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Comparison of methods to extract PCR-amplifiable DNA from fruit, herbal and black teas

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Abstract: The success of polymerase chain reaction (PCR) assay depends on template deoxyribonucleic acid (DNA) being sufficient with respect to both quantity and quality. Some biological materials contain compounds which inhibit the functioning of DNA polymerase and thus need to be removed as part of the DNA extraction procedure. The aim of the present experiments was to optimise the process of DNA isolation from various types of black, fruit and herbal teas. A comparison was made between two cetyltrimethylammonium bromide (CTAB)-based protocols and two commercially available DNA purification kits. The yield and integrity of the extracted DNA were monitored both spectrophotometrically and using agarose gel electrophoresis. The presence/absence of inhibitors in the DNA preparations was checked by running quantitative real-time PCRs. The optimal protocol was deemed to be the CTAB method described in ISO 21571:2005, so this method is recommended for the routine sample analysis of tea products.

Keywords: CTAB; DNA extraction; PCR amplification; tea; tRNA-Leu

The adulteration of food and beverage products, whether by accident or design, is a perennial problem. In the case of teas, adulteration can take the form of admixture with material from plant species other than those declared as present. Robust and practical methods able to detect such adulteration are particularly important in the case of herbal teas claiming to have medicinal properties (Xanthopoulou et al. 2016). In principle, diagnostic assays can target metabolites, proteins or deoxyribonucleic acid (DNA) (Efenberger-Szmechtyk et al. 2018; McGrath et al. 2018); the latter are particularly suitable because they are so readily targeted to a specific adulterant(s); furthermore, unlike both metabolites and proteins, DNA is a very stable compound largely unaffected by processing or the environment under which the plants had been grown (Granato et al. 2018; Hrbek et al. 2018). Of the various DNA-based assays which have been developed, those based on the polymerase chain reaction (PCR) are favoured thanks to their simplicity, robustness and potential to be standardised. Mostly, only a very small quantity of DNA is required to provide the template containing the target sequence(s) (Bernardo et al. 2007; Gryson 2010; Sajali et al. 2018).

Many methods have been elaborated to extract DNA from plant material (Dellaporta et al. 1983; Doyle and Doyle 1990). ISO standard 21571:2005 describes several such protocols, including a widely used one based on the quaternary ammonium surfactant cetyltrimethylammonium bromide (CTAB). Numerous CTAB-based protocols have been presented in the literature, varying with respect to the concentration of the components of the extraction solution. Effective CTAB-based protocols have been described to obtain DNA from soybean,

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maize (Demeke and Jenkins 2010), various cereal grains, oilseed rape (Park et al. 2010; Li et al. 2013), many of the fruits used to produce infusions (e.g. apple, pear, apricot and raspberry) (Lodhi et al. 1994), medicinal and aromatic plants and even from herbarium samples (Cota-Sánchez et al. 2006; Attitalla 2011). As an alternative, a number of commercial DNA isolation kits have been developed which rely on either anion exchange chromatography or silica-gel membrane (Gaikwad 2002). While such kits are convenient to use, they are relatively costly; furthermore, optimising their use to a specific situation is not generally possible because the accompanying reagents are proprietary. While a number of authors have recommended various of these kits for extracting DNA from plant materials (Peano et al. 2004; Dimitrijević et al. 2013), others maintain that conventional methods are superior (Akkurt 2012). The aim of the present investigation was to assess the effectiveness of two CTAB-based extraction protocols and two commercial extraction kits for acquiring PCR-amplifiable DNA from a number of black, fruit and herbal teas.

MATERIAL AND METHODS

Tea samples. The samples of black, fruit and herbal teas purchased from local shops in the Czech Republic, are given in Table 1. The samples were kept dry at room temperature. Prior to the DNA extraction procedure, the entire contents of tea bags were snap-frozen in liquid nitrogen and pulverised in a mortar. At least three different bags of each sample were analysed. Loose teas (50 g package) were firstly homogenised in a blender (IKA A10; IKA-Werke, Staufen im Breisgau, Germany); 5 g of the mixture were treated in liquid nitrogen as portioned teas.

