

E. coli O157:H7 WDCM 00014 was used for the initial development and evaluation of the methodology. This strain was selected for safety reasons (*stx1/stx2* negative). Strain AMC 76 provided by ASMECRUZ (*stx1/stx2* positive) was also used. Fresh cultures were prepared by resuspending one colony in 4 mL of Nutrient Broth (NB, Biokar diagnostics S.A., France) and incubated overnight at 37 °C. After incubation, reference values of viable bacteria were obtained by plating on Tryptic Soy Agar (TSA, Biokar diagnostics S.A., France) and incubation at 37 °C overnight.

Sample enrichment was performed in modified Trypticase Soy Broth (mTSB, Biokar diagnostics S.A., France) supplemented with 16 mg/L of

novobiocin (Biokar diagnostics S.A., France) and 12 mg/L of acriflavin (Sigma-Aldrich, St. Louis, MO, USA), from now on mTSB_{NA} (incubation conditions are detailed below). STEC growth confirmation in spiked samples was performed by streaking the enriched cultures on ChromAgar™ O157 and STEC (CHROMagar Microbiology, Paris, France) and incubated overnight at 37 °C.

2.2. Sample inoculation for initial COF screening

A total of 25 g of RTE salad leaves, purchased from local suppliers, were mixed with 225 mL of mTSB_{NA}. Ten microliters of a fresh AMC 76 bacterial culture were added and samples were mixed for 30 s in a Stomacher 400 Circulator (Seward Limited, West Sussex, UK). One milliliter was taken for DNA extraction.

The comparative study was based on the concentration of the DNA, measured with a Qubit™4 Fluorometer (Invitrogen™, Carlsbad, CA, USA), its quality (260/280 and 260/230 absorbance ratios) measured in a NanoVue Plus™ Spectrophotometer (GE Healthcare Europe GmbH, Portugal), and qPCR Cq values.

2.2.1. DNA extraction

The aliquot collected was centrifuged at 900×g for 1 min to pellet large food particles. The supernatant was transferred to a clean tube and centrifuged at 16000×g for 2 min. Pellet was resuspended in 1 mL of TE 1X (Tris-HCl 10 mM and EDTA 1 mM) and centrifuged again under the same conditions. The supernatant was removed and pellets were used for DNA extraction.

2.2.1.1. Reference method (Chelex 6%). Chelex 6% (Bio-Rad Laboratories, Inc., USA) was selected as the reference DNA extraction method as it is based on adsorption/chelation of the contaminants of the sample. Bacterial pellet was resuspended in 200 µL of Chelex 6%, heated at 56 °C for 15 min and then, the bacteria were thermally lysed at 99 °C for 10 min. Both heat treatments were performed under constant agitation (1400 rpm). The lysates were finally centrifuged at 16000×g for 2 min at 4 °C. The DNA extracts were stored at −20 °C until needed.

2.2.1.2. COF screening conditions. TpBD-Me₂, TpBD-(CF₃)₂, and TpBD-(NO₂)₂ COFs were synthesized from Tp and the corresponding commercially available benzidine derivatives (for more details, see Scheme S1 in Supporting information) [16,17]. Then, by reduction of the nitro functional groups of TpBD-(NO₂)₂, TpBD-(NH₂)₂ was obtained (for more details, see Supporting information; full characterization of all COFs in Table S3 and Figs. S1–S26) [16]. As assessed by small-angle X-ray scattering (SAXS), an ordered porous structure of TpBD-Me₂, TpBD-(CF₃)₂, TpBD-(NO₂)₂, and TpBD-(NH₂)₂ was successfully obtained. The N₂ sorption measurements at 77 K of the crystalline COF materials gave a type I isotherm typical of microporous materials, with a Brunauer-Emmett-Teller (BET) surface area in the range of 391–987 m² g^{−1}. The pore size distribution calculated using quenched-solid density functional theory (QSDFT) showed a large contribution of micropores, as well as the presence of mesopores at 1.1 and 1.5 nm for TpBD-Me₂, at 0.5, 1.2, and 2.0 nm for TpBD-(CF₃)₂, at 1.5 and 2.3 nm for TpBD-(NO₂)₂, and at 1.8 and 2.7 nm for TpBD-(NH₂)₂. Scanning electron

microscopy evidenced TpBD-Me₂ and TpBD-(CF₃)₂ to feature a granular morphology, and TpBD-(NO₂)₂ and TpBD-(NH₂)₂ a wire-like morphology (Fig. S27–S30).

