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Determining the Optimal Method for DNA Isolation from Fruit Jams

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Abstract

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DNA extraction is a crucial step in PCR analysis especially when analysing food samples that can be degraded and can potentially contain PCR-inhibiting substances. In this study, we compared the suitability of three DNA extraction methods – two kits: DNeasy® Plant Mini Kit and NucleoSpin® Food, and the CTAB method – for DNA extraction from commercial fruit jams. Fourteen jams with different contents of fruit, sugar and other additives were extracted in triplicate using the above-mentioned methods directly and after a washing step. The concentration and optical density were analysed using UV spectrophotometry and the amplifiability of the obtained DNA was evaluated using a PCR assay targeting a sequence coding for chloroplast tRNA-Leu. Samples isolated using the NucleoSpin® Food kit contained non-amplifiable DNA in eight cases, and samples isolated using the CTAB method could not be quantified. The DNeasy® Plant Mini Kit thus proved to be the most suitable method, since well-amplifiable DNA was obtained for all the analysed samples.

Keywords: DNA amplifiability; DNA extraction; food analysis; food quality

With the rapid development and globalisation of the international food market, food quality control has become a high priority. It is necessary to ascertain that consumers are correctly informed about the origin and composition of available products, and that the safety and authenticity have been verified. Apart from physical and chemical methods, DNA-based techniques, such as the polymerase chain reaction (PCR) and its variants like quantitative PCR, are also basic tools in food quality and safety assurance (Renault *et al*. 2004; Deer *et al*. 2010).

This study was aimed at DNA isolation from fruit jams that are also subject to adulteration, and thus definitions and labelling requirements for jams are specified by legislation (Council Directive 2001/113/EC, US FDA Code of Federal Regulations). Fruit jams are usually defined as a mixture of sugars, pulp and/or purée of one or more kinds of fruit and water (Council Directive 2001/113/EC); moreover, various preservatives, colorants and aromas can also be added. Fruits are the most expensive of the above-mentioned ingredients and thus the most convenient adulteration method is their partial replacement with cheaper ingredients (such as alternative fruits, vegetable matter or sugar) or the addition of more artificial additives (Defernez & Wilson 1995; Fügel *et al.* 2005). Physical and chemical methods, such as analysis of sugar profiles using electrophoresis (Navarro-Pascual-Ahuir *et al.* 2015) or evaluation of Fourier-transform infrared spectra of jams (Defernez & Wilson 1995), can be used to monitor the adulteration of fruit jams. However, metabolite profiles can sometimes be influenced by external factors (e.g., light and other storage conditions) and

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can even alter within the same species (Mafra *et al.* 2008; Jaakola *et al.* 2010). It is also sometimes necessary to distinguish between very closely related fruit species, such as berries within the *Vaccinium* sp. (Jaakola *et al.* 2010). Therefore, DNA-based authentication methods are of interest (Mafra *et al.* 2008; Jaakola *et al.* 2010).

One of the crucial steps in a PCR analysis is to obtain a good quality sample with easily amplifiable DNA, free of PCR inhibitors and other contaminants that could skew the result of the analysis (di Pinto *et al.* 2007). Food samples are often difficult in this aspect as food production often includes thermal and other aggressive treatments that can degrade the DNA. Moreover, the matrices of food samples are rich in PCR inhibitors such as polysaccharides, phenolic substances, proteins and humic acids (Chapela *et al.* 2007; di Pinto *et al.* 2007; Turci *et al.* 2010). There are several methods of DNA isolation that differ in their principles, number of purification steps and other parameters, and it is crucial to choose the right method for the sample in question to obtain DNA of optimal quality for the PCR analysis (Chapela *et al.* 2007; di Pinto *et al.* 2007; Turci *et al.* 2010).