DNA isolation and quality assessment. At first, dried pulverised samples were rehydrated. For the two kit-based procedures, this step involved suspending 200 mg of powdered sample in 0.4 mL nuclease-free water and holding for 10 min at room temperature; thereafter, the samples were processed as described in the protocol provided by the manufacturer. One of these kits was a DNeasy plant mini kit (Qiagen, Hilden, Germany) and the other a Nucleospin food kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Because of the relatively low recovery of DNA from rehydrated material of some of the samples recorded when the CTAB-based protocol followed the ISO standard 21571:2005 method (hereafter referred to as ISO CTAB), a 5 min rehydration in 0.4 mL nuclease-free water at room temperature was followed by a 12 h incubation at 60 °C in 0.6 mL of extraction buffer [MB-102 ThermoCell, Hangzhou Bioer Technology Co. Ltd. (BIOER), Hangzhou, China]. The second CTAB method (2% CTAB) was a slightly modified version of the standard procedure (Doyle and Doyle 1990), in which a 0.2 g aliquot of pulverised tea was suspended in 0.7 mL CTAB extraction buffer with added 10 mg of polyvinylpyrrolidone (PVP). The mixture was held 30 min at 60 °C and then cooled on ice for 5 min [MB-102 ThermoCell, Hangzhou Bioer Technology Co. Ltd. (BIOER), Hangzhou, China]. DNA was precipitated by adding 750 µL of cold 99% v/v ethanol. The precipitated DNA was rinsed for 1 h at 4 °C in 0.5 mL of 75% v/v ethanol, held overnight at 4 °C in a fresh volume of 75% v/v ethanol (Liebherr MediLine, Liebherr GmbH, Bulle, Switzerland) and finally dissolved in 100 µL of TE buffer as described in the protocol. For those extractions that resulted in a pigmented viscous DNA solution, a re-extraction step was introduced in which 100 µL of TE buffer and 200 µL of a mixture of chloroform and isoamyl alcohol (ratio of 25; e.g. 24 : 1) were added to the DNA solution, after which DNA was precipitated from the aqueous phase by the addition of 1 : 10 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 99% v/v ethanol, as recommended elsewhere (Porebski et al. 1997; Sharma and Purohit 2012). Incubating the viscous solutions with 2 mg of pectinase (> 1 unit mg^{-1}) for 2 h at 45 °C [MB-102 ThermoCell, Hangzhou Bioer Technology Co. Ltd. (BIOER), Hangzhou, China] was also tested as a means of correcting the viscosity problem. The concentration and integrity of all of the resulting DNA samples were checked both spectrophotometrically using a NanoPhotometer® Classic (Implen GmbH, Munich, Germany) and by horizontal 1% agarose gel electrophoresis.

Real-time polymerase chain reaction (qPCR). The DNA acquired from each tea sample was subjected to PCR in triplicate. Each 25 µL PCR contained 12.5 µL of TaqMan® 2x Universal PCR MasterMix (Applied Biosystems, Foster City, California, USA), 300 nM of each of the forward and reverse primers (Generi Biotech, Třebeš, Czech Republic), 200 nM of the probe (Generi Biotech, Třebeš, Czech Republic), 0.2–100 ng DNA template and nuclease-free water (Sigma, Seelze, Germany). The PCRs targeted the chloroplast gene encoding tRNA-Leu, using primer and probe sequences published elsewhere (Taberlet et al. 1991); the expected length of the amplicon was ~90 base pair (bp). The amplification regime comprised 2-min incubation at 50 °C, 10-min incubation at 95 °C, followed by 40 cycles of 95 °C/15 s and 60 °C/60 s. The reaction prod-

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Table 1. The identity and declared composition of the tested tea products