For the initial screening the different COFs were added to each sample at a final concentration of 1 mg/mL. The material providing the best results was also tested at higher concentrations, up to 5 mg/mL, to evaluate if there was a concentration-dependent response.

In order to be able to perform a direct comparison with the reference method, chelex 6%, the same protocol, to extract and purify the DNA, was followed. In this sense, the bacterial pellet was resuspended in 200 µL of the corresponding COF, it was also heated at 56 °C for 15 min at 1400 rpm, and finally, the bacteria were lysed at 99 °C for 10 min at 1400 rpm. The last step consisted on a centrifugation at 16000×g for 2 min at 4 °C, and the DNA extracts were stored at –20 °C analyzed.

2.3. Multiplex qPCR

STEC detection was performed by multiplex qPCR targeting their major virulence genes *stx1*, *stx2*, *eae* [18,19], and *rfbE* which encodes for the “O157” antigen, as this serogroup is the one most commonly implicated in STEC infections [20]. A non-Competitive Internal Amplification Control (NC-IAC) was also included. Primers and probes sequences are provided in Table 1. The final reaction volume was 20 µL, containing 10 µL of NZYSupreme qPCR Probe Master Mix (NZYTech, Lisbon, Portugal) 3 µL of template DNA, 200 nM of each primer, 150 nM of probes; 100 nM of NC-IAC primers and probes, and 685 copies of NC-IAC DNA. The remaining volume was filled with sterile, DNase/RNase-free water.

All experiments were performed in a QuantStudio 5 Real-Time PCR System with the QuantStudio™ Design and Analysis Software v1.4.3 (Applied Biosystems™, Foster City, CA, USA). The thermal profile consisted of a hot-start step of 5 min at 95 °C followed by 50 cycles of Dissociation at 95 °C for 15 s and Annealing-Extension at 60 °C for 30 s.

Table 1
STEC multiplex qPCR primers and probes.

Primer	Sequence 5' → 3'	Modifications	Reference
stx1-P3F	TGT CGC ATA GTG GAA CCT CAC	–	This study
stx1-P3R	CAG CTG TCA CAG TAA CAA ACC G	–	
stx1-P3P	ACG CAG TCT//GTG GCA AGA GCG ATG T	FAM/ZEN/ IABkFQ	
stx2-P3F	AAC GGT TTC CAT GAC AAC GG	–	This study
stx2-P3R	CAG TGA GTG ACG ACT GAT TTG C	–	
stx2-P3P	TGC AAC GTG TCG CAG CGC TGG	ATTO550 N/ IABRQSp	
eae-P3F	TGA CGG TAG TTC ACT GGA CTT C	–	This study
eae-P3R	TGA CCC GCA CCT AAA TTT GC	–	
eae-P3P	TGG TCA GGT CGG AGC GCG TTA CA	TexRd-XN/ IABRQSp	
O157- rfbE-F	TCA ACA GTC TTG TAC AAG TCG AC	–	(Garrido-Maestu et al., 2020)
O157- rfbE-R	ACT GGC CTT GTT TCG ATG AG	–	
O157- rfbE-P	AC TAG GAC CGC AGA GGA AAG AGA GGA A	Cy5/IABRQSp	
NC-IAC-F	AGT TGC ACA CAG TTA GTT CGA G	–	(Garrido-Maestu et al., 2019)
NC-IAC- R	TGG AGT GCT GGA CGA TTT GAA G	–	
IAC-P	AGT GGC GGT//GAC ACT GTT GAC CT	YY/ZEN/IABkFQ	(Garrido-Maestu et al., 2018)

YY (Yakima Yellow), IABkFQ (Iowa Black®FQ), IABRQSp (Iowa Black®Sp) and ZEN (secondary, internal quencher) are trademarks from IDT.