In this study, we evaluated three DNA isolation techniques – two kits, DNeasy® Plant Mini Kit and NucleoSpin® Food, and the CTAB method – for the analysis of fruit jams. Fruit jams are complex samples subjected to high temperatures and pressure during production and can contain many additives (polysaccharides such as pectin, preservatives, colorants and aromas) which could complicate DNA extraction and subsequent PCR analysis. As an indicator of DNA quality and amplifiability, a PCR assay targeting a sequence coding for chloroplast tRNA-Leu was chosen; this basic PCR assay is often used to check DNA isolated from plant-based materials (Taberlet *et al.* 1991; Meyer 1999) and has already been used in studies aimed at fruits and fruit-derived products (Yamamoto *et al.* 2006; di Pinto *et al.* 2007; Clarke *et al.* 2008).

MATERIAL AND METHODS

Samples. Fourteen samples of fruit jams with different contents of fruit, sugar and additives (Table 1) were purchased in local supermarkets and stored (dark, 4°C) until needed.

DNA extraction. DNA was extracted using two kits: DNeasy® Plant Mini Kit (DPM; Qiagen, Germany) and NucleoSpin® Food (NF; Macherey-Nagel, Germany), and the CTAB (cetyltrimethylammonium bromide) method. The kits were used according to the manufacturers' instructions without any modifications. The CTAB method was performed according to the ISO 21571:2005 standard without any modifications. Each sample was extracted both (1) directly without any pre-treatment and (2) after a washing step. In the washing step, 10 g of each sample were dissolved in approx. 40 ml of double-distilled sterile water by vigorous shaking and centrifuged for 5 min at 4000 *g* (Ganopoulos *et al.* 2011). The supernatant was carefully discarded and the washing step was repeated two more times. In order to determine the amount of solid insoluble fraction in the samples, another set of samples were washed, dried (60°C, overnight) and reweighed. All samples (both un-washed jams and pellets obtained after the last washing step) were then homogenised in liquid nitrogen and extracted in triplicate. The concentration and purity of the DNA was determined using UV spectrophotometry (NanoPhotometer P300; Germany).

PCR assay. The PCR assay targeted a sequence coding for chloroplast tRNA-Leu using forward primer 5'-ATT GAG CCT TGG TAT GGA AAC CT-3' and reverse primer 5'-GGA TTT GGC TCA GGA TTG CC-3' primer (Taberlet *et al.* 1991). The reaction was performed in a final volume of 25 µl using 2.5 µl of 10 × PCR Buffer, 800 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 IU TaqDNA Polymerase (Applied Biosystems, USA), 0.2 µM forward primer, 0.2 µM reverse primer and 5 µl of the DNA sample. The DNA samples were diluted to a concentration of 20 ng/µl; when the initial sample concentration was lower, the sample was added to the reaction mixture directly without dilution. If the PCR result was negative, the reaction was repeated with serial dilutions of the sample to identify potential inhibition. The PCR was performed in a Mastercycler® nexus (Eppendorf AG, Germany) with an initial denaturation step of 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s; final extension was at 72°C for 6 minutes.

The PCR products were run on 2% (w/v) agarose gels stained with ethidium bromide (0.1 µg/ml) for approx. 1 h in 1 × TAE buffer at room temperature using a constant voltage of 60 V and a 100-bp ladder size standard (Applied Biosystems, UK). Bands were visualised using UV light. DNA isolated from the fresh leaves of lingonberry (*Vaccinium vitis-idea* L.) was used as a positive control.