Sample	No.	Ingredients
Immunity with *Ginkgo biloba*	1	echinacea leaves, *Ginkgo biloba* leaves, leaves of mint, St. John's wort leaves, buckthorn fruit, rosehip fruit
Fantastic tea – apple and echinacea	2	apple fruit, brier fruit, apple peel, blackberry leaf, echinacea flower, lemon rind, hibiscus flower, chicory root, cinnamon bark, aroma
Pineapple and papaya	3	rosehip fruit, apple fruit, hibiscus flower, blackberry leaves, buckthorn fruit, aroma, marigold flower, concentrated pineapple juice, anise fruit, concentrated papaya juice
Cranberry and raspberry	4	hibiscus flower, apple fruit, blackberry leaf, aroma, rosehip fruit, citric acid, cranberry fruit, raspberry fruit
Cherry, blend No. 36 Ceylon tea	5	black tea, natural aroma (cherry)
Apricot, blend No. 31 Ceylon tea	6	black tea, natural aroma (apricot)
Raspberry, blend No. 38 Ceylon tea	7	black tea, natural aroma (raspberry)
Cranberry and raspberry	8	hibiscus, apple, rosehip, cranberry aroma, blackberry leaves, orange peel, raspberries aroma, raspberries, cranberry, elderberry
Urological tea with cranberries	9	nettle leaves, peppermint, lemon grass, bearberry, cranberry, rosemary leaf, dandelion (root and flower), basil, angelica root
Cranberry; fruit fusion with cranberry taste	10	hibiscus, apple, sweet blackberry leaves, flavour (cranberry), rosehip, orange peel, blackberry leaves, cranberry
Ginkgo leaf	11	ginkgo leaves
Ginkgo	12	*Ginkgo biloba* leaf
Genius tea	13	*Ginkgo biloba* leaf, Japanese pagoda tree flower, Chinese tea (true green tea), eglantine flower, white willow bark, sage leaf
Cranberry and ginger	14	hibiscus flower, ginger, apple, aronia fruit, roasted root of chicory, blackberry leaves, licorice root, aroma, rhubarb, cranberry fruit
Fruit fusion cranberry and raspberry	15	hibiscus, apple, apple pulp, pieces of fruit (raspberry pulp, cranberry pulp), aroma, licorice root, rosehip
Bora Bora	16	raisins (raisins, sunflower oil), elderberry, papaya (papaya, sugar, firming agents calcium chloride, acidity regulator citric acid), apples, hibiscus, black currant, aroma, sunflower, cornflower, raspberries, strawberries
Tropical storm	17	apples, rosehip, banana (banana, honey, sugar, banana aroma, coconut oil), ananas (ananas, acidity regulator citric acid), orange peel, beetroot, aroma
Oma's garten	18	Lemon grass, hibiscus, chicory, eglantine, grapefruit peel, lemon peel, sweet blackberry extract, apple pomace, aroma

ucts were analysed using a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, California, USA), and the data were analysed using StepOne v2.1 software (Applied Biosystems, Foster City, California, USA). The cycle threshold (*Ct*) values were determined for each qPCR.

Presence of inhibitory compounds in DNA extracts. To assess the extent of inhibition of the PCR by contaminants present in the template, the verification procedure (European Network of GMO Laboratories 2011) was used. In brief, the template isolated using the ISO CTAB protocol was diluted to 4 ng μL^{-1}, from which a series of four consecutive 4× dilutions was prepared. An aliquot of each template (5 µL) was provided to each PCR, and the PCR yield of each dilution was plotted. The slope of the resulting linear regression and the coefficient of determination (R^2) were used as acceptability criteria: the slope was required to be between –3.6 and –3.1, and the R^2 parameter to be at least 0.98. Inhibition was also tested by comparing the *Ct* values of the qPCRs involving a 20 ng and a 2 ng template.

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RESULTS AND DISCCUSION

The yield and integrity of the extracted DNA

DNeasy plant mini kit. At least 1.7 µg was obtained from every sample; the A_{260}/A_{280} ratio of the extract ranged from 0.6 to 2.0, and the A_{260}/A_{230} ratio from 0.2 to 1.6 (Table 2). These absorbance ratios indicate the presence of some contamination in most of the samples both by compound(s) absorbing at 280 nm (probably proteins) and/or at 230 nm [ethylenediaminetetraacetic acid (EDTA) or carbohydrates]. The extraction procedure did not remove the pigmented compounds, proteins or carbohydrates. The A_{260}/A_{230} ratio can also be reduced by residual guanidine, which is often used in column-based kits. Imperfect removal of additives from samples can cause an overestimation of the DNA concentration (Wilfinger et al. 1997). This is evident, for example, in sample No. 18, where the highest concentration was measured, but both, the absorbance ratio and the qPCR results, did not correspond to this.