2.4. Low-cost sample treatment for the final method

RTE salad, 25 g, was weighted in a stomacher BagFilter XF (filter size <20 µm, Interscience, Saint Nom, France), mixed with 25 mL of mTSB, and homogenized as described above. Samples were incubated at 37 °C for 1 h with constant agitation (120 rpm). After this first enrichment, all the whole liquid was recovered, passed through a 0.45 µm filter of mixed cellulose esters (Merck Millipore, Burlington, MA, USA), 1 mL washing buffer [21] was passed through as well to rinse the funnel. The filter was placed in a 50 mL tube containing 4 mL of mTSB_{NA}, and reincubated for another 3 h under the previously indicated conditions. Once completed this second enrichment, 1 mL was taken for DNA extraction by the Chelex and COF methods. In addition, direct thermal lysis after resuspending the bacterial pellet in 200 µL of TE 1X was also included to evaluate the capacity of the sample treatment to aid in the removal of qPCR inhibitors. After the secondary enrichment a loopful was streaked on Chromagar STEC and Chromagar O157 for result confirmation purposes. The overall protocol is summarized in Fig. 1.

2.5. Determination of the Limit of Detection (LoD) and evaluation of the method

The LoD of the novel method implementing the low-cost sample treatment, along with the DNA extraction with the COF, and Chelex reference method, was statistically calculated with the mathematical model described by Wilrich & Wilrich [22]. For the model to work, a concentration where positive and negative samples are obtained must be reached. For calculation, all the samples spiked below 10² CFU were included. The samples were classified as “positive” if any of the target genes, namely *rfbE*, *eae*, *stx1*, or *stx2* were positive, and as “negative” when all the targets were negative while the IAC was positive.

Having determined the LoD, all the samples above this value were classified as Positive or Negative Agreement (PA/NA) if the result obtained by the alternative method, multiplex qPCR, matched that of the reference culture-based one. Likewise, the samples were classified as Positive or Negative Deviations (PD/ND) if the results did not match. These parameters were used to determine the relative sensitivity, specificity and accuracy (SE, SP and AC) along with the Cohen's kappa (k) following the formulae described by NordVal [23].

2.6. Performance on stressed microorganisms

The low-cost sample treatment and the different DNA extraction materials were tested with a panel of samples including stressed bacteria. The procedure consisted of the inoculation of 25 g of RTE salad with 10–10² CFU, and after inoculation the samples were stored for 24 h at 4 °C. After the cold treatment, the samples were analyzed following the procedure indicated in Section 2.4.

3. Results & discussion

3.1. COF selection

We chose COFs based on triformylphloroglucinol [24](Tp) building block, since these materials feature remarkable water stability at a wide range of pH conditions [12,25]. Thermogravimetric analysis (TGA) data evidenced the materials to be thermally stable at least up to ~300 °C (Fig. S17–S24). The chosen COFs feature functional groups that could undergo hydrogen bonding as acceptors (TpBD-(NO₂)₂) or donors (TpBD-(NH₂)₂), as well as very different hydrophobicity, as evidenced by water contact angle measurements (Figs. S25 and S26, Table S1). A value of 133° was found for TpBD-(CF₃)₂, highlighting the higher lipophilicity of this COF as compared to the more hydrophilic TpBD-Me₂, TpBD-(NO₂)₂, and TpBD-(NH₂)₂ with values of 18°, 15°, and 6.3°, respectively.