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RESULTS AND DISCUSSION

The aim of this study was to compare three DNA isolation methods. Since its development in the 1980s, the CTAB method has become a fundamental method for the extraction of DNA from plant-based material (Murray & Thompson 1980; Allen *et al.* 2006). The procedure does not require any special chemicals or equipment and is thus inexpensive; it produces good quality DNA in sufficient quantity for most molecular biological techniques (Allen *et al.* 2006). The method is also ISO-standardised which facilitates its use in accredited labs (ISO 21571:2005). However, as it consists of a series of centrifugation and organic solvent extraction steps the method can be lengthy even though modifications of the original protocol have reduced the duration of the process (Allen *et al.* 2006). On the other hand, the two ready-to-use kits, both intended for isolating plant- (DPM) or food-based samples (NF), offer a rapid isolation involving only five to six steps. However, the price per sample is higher compared to the CTAB method and the composition of the used solutions is not disclosed (DNeasy® Plant Handbook, Genomic DNA from food – User manual -–NucleoSpin® Food). The kits are also optimised for a limited amount of sample – typically up to 200 mg, maximum 1 g (DNeasy® Plant Handbook, Genomic DNA from food – User manual – NucleoSpin® Food), while the CTAB method can be used for a several-fold higher quantity; e.g., 2 g (Mekuria *et al.* 1999) or even 5 g (Qiu *et al.* 2005). Moreover, the capacity of the silica membrane which is used in the kits to extract DNA is limited and thus obtaining a larger quantity of DNA can be a challenge (DNeasy® Plant Handbook; Genomic DNA from food – User manual, NucleoSpin® Food).

The results of DNA isolation and the PCR assay are presented in Table 1. Using both kits (DPM and NF), DNA was successfully isolated from all 14 samples. Compared to DPM, samples isolated using the NF kit generally had higher DNA yield and also better DNA quality (based on A_{260}/A_{280} and A_{260}/A_{230} values). The NF kit uses twice as much initial sample for isolation compared to DPM (200 vs. 100 mg) but the produced DNA quantity was more than two times larger in most of the samples which might point to a better efficiency of the NF kit. The washing step, which helps in eliminating sugar and other additives and solid fruit constituents that have accumulated, increased the quantity of isolated DNA in the large majority of samples for both kits; however, the purity of the DNA was improved only in a third of the samples. Overall, the quality of the DNA samples was not ideal with only one sample with an A_{260}/A_{280} value within the optimal range of 1.7–1.9 (sample J, washed, isolated with the NF kit); moreover, the A_{260}/A_{230} values were very low (below 0.601; optimally above 1.8) for all of the samples and both treatments. This might point to the presence of contaminants (such as proteins, phenolic compounds or polysaccharides) that could cause inhibition of the PCR (Varma *et al.* 2007). Higher quantities of DNA were isolated from better quality jams with a higher solid fraction (Table 1) containing large fruit fragments and seeds (samples A–I) and also fewer additives compared to other jams of lower quality that did not contain any fruit fragments but did contain additives such as colorants and aromas.

Compared to the kits, it was not possible to detect the concentration or purity of DNA samples isolated using the CTAB method, since the spectrophotometer gave no signal. However, when the CTAB-isolated samples were used in the PCR assay (undiluted samples were used), the target sequence was amplified in almost all samples including the unwashed ones. The sole exception was the jam produced from blackcurrant (sample H). Currants are fruits with a generally higher content of pectins compared to other fruits (Voragen *et al.* 2009). Pectins have been shown to cause PCR inhibition (Pandey *et al.* 1996; Peist *et al.* 2001), which could have been the cause of the negative result. The washing step, however, removed the potential inhibitors and the target sequence was detected in the PCR assay.

On the other hand, the samples isolated using the NF kit, which were consistently of good yield and quality, proved not to be amplifiable in the PCR assay. For seven samples, the PCR assay did not give a positive result even after the washing step and after serial dilution. In the case of the DPM kit, the washed samples were positive in all cases, although in four samples dilution was required to relieve the inhibition. Both kits are based on solid-phase extraction. The DNA is bound on a silica membrane, washed by buffers to remove contaminants and inhibitors and eluted using low-salt buffer or water. However, the kits differ in the steps preceding the DNA binding. While NF uses proteinase to digest proteins which are then removed by simple centrifugation together with cell debris, DPM eliminates proteins and other contaminants such as detergents and polysaccharides

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Table 1. Summary of properties of 14 fruit jams, and concentration and optical density ranges