NucleoSpin Food kit. The DNA yield in each case was > 825 ng, which was sufficient to provide the template for subsequent PCRs. The quality of the DNA was generally similar to that obtained using the DNeasy plant mini kit, although for some samples, the absorbance ratios lay outside the commonly accepted range (Table 2). The DNA solutions were slightly pigmented again.

2% CTAB. At least 5.6 µg was obtained from every sample. The quality of the DNA produced by this extraction method was comparable with that produced by either of the commercial kits, also failing to remove some pigmented compounds in most of the samples. Some of the extractions (particularly samples No. 2 and No. 18) resulted in a viscous solution, probably due to contamination with polysaccharides and/or polyphenols, even though both salt and PVP had been included in the extraction solution to reduce the amount of these impurities. When the viscous, pigmented DNA solutions were re-extracted with chloroform/isoamyl alcohol and re-precipitated with sodium acetate/ethanol, the pigmentation remained, and there was no improvement in viscosity. The pectinase treatment did produce a slight decrease in viscosity, but the pellets proved hard to dissolve, and the absorbance ratios were not measurably improved. Overall, the 2% CTAB method proved to be the least satisfactory for extracting DNA from tea samples.

ISO CTAB. Spectrophotometry results suggested that among the four extraction methods compared, the ISO CTAB method was the least effective in terms of DNA yield. For example, the amount of DNA recovered from samples No. 4 and No. 8 was at least 20 times lower than the yield from either of the two commercial kits. However, the quality of these DNAs was superior to that of DNA obtained using any of the other three extraction methods (Table 2). Using 20 ng DNA as a template, the target sequence was successfully amplified from each of the samples. However, a higher amount of DNA in the reaction may increase the chances of its detection. As the yield was low for some of the samples, some optimisation of the isolation method was attempted; the best results were obtained by combining the rehydration step with the lysis step: the samples were incubated overnight in 0.6 mL of the extraction buffer. Although the method still resulted in a lower DNA yield than the 2% CTAB method, the quality of the DNA was higher (Figure 1). From the results obtained, it appears that the ISO CTAB method was the best way to remove possible contaminants that may spuriously increase the spectrophotometrically measured DNA concentration.

Overall, the best quality DNA was obtained from black tea samples, irrespective of the extraction method used (Figure 1).

In summary, this study demonstrates the efficiency of using various DNA isolation methods from teas based on spectrophotometry and qPCR data. Spectrophotometric measurement is the most available method of measuring DNA concentration in a routine laboratory; however, it may be over- or underestimated due to the presence of contaminants that absorb highly at or near the 260/280 nm field. For example, plant secondary metabolites, ribonucleic acid (RNA) contamination, carbohydrate carryover, residual guanidine (when using commercial kits) or CTAB in the isolate may affect spectrophotometric measurements. The DNA concentration may be measured more accurately fluorometrically (Wilfinger et al. 1997; Csaikl et al. 1998; Sovová et al. 2018). Unfortunately, the fluorometric measurement would not provide information on the purity of the isolate, which is important for this type of sample containing many possible inhibitors (e.g. dyes, aroma). Therefore, in this case, we decided to use a spectrophotometric assay followed by verification of a possible overestimation of the DNA concentration in the isolate and its amplifiability by qPCR assay. Only in a few samples, the A_{260}/A_{280} ratio ranged between 1.6 and 2.0 (1.8 ± 0.2); for ISO CTAB method, 44% of the samples, in the case of both kits 33% and for 2% CTAB only 5% of the samples. The A_{260}/A_{230} ratio was in no case in the expected range of 2.0–2.2. The best results were also obtained by the ISO CTAB method,