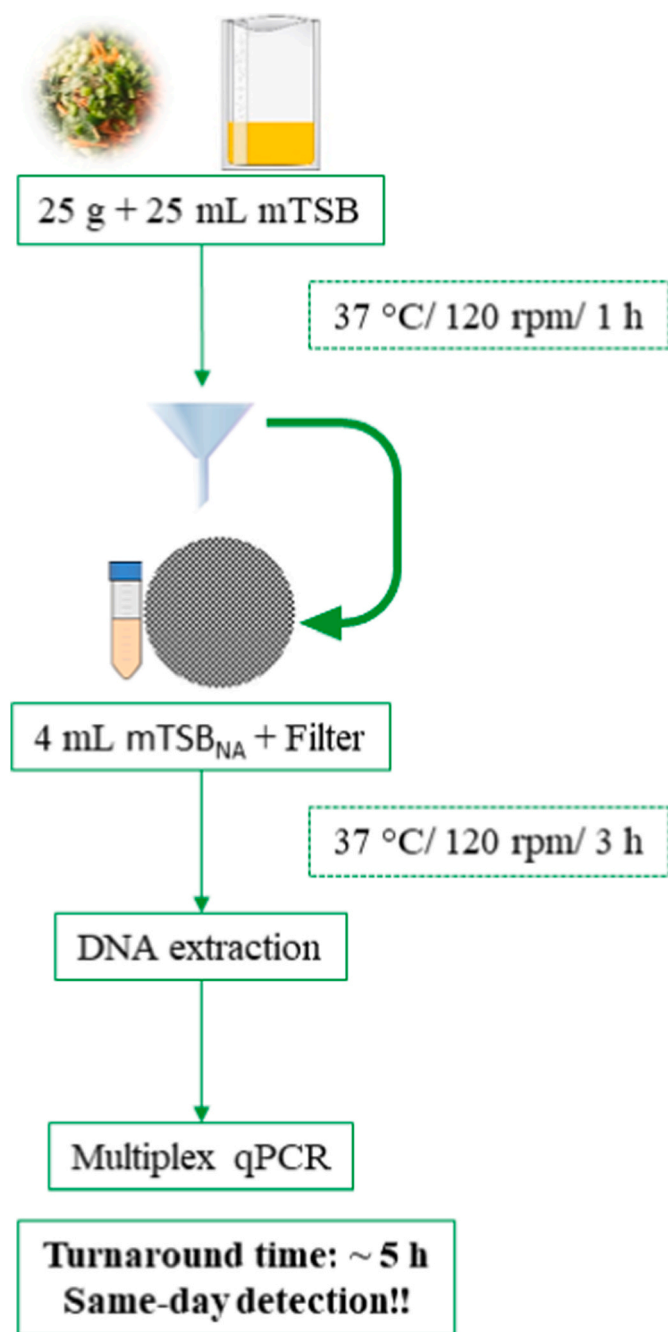


Fig. 1. Workflow of the low-cost sample treatment protocol.

3.2. Initial COF screening

For the initial COF screening step, DNA concentration and purity, attending to the ratios 260/280 and 260/230, were considered. This approach was discarded due to inter-sample variation (see Table S1). The physico-chemical properties of the COFs affected their handling during the screening, with e.g. the high hydrophobicity of the fluorine-containing TpBD-(CF₃)₂ rendering it harder to disperse in water, more difficult to pipette due to the formation of aggregates, and finally harder to separate by centrifugation under the standard conditions tested. These features, along with certain variation in the initial bacterial spiking level, were most likely behind standard deviations observed for the DNA concentration as well as the absorbance ratio 260/280. The values obtained for 260/230 are most likely associated to the presence of some COF remaining in the final DNA extract, as Tp-containing COFs can

be expected to absorb around this wavelength [26]. Therefore, the comparison was carried out based on the multiplex qPCR results, more specifically, in the average Cq value obtained for all the genes reporting a positive result. Considering the overall data, the COF providing the best results was found to be TpBD-Me₂, as it was the only one providing reproducibly positive qPCR results. In Table 2 a summary of the Cq values obtained is provided.

In order to confirm the stability of the COF under aqueous condition, TpBD-Me₂ was subjected to ultrapure water for 7 d at room temperature. As confirmed by SAXS, no loss in crystallinity was found as compared to the as-synthesized material (Fig. S31).