Sample	Fruits	Total sugar content (g/100 g final product)	Additives	Solid fraction (g/100 g jam)	Extraction method	No pre-treatment				Water wash			
						DNA concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	PCR	DNA concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	PCR
A	lingonberry	57	sugar, pectin, citric acid	9.3 ± 0.4	DPM	9.5–15.5 (12.3)	0.795–0.826 (0.845)	0.253 (0.196–0.368)	+	11.5–17.5 (14.2)	0.821–0.972 (0.870)	0.183 (0.146–0.268)	+
					NF	20.5–27.0 (23.8)	0.897–0.900 (0.898)	0.237 (0.206–0.289)	–	11.5–40.5 (30.5)	0.920–0.953 (0.949)	0.260 (0.216–0.316)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
B	lingonberry	54	sugar, pectin, lemon juice	11.1 ± 0.4	DPM	11.5–13 (12.2)	0.793–0.826 (0.811)	0.299 (0.205–0.437)	+	5.5–7.5 (6.3)	0.647–0.938 (0.778)	0.236 (0.165–0.367)	+
					NF	20–56 (38.7)	0.889–0.926 (0.910)	0.250 (0.200-0.312)	–	29.5–49 (37.7)	1.017–0.867 (0.935)	0.294 (0.171-0.325)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
C	lingonberry	45	sugar, pectin, citric acid	8.4 ± 0.1	DPM	9.5–10 (9.7)	0.741–0.905 (0.834)	0.261 (0.157–0.324)	+	9.5–19 (14.2)	0.76–0.884 (0.794)	0.251 (0.167–0.421)	+
					NF	18.5–30.5 (24.7)	0.910–0.925 (0.918)	0.257 (0.223–0.249)	–	31.5–44.5 (39.2)	0.840–0.967 (0.935)	0.213 (0.149–0.379)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+
D	cranberry, bilberry	52	pectin, fruit juice	7.5 ± 0.1	DPM	11.5–12 (11.8)	1.043–1.045 (1.044)	0.275 (0.182–0.367)	+	9–15.5 (12.7)	1–0.912 (0.959)	0.313 (0.267–0.391)	+
					NF	26–65.5 (43.8)	0.900–1.020 (0.979)	0.209 (0.189–0.220)	–	50–72.5 (60.5)	0.952–0.918 (0.941)	0.259 (0.175–0.251)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+
E	strawberry	47	sugar, pectin, citric acid, glucose syrup	5.3 ± 0.1	DPM	4.5–6.5 (5.0)	1.286–1.300 (1.292)	0.344 (0.289–0.407)	+	3.5–7.5 (5.8)	1–1.364 (1.149)	0.369 (0.246–0.430)	+
					NF	28.5–29.5 (29.0)	1.295–1.311 (1.303)	0.497 (0.292–0.855)	–	32.5–60.5 (42.3)	1.912–1.142 (1.462)	0.345 (0.274–0.406)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
F	bilberry	43	sugar, pectin, citric acid, glucose syrup	2.6 ± 0.1	DPM	6.0–6.5 (6.2)	1.200–1.444 (1.336)	0.357 (0.204–0.477)	+	6.5–8.5 (7.2)	1.182–1.214 (1.159)	0.409 (0.316–0.499)	+
					NF	33–33.5 (33.2)	1.179–1.196 (1.186)	0.322 (0.312–0.339)	–	41–68.5 (55.5)	1.439–1.412 (1.397)	0.305 (0.260–0.365)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
G	raspberry	57	sugar, pectin, lemon juice	8.2 ± 0.1	DPM	6.5–9.5 (7.7)	1.182–1.357 (1.237)	0.275 (0.182–0.367)	+*	11.5–16.5 (14.0)	1.211–1.222 (1.217)	0.313 (0.267–0.391)	+*
					NF	35.5–47 (41.3)	1.516–1.617 (1.572)	0.209 (0.189–0.220)	+	48.5–262 (165.8)	1.644–1.959 (1.785)	0.259 (0.175–0.251)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
H	black currant	32	sugar, pectin, citric acid	5.6 ± 0.1	DPM	17–18.5 (17.7)	1.370–1.417 (1.398)	0.181 (0.171–0.203)	+*	82–99.5 (89.0)	1.271–1.206 (1.227)	0.225 (0.154–0.288)	+*
					NF	32.5–59.5 (46.0)	1.190–1.226 (1.205)	0.213 (0.190–0.236)	–	103–246 (151.3)	1.192–1.019 (1.092)	0.270 (0.257–0.361)	–
					CTAB	ND	ND	ND	–	ND	ND	ND	+