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Table 2. The yield and quality of DNA obtained from the tea samples, and their effectiveness as template in qPCRs primed to amplify the chloroplast gene for tRNA--leucine (tRNA-Leu) (mean ± SD; n = 3)

No.	Extraction method											
	ISO CTAB			2% CTAB			Nucleospin Food kit			DNeasy Plant kit		
	yield (ng)	A_{260}/A_{280}	*Ct* (tRNA-Leu)	yield (ng)	A_{260}/A_{280}	*Ct* (tRNA-Leu)	yield (ng)	A_{260}/A_{280}	*Ct* (tRNA-Leu)	yield (ng)	A_{260}/A_{280}	*Ct* (tRNA-Leu)
1	11 267 ± 4 654	1.75	20.7 ± 3.0	10 388 ± 2 522	0.91	30.9 ± 1.9	4 617 ± 1 475	0.99	27.3 ± 1.3	6 825 ± 1 175	1.03	23.6 ± 0.3
2	475 ± 355	1.20	23.1 ± 1.4	26 483 ± 11 483	0.91	29.8 ± 4.4	4 619 ± 954	0.73	32.2 ± 0.5	4 300 ± 760	0.71	26.1 ± 0.2
3	200 ± 94	1.38	22.2 ± 1.4	7 183 ± 3 248	0.84	35.5 ± 0.2	2 250 ± 1 167	0.83	32.2 ± 0.5	3 375 ± 216	0.71	24.5 ± 0.2
4	125 ± 35	1.36	24.5 ± 0.2	12 267 ± 4 445	1.07	31.3 ± 3.2	3 375 ± 719	0.91	33.4 ± 0.7	2 917 ± 680	1.20	25.4 ± 0.1
5	16 900 ± 4 451	1.85	20.6 ± 0.2	47 300 ± 5 267	1.15	24.2 ± 2.1	38 200 ± 5 864	1.99	23.6 ± 0.4	6 025 ± 625	1.80	18.4 ± 0.6
6	19 063 ± 2 370	1.85	19.9 ± 0.1	41 233 ± 13 389	1.37	21.4 ± 2.8	22 525 ± 5 025	2.01	23.1 ± 0.1	2 875 ± 2 075	1.66	20.2 ± 1.0
7	17 013 ± 3 603	1.85	20.5 ± 0.3	43 775 ± 6 175	1.42	23.8 ± 1.6	44 400 ± 9 296	1.94	24.2 ± 0.1	14 750 ± 4 200	1.83	20.5 ± 0.0
8	125 ± 43	1.04	24.8 ± 0.0	7 533 ± 2 384	0.72	34.9 ± 0.0	2 675 ± 300	0.87	34.9 ± 0.7	2 719 ± 747	0.74	28.0 ± 1.0
9	8 863 ± 1 415	1.83	21.3 ± 0.8	10 325 ± 9 825	1.00	24.2 ± 0.5	38 467 ± 4 514	1.87	22.1 ± 0.0	8 483 ± 2 322	1.61	23.6 ± 0.6
10	492 ± 139	1.31	30.9 ± 1.0	5 625 ± 1 956	0.90	34.6 ± 0.9	2 317 ± 663	0.82	33.2 ± 0.9	1 700 ± 400	0.94	23.9 ± 0.6
11	208 ± 24	1.64	28.6 ± 0.8	12 950 ± 5 500	1.61	32.9 ± 1.1	2 013 ± 63	1.59	28.9 ± 0.7	2 363 ± 563	1.96	ND
12	1 181 ± 250	1.78	27.4 ± 0.2	10 675 ± 925	1.40	30.1 ± 5.3	1 538 ± 290	1.68	29.6 ± 0.3	3 542 ± 676	2.02	27.9 ± 0.1
13	2 206 ± 800	1.65	19.7 ± 0.9	28 400 ± 4 000	0.93	34.3 ± 2.2	3 781 ± 1 143	0.95	22.7 ± 0.1	4 513 ± 1 388	0.98	20.7 ± 0.0
14	325 ± 82	1.24	29.7 ± 0.5	15 750 ± 250	1.00	36.9 ±0.8	2 942 ± 715	0.80	34.9 ± 0.4	3 613 ± 288	1.11	24.9 ± 0.6
15	531 ± 451	1.20	29.3 ± 0.2	13 950 ± 1 400	0.87	34.8 ± 1.7	825 ± 74	0.86	32.9 ± 0.6	2 525 ± 850	1.19	25.0 ± 0.1
16	475 ± 450	1.27	24.5 ± 1.4	13 550 ± 5 000	1.01	30.2 ± 1.7	3 000 ± 304	1.16	30.4 ± 0.3	5 642 ± 1 433	1.32	21.9 ± 1.0
17	469 ± 174	1.12	23.3 ± 0.3	11 625 ± 75	0.60	31.2 ± 1.0	2 583 ± 693	0.70	30.2 ± 1.2	1 985 ± 333	0.66	24.6 ± 0.8
18	3 075 ± 225	0.79	27.9 ± 0.2	26 200 ± 6 600	0.73	22.3 ± 0.0	2 758 ± 597	0.64	31.9 ± 1.0	9 070 ± 1 925	0.64	28.7 ± 0.4