3.3. COF concentration effect

The effect of TpBD-Me₂ COF concentration on its performance was tested. A similar observation as described above related to DNA concentration and purity was observed (see Table S2). Once more, the evaluation was based on multiplex qPCR results. A concentration-dependent effect was indeed observed (Table 3). However, additional experiments showed that these results were not consistently reproducible. This could be associated with the fact that concentrations higher than 1 mg/mL were more difficult to manipulate and separate, resulting in the transfer of minute amounts of COF into the qPCR reaction, which may hinder the overall performance of the assay. A more effective COF separation procedure, such as centrifugation at higher speed for a longer time or growth of the COF on magnetic nanoparticles giving access to separation through external magnetic field [27,28] is envisioned to improve the procedure. Consequently, 1 mg/mL was selected as the concentration for the final application.

3.4. Determination of the LoD and evaluation of the method

The utility of a filtration step for the removal of certain PCR inhibitors and for concentrating bacteria was already reported by Murakami. However, vegetables, which are highly problematic, were not included in the evaluation [29]. Indeed, in a study from Garrido-Maestu et al., even the implementation of a plant-specific DNA extraction kit was not able to fully eliminate all the qPCR inhibitory compounds present in this type of samples, and an additional DNA dilution was reported to be needed with certain samples [30]. The LOD₅₀, which represent the bacterial concentration with a 50% chance of being detected, was calculated with the model described by Wilrich & Wilrich as recommended by different international bodies [22,23,31,32]. As it may be observed in Fig. 2, the LOD₅₀ was 8.7 CFU/25 g, lower than that reported by Murakami (1 CFU/g). Also, the results obtained are in agreement to those reported by Kim et al. who followed a similar procedure including vegetables [33]. Also, the LOD₅₀ was similar to those obtained by other “same-day detection methods. For *E. coli* O157 was obtained a LOD₅₀ of 4 CFU/25 g in ground meat by Recombinase Polymerase Amplification, along with naked-eye observation [34], or 1 CFU/25 g in leafy greens [30], and 8.8 CFU/25 g when targeting *Salmonella* spp. in meat samples [35,36]. In addition to these studies, a few

Table 2
Multiplex qPCR average Cq values for the COF initial screening.

Material	Average Cq ± SD
Chelex 6%	26.33 ± 1.23
CF3	34.28 ± 1.28
TpBD-Me ₂	30.92 ± 4.42
NO2	34.21 ± 11.07*
NE2	34.77**

All the COFs were added at a final concentration of 1 mg/mL. SD: Standard Deviation. *Positive for 3 out of the 4 target genes (*rfbE* negative). ** Positive for 1 out of the 4 target genes (*rfbE*, *stx1* and *stx2* negative).

Table 3
Multiplex qPCR average Cq values for the COF initial screening.

Material	Average Cq $\pm$ SD
Chelex 6%	15.82 $\pm$ 0.66
TpBD-Me ₂ 1 mg/mL	21.53 $\pm$ 1.27
TpBD-Me ₂ 2 mg/mL	21.42 $\pm$ 1.22
TpBD-Me ₂ 3 mg/mL	20.76 $\pm$ 1.35
TpBD-Me ₂ 4 mg/mL	19.70 $\pm$ 1.51
TpBD-Me ₂ 5 mg/mL	19.23 $\pm$ 1.40

The average Cq values provided correspond to all the values of all the genes, *rfbE*, *eae*, *stx1* and/or *stx2*, reporting a positive result. SD: Standard Deviation.