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Table 1 to be continued

Sample	Fruits	Total sugar content (g/100 g final product)	Additives	Solid fraction (g/100 g jam)	Extraction method	No pre-treatment				Water wash			
						DNA concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	PCR	DNA concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	PCR
I	fig	52	pectin, lemon juice, fruit juice	6.3 ± 0.1	DPM	3–4 (3.6)	1.500–1.333 (1.359)	0.523 (0.467–0.578)	+	1.5–3.5 (2.5)	1.5–1.75 (639)	0.403 (0.392–0.459)	+
					NF	12–21.5 (17.5)	1.483–1.500 (1.488)	0.350 (0.287–0.436)	+	9–29.5 (16.2)	1.5–1.475 (1.492)	0.393 (0.289–0.464)	+
					CTAB	ND	ND	ND	+	ND	ND	ND	+
J	strawberry, apple	59	sugar, pectin, citric acid, sulphur dioxide, synthetic aroma, carmine (cochineal)	0.2 ± 0.1	DPM	7.5–8.5 (8.1)	1.214–1.250 (1.238)	0.318 (0.172–0.532)	–	6–10.5 (7.7)	1.091–1.167 (1.114)	0.306 (0.173–0.451)	+*
					NF	13–17 (15.3)	1.182–1.214 (1.198)	0.216 (0.204–0.230)	–	9.5–130 (81.2)	1.026–1.016 (1.008)	0.301 (0.252–0.319)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+
K	apricot, apple	59	sugar, pectin, citric acid, synthetic aroma	1.3 ± 0.2	DPM	8–22 (16.3)	0.696–1.630 (1.326)	0.331 (0.273–0.479)	+	2–4.5 (3.2)	1–1.125 (1.108)	0.421 (0.362–0.454)	+
					NF	18–21.5 (19.2)	1.200–1.229 (1.216)	0.265 (0.191–0.338)	–	19.5–35.5 (28.7)	0.867–1.268 (1.056)	0.306 (0.263–0.396)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+
L	apple, black currant, raspberries, elderberry	57	sugar, pectin, citric acid, synthetic aroma, sulphur dioxide	1.7 ± 0.9	DPM	7.5–14.5 (10.7)	1.250–1.318 (1.292)	0.301 (0.250–0.367)	+*	3.5–7 (5.2)	1.167–1.273 (1.123)	0.393 (0.257–0.468)	+*
					NF	25–38 (29.7)	1.246–1.282 (1.264)	0.258 (0.209–0.284)	–	31–82 (53.3)	1.069–1.215 (1.153)	0.303 (0.267–0.372)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+
M	apricot	34	sugar, pectin, citric acid	0.8 ± 0.3	DPM	1.5–3.5 (2.8)	1.400–1.427 (1.411)	0.231 (0.156–0.563)	+*	1.5–3 (2.0)	1.5–1.5 (1.333)	0.461 (0.164–0.528)	+
					NF	6–11 (8.5)	1.571–1.714 (1.646)	0.290 (0.274–0.306)	–	36.5–67.5 (49.7)	1.123–1.184 (1.169)	0.364 (0.301–0.467)	–
					CTAB	ND	ND	ND	+*	ND	ND	ND	+
N	sour cherry	34	sugar, pectin, citric acid	3.3 ± 0.4	DPM	2.5–22.5 (14.3)	1.000–1.154 (1.095)	0.370 (0.267–0.467)	+*	3.5–8 (5.7)	0.778–0.941 (0.855)	0.222 (0.199–0.269)	+
					NF	5.5–11 (8.3)	1.158–1.222 (1.182)	0.227 (0.205–0.261)	–	27–65.5 (42.0)	0.947–0.929 (0.949)	0.392 (0.266–0.420)	–
					CTAB	ND	ND	ND	+	ND	ND	ND	+