SD – standard deviation; N – not detected; C_t (tRNA-Leu) – cycle threshold values obtained by qPCR with primers for leucine tRNA

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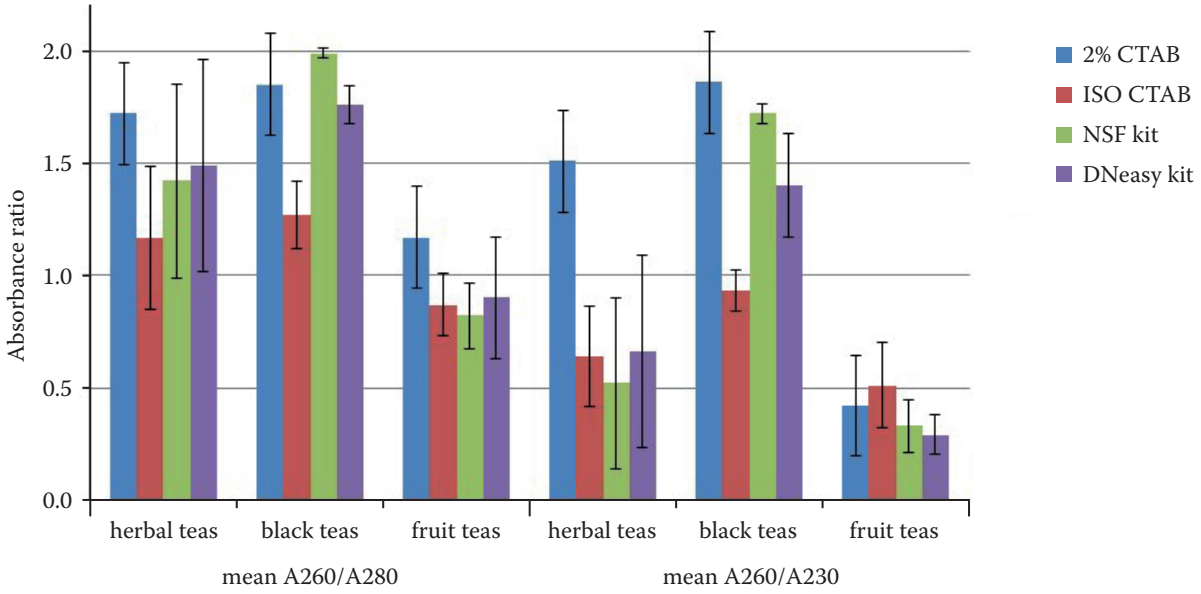

Figure 1. Comparison of the quality of the DNAs extracted from three categories of tea (black, fruit, and herbal), using four different extraction procedures

Absorbance (A) was measured spectrophotometrically at 230, 260, and 280 nm; DNA – deoxyribonucleic acid; CTAB – cetyltrimethylammonium bromide

where six samples provided a value higher than 1.6. In general, the worst results were obtained for fruit teas; the absorbance ratio was < 1.0. These very low values may suggest the presence of contaminants in the sample. *Ct* values, which are generally the best for the used ISO CTAB method, confirmed our assumptions about overestimation of DNA concentrations, and in most samples, they corresponded to the spectrophotometric data indicating the quality of the isolate.