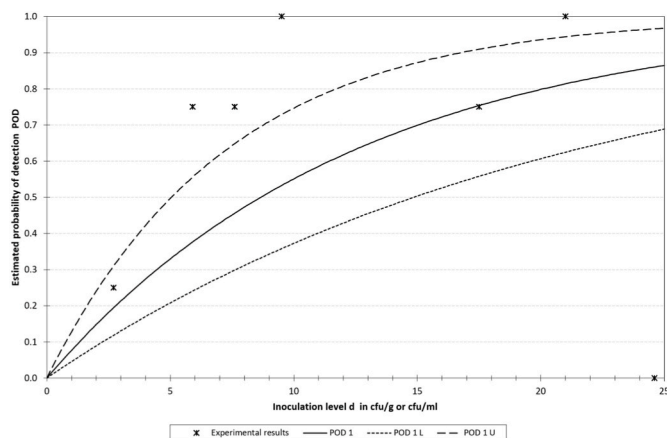


Fig. 2. Graphical representation of the LoD calculated with the mathematical model described by Wilrich & Wilrich. In the graph, “p 1(d)” represents the probability of detection (POD) and “POD 1 L”/“POD 1 U” represent the Lower and Upper limit of the 95% confidence interval respectively.

others, targeting other microorganisms or implementing other detection strategies, have been reported, like the ones from Milton et al. or Leach et al. [37–39]. In Table 4 a direct comparison of the reported method against these previous studies is provided. The fact that all these various methods reached similar LOD₅₀

No differences in terms of the LoD were detected upon the application of the different DNA methods, Chelex, COF, and TE. Any given sample positive by the reference extraction method was also positive with the others, likewise, samples negative by the reference were also negative by the other two approaches.

The fact that even the simplest DNA extraction procedure, thermal lysis with TE, did not exhibit any kind of qPCR inhibition, as no variations were observed in the Cq values of the IAC, indicates that the sample treatment procedure applied was enough to efficiently remove any potential inhibitor associated to the vegetable samples. In the study of Delbeke et al. DNA had to be diluted in order to overcome this issue

Table 4
Comparison against other existing methods.

Microorganism	Media	Targets	Turnaround time (h)	Tested in vegetables	Technique	LOD50*	Stressed bacteria	Reference
STEC	mTSB/ mTSB _{NA}	<i>eae/stx1/stx2</i> / IAC	5	Yes	qPCR	8.7	Yes	Present study
<i>E. coli</i> O157	mTSB _N	<i>rfbE</i>	5	No	RPA	4	No	[34]
<i>E. coli</i> O157	mTSB _N	<i>rfbE</i>	5	Yes	qPCR	1	No	[30]
<i>Salmonella</i> spp.	BPW	<i>ttrRSBCA</i>	5	No	qPCR	8.8	Yes	[35,36]
<i>E. coli</i> O157:H7	2 × mBPWP	–	6.5–10	Yes	ECL – CBA**	2.5	No	[38]
<i>C. coli</i>	Bolton broth	<i>ceuE</i>	14	No	PSR	2.5 × 10 ³	Yes	[37]
<i>E. coli</i> O157/ <i>Salmonella</i> spp./ <i>L. monocytogenes</i>	TSB	<i>rfbE/invA/plcA</i>	9	No	LAMP	0.4–18.3	No	[39]

*LOD expressed in “CFU/25 g”. **Electrochemiluminescent and Cytometric bead array.

[40]. Although similar DNA concentration and purity were obtained among all the methods, Cq values of the target genes were lower when using Chelex 6%. This is in line with previous observations, leading to the postulation that this resin has a protective effect over DNA during heat treatment, in addition to the known feature of protecting DNA from degradation due to the chelation of divalent cations [41].

Overall, a total of 38 samples were analyzed with the rapid, low-cost method implementing the Chelex, COF, or TE DNA extraction protocols. This panel of samples included 8 inoculated with >10³ CFU of strain AMC 76, 19 inoculated with WDCM 00014 (11 samples >10³ CFU, 4 with 10–10² CFU and 4 with 1–10 CFU) and 11 with WDCM 00014 analyzed after cold stress treatment (3 with 10–10² CFU and 8 with 1–10 CFU). All the samples inoculated with >10³ CFU were positive by the culture-based method and multiplex qPCR regardless the DNA extraction protocol selected. In the intermediate inoculation range, 10–10² CFU, 1 sample inoculated with AMC 76 was negative for all the genes regardless the DNA extraction protocol: This also happened with 1 sample inoculated with WDCM 00014. Lastly, in the low inoculation range 1–10 CFU, for samples inoculated with WDCM 00014, 2 were negative when using Chelex 6%, while 3 with the COF and the TE protocols (results for stressed bacteria are commented in the section below). These results are summarized in Table 5.