Mean values in brackets (n = 3), of DNA samples obtained from those jams using three DNA extraction methods (two kits – DNeasy® Plant Mini Kit (DPM) and NucleoSpin® Food (NF) – and the CTAB method). The DNA samples were subsequently used in a PCR reaction targeting a sequence coding for chloroplast tRNA-Leu: $^{+}$the sequence was detected; $^{+*}$the sequence was detected after diluting the DNA sample, $^{-}$the sequence was not detected even after dilution. The extractions and PCR reactions were performed in triplicate.

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by salt-precipitation; the precipitate along with cell debris is then removed by a filtration membrane. It is possible that the filtration step in the DPM kit is more efficient in removing inhibitors present in the jam matrix, which is why DPM produced a better amplifiable DNA even though the yield and purity of the DNA was worse than for the NF kit. It is, however, well known that the optical density of a DNA sample is not directly linked to its amplifiability (Hedman & Rådström 2013). In addition, the determination of DNA concentration using UV spectrophotometry can overestimate the DNA quantity as it does not distinguish between double-stranded and single-stranded DNA, single nucleotides and RNA (Olexová *et al.* 2004; Holden *et al.* 2009). As the DPM kit, unlike NF, by default contains an RNase lysis step, overestimation due to the presence of RNA is possible. Moreover, as the removal of inhibitors differs between the kits, the NF extracts might contain additional non-DNA substances that also absorb at 260 nm.

Our results are consistent with other studies evaluating DNA isolation from fruit products. Ganopoulos *et al.* (2011) did not find NF to be a suitable extraction method for cherry jams (both commercial and laboratory-made) while di Pinto *et al.* (2007) successfully isolated DNA from cherry jam using DPM. The CTAB method was also used in a number of studies; however, the method was modified to account for the specific characteristics of different processed fruit samples. Several studies have combined the CTAB method with a solid-phase extraction column from a commercial kit (Siret *et al.* 2000; di Pinto *et al.* 2007; Clarke *et al.* 2008; Ganopoulos *et al.* 2011; Arleo *et al.* 2012). For extraction of fig jam, di Bernardo *et al.* (2005) added a 10% solution of *n*-phenacylthiazolium bromide to the extraction mixture. Its role is to cleave sugar-derived cross-links between proteins and DNA, that might have appeared in the sample due to high temperature during fruit processing and that could prevent DNA extraction and/or amplification. In the study by Clarke *et al.* (2008), the low pH values of samples of fruit juices were adjusted to pH 8 using NaOH before CTAB extraction; this could be of relevance to fruit jams too, as their pH is also acidic (Egbekun *et al.* 1998; Anvoh & Bi 2009).

Overall, the washing step improved the yield and amplifiability of the DNA samples. Thus, if a sufficient amount of jam is available, it is always worth performing it. From the three evaluated isolation methods, the DPM kit proved to be the most suitable. It is, however, always necessary to incorporate the washing step; otherwise, there is a risk that the DNA sample is non-amplifiable especially when isolating from a lower quality jam not made from whole fruits. The low yield of the kit can be overcome by pooling different samples, using a lower volume of buffer to elute the silica membrane or using the DNeasy® Plant Maxi Kit, which uses a larger quantity of initial sample (up to 1 g). The CTAB method would also be a suitable method (based on both the amplifiability of the produced DNA even in the unwashed samples and the lower price per sample compared to kits) if it were not for the inability to quantify the obtained DNA samples. This could be overcome by including a further purification step in the process.

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