Effectiveness of DNA as PCR template

Except for the extract from *Folium ginkgo*, all of the templates produced using the DNeasy plant mini kit were PCR-amplifiable. All three replicated extractions of the template prepared from the *Ginkgo biloba* L., folium failed to amplify, even after diluting the template fourfold. The samples most readily amplified were those extracted from black tea samples, for which the *Ct* ranged from 18 to 20 (Table 2). The templates extracted using the NucleoSpin Food kit all successfully amplified the target sequence in reactions provided with 20 ng of template. However, the *Ct* for a number of samples was higher than was obtained using DNA extracted with the DNeasy plant mini kit (Table 2). The least effective templates were those extracted using the 2% CTAB method across all sample types (Table 2). The best results obtained from extracts of the herbal tea samples were obtained using the ISO CTAB method, which also provided the second-best performance for the black and fruit teas.

Presence of PCR inhibitors in DNA templates

The presence of PCR inhibitors was tested by subjecting template solutions derived from ISO CTAB extractions to a dilution series, following published recommendations (European Network of GMO Laboratories 2011). Inhibitors were detected in over a half of the samples, notably in samples No. 5, 7, and 10–18. For samples No. 5 and No. 7 (aromatic black teas), the slope of the regressions (–3.7 and –3.8) fell outside the recommended range, indicative of a low reaction efficiency (82–84%). The R^2 statistic was > 0.99, and the difference in *Ct* values did not exceed 0.5 in either case. Samples No. 12 (herbal tea), 16, and 17 (fruit teas) all failed to pass the same two acceptability criteria: the efficiency of their reactions was > 110%, and the regression slope was higher (–2.3 to –2.8) than desirable. For the other samples, the presence of inhibition was inferred from their non-compliance with all three acceptability criteria (R^2, regression slope and reaction efficiency). The ΔCt values between the two final dilutions in samples No. 10, 14, and 15 (fruit teas) were all < 1, and the same was the case between the second and third dilution of samples No. 10 and No. 14. In some cases, the difference between the $Ct_{theoretic}$ and $Ct_{average}$ values was exceeded; since the DNA concentrations in these

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cases were low, the two *Ct* values may have differed as a result of exceeding the quantification limit, or they may be around the detection limit. There was no evidence for any inhibition to the PCRs caused by the template of samples No. 1–4, 6, 8, 9, or 13, although PCRs from sample No. 8 DNA produced an R^2 of only 0.94. Reactions using the template from samples No. 2–4 and No. 8 (fruit teas) recorded a fluorescence signal (ΔRn) which was a little over 50% of the parameter value in reactions based on the template from the other samples. The ΔRn decrease in amplification curves may also be an indicator of the presence of inhibitors.

The way in which the raw material used for manufacturing infusions is processed can also reduce the PCR amplification efficiency: an example is the presence of residual sugar in extracts of teas containing candied fruit. During the preparation of black teas, leaves have to be heated to a high temperature (Valter 2010), but it has been well established that DNA sequences are not readily disrupted by temperatures up to 100 °C (Hrnčírová et al. 2008; Karni et al. 2013). However, the quality of the DNA can be compromised if the tea is stored improperly, allowing for its degradation by microorganisms. The tea used to produce tea bags is also typically of poor quality, consisting of damaged leaves and tea dust.

CONCLUSION

To the best of our knowledge, this is the first study focused on isolating DNA from black, fruit and herbal teas. The four DNA extraction protocols compared all produced enough DNA from these samples for a PCR assay, but its quality varied: three of the protocols were unable to fully exclude pigmented compounds and other additives, some of which may affect the spectrophotometrically measured concentration and/or have been responsible for their reduced efficiency as a PCR template. The ISO CTAB method produced a colourless extract; while spectrophotometry results suggested that its DNA yield was the lowest of the four methods, the recommendation is that it represents the method best suited for extracting DNA from teas.

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