3.5. Performance on stressed microorganisms

Stressed microorganisms may not grow under standard laboratory conditions, particularly in selective media [42,43]. To overcome this limitation, a short primary enrichment in less selective mTSB was performed, followed by bacterial concentration in a filter to remove qPCR inhibitors, and secondary fully selective enrichment in mTSB with novobiocin and acriflavin. This approach was tested in a panel of 11 samples previously subjected to cold stress (24 h at 4 °C). Overall, the same observations were made for the non-stressed bacteria also after the cold treatment, evidencing that the low-cost sample procedure effectively removed the qPCR inhibitors, and it was possible to detect the pathogen by directly lysing the aliquots in TE. However, lower Cq values were obtained with Chelex.

Two out of the 11 samples returned negative results, one inoculated with 5.9 CFU/25 g and the other with 7.6 CFU/25 g. These deviations are speculated to be associated with the fact that these samples were very close to the LOD₅₀, which, combined with the cold stress and the selective enrichment, may have hindered the successful recovery. It is worth to note that typical colonies were not observed either on ChromAgar STEC or on ChromAgar O157 in the sample spiked with 5.9 CFU/25 g, and thus, this sample was not considered as a false negative result.

4. Conclusions

It was successfully demonstrated that COFs may serve for simple DNA extraction and purification, and although the ones tested in the

Table 5

Method evaluation summary for spiked samples.

DNA extraction material	N	PA	NA	PD	ND	SE	SP	AC	k
Chelex 6%	38	29	9	0	0	100.0	100.0	100.0	1.00
TpBD-Me ₂ COF	38	26	9	0	3	89.7	100.0	92.1	0.87
TE	38	27	9	0	2	93.1	100.0	94.7	0.92

N: number of samples. LOD: Limit of Detection. PA: Positive Agreement. PD: Positive Deviation. NA: Negative Agreement. ND: Negative Deviation. SE: relative sensitivity. SP: relative specificity. AC: relative accuracy. k: Cohen's kappa, interpreted as "substantial agreement" (0.61–0.8) and "almost complete concordance" (0.81–1.00) according to previous references (Anderson et al., 2011; DG, 1991).

current study did not outperform the reference protocol, Chelex 6%, the fact that these materials can be synthesized with different pore sizes and functionalities what opens the door for future improvements for the specific removal of a given panel of contaminants. In addition, novel multiplex qPCR assay coupled with a low-cost sample treatment protocol allowed to perform same-day detection of STEC, successfully eliminating vegetable-associated qPCR inhibiting compounds.

CRediT authorship contribution statement

Ana Costa-Ribeiro: Investigation, and revision. **Sarah Azinheiro:** Investigation, and revision. **Soraia P.S. Fernandes:** synthesis of nano-materials, revision and edition of the manuscript. **Alexandre Lamas:** revision and editing of the original manuscript. **Marta Prado:** Funding acquisition, revision and editing. **Laura M. Salonen:** Funding acquisition, Conceptualization, Methodology, Supervision, revision and edition of the manuscript. **Alejandro Garrido-Maestu:** Funding acquisition, Conceptualization, Methodology, Supervision, Validation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the INL for funding of the project through the INL Seed Grant. SPSF and SA acknowledge the FCT – Fundação para a Ciência e Tecnologia for the Ph.D. scholarships SFRH/BD/131791/2017 and SFRH/BD/140396/2018 respectively. AGM acknowledges funding from the Fundação para a Ciência e a Tecnologia through the Scientific Employment Stimulus Program (2021.02810. CEECIND). LMS acknowledges financial support from the Spanish Ministry of Science through the Ramón y Cajal grant RYC2020-030414-I.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2023.341357>.

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