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Comparison of Several Methods for the Extraction of DNA from Potatoes and Potato-Derived Products

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Eight methods were compared for the extraction of DNA from raw potato tubers, and nine methods were evaluated for the extraction of DNA from dehydrated potato slices, potato flakes, potato flour, potato starch, and two ready-to-eat potato snack foods. Extracts were assessed for yield using a fluorescence-based DNA quantification assay. Real-time amplification of an endogenous gene, sucrose synthase (*sus*), was used to assess extract and template quality. A CTAB-based method extracted the highest DNA yields from the tuber material. An in-house method, which utilized the Kingfisher magnetic particle processor, yielded the highest template quality from the tubers. For most of the tuber samples, the Kingfisher and CTAB methods recovered the highest levels of amplifiable *sus*. DNA yields for potato-derived foods generally decreased with the extent that the product had been processed. The methods that utilized the magnetic particle processor delivered the highest template quality from one of the snack products that was particularly high in fat. For most of the remaining processed products, the levels of amplifiable target DNA recovered were roughly correlated with total DNA recovery, indicating that overall yield had greater influence over *sus* amplification than template quality. The Wizard method was generally the best method for the extraction of DNA from most of the potato-derived foods.

KEYWORDS: ***Solanum tuberosum*; potato; DNA extraction; DNA detection; GMO; qPCR**

INTRODUCTION

DNA amplification methods are emerging as useful tools in food inspection and regulation. Such methods have been developed to detect and quantify the adventitious mixing of unwanted genetically modified ingredients in a variety of matrices (*1*−*4*). The steady growth in the acreage and diversity of genetically modified crops planted has also driven a demand for "farm-to-fork" traceability to ensure the integrity and safety of the food supply (*2*). DNA amplification methods have also been used to detect the fraudulent misdescription or adulteration of food products (*5*). Furthermore, amplification-based detection of DNA markers in various crops can be useful for determining phylogenetic relationships as well as establishing and tracking breeding pedigrees in crops (*6*). In potato (*Solanum tuberosum* L.), sequence-tagged microsatellite analysis was used to differentiate 50 commonly grown cultivars (*7*).

Regulatory laboratories must employ methods with sufficient validity and robustness to support enforcement action. DNA extraction methods should ideally be simple, rapid, efficient, and consistent while minimizing the potential for cross-contamination. Safety and cost are also primary considerations. DNA quality is a critical factor for most amplification-based analyses, because the amplification of DNA is influenced by the presence of copurifying inhibitors from the matrix or the extraction reagents, which can reduce the efficiency of the polymerase chain reaction (PCR). DNA damage may also occur during the extraction through oxidation and enzyme hydrolysis, so extraction buffers should be carefully formulated.

Potatoes are a staple crop in much of the world, and they are processed in a variety ways to eliminate waste, prevent spoilage, preserve nutrients, and increase their value (*8*). Like many plant tissues, potato tubers can present problems for some DNA analyses. Tuber tissues contain acidic polysaccharides and various polyphenolics that are known to inhibit nucleic acid amplification (*9, 10*). Processed potatoes present even greater challenges, as the mechanical, thermal, and chemical processes that are used to refine potatoes and other food commodities may damage DNA through endogenous enzyme hydrolysis, shearing, depurination, cross-linking, acid hydrolysis, and oxidation (*11*). Damaged or fragmented template DNA may severely reduce amplification efficiency in PCR or may render target sequences undetectable (*3*). Fats, salts, acids, and other additives in the food matrix may also contribute to PCR inhibition (*11*).

In this study, eight methods were compared for the extraction of DNA from raw potato tubers, and nine DNA extraction methods were compared for several processed potato foods and food ingredients. The methods that were chosen for this study are shown in **Table 1**, which also lists an approximate estimate of cost per sample (materials only) and the special advantages

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Table 1. General Description of Each DNA Extraction Method Compared in This Study

method name	description	cost per sample[a] ($)	basis and format	special advantages
Wizard	columns and Wizard resin purchased from Promega; other reagents prepared in-house[b]	2.15	silica binding; vacuum manifold format	better scalability; more economical than most spin-column kit methods
DNeasy	DNeasy Plant Mini kit (Qiagen)	3.70	silica binding.; spin-column format	rapid, convenient; widely used
Roche l	DNA Isolation Kit for Cells and Tissues (Roche)	5.90	solution-based; selective precipitation of DNA	convenient; excellent scalability and flexibility
Hi-Pure	Hi-Pure PCR Template Preparation Kit (Roche)	2.50	silica binding; spin-column format	lower cost alternative to DNeasy and Nucleospin kits
CTAB[c]	reagents prepared in-house[b]	0.50	solution-based; selective precipitation of DNA	economical; widely used
Mo-Bio	UltraClean Plant DNA Kit (Mo-Bio)	3.20	cell lysis achieved with bead-grinding; silica binding; spin-column format	rapid, convenient method of cell disruption
Kingfisher	Kingfisher instrument used with in-house reagents[b]	2.50	silica binding; magnetic separation	convenient; semiautomated
Magnesil KF	Kingfisher instrument used with MagneSilKF Genomic System (Promega)	4.40	silica binding; magnetic separation	convenient; semiautomated
Nucleospin	NucleoSpin Food Kit (Machery-Nagel)	4.20	silica binding; spin-column format	rapid and convenient
PM Food	Wizard Magnetic DNA Purification System for Food (Promega)	3.70	silica binding; magnetic separation	very rapid lysis and digestion step

[a] Cost for kit, reagents, and special required consumables in Canadian dollars. Does not include additional costs for equipment or labor. [b] See **Table 2** for formulations. [c] Cetyltrimethylammonium bromide.

that each method offers. Methods were chosen on the basis of potential applicability in the regulatory diagnostic laboratory; procedures that included the use of liquid nitrogen were avoided due to safety and cross-contamination concerns. Both commercial kit methods and methods utilizing in-house reagents were evaluated in this study. Kits offer considerable convenience but sometimes lack the flexibility or scalability that in-house methods can provide.

The methods were evaluated on the basis of DNA yield and quality. Because coextraction of impurities and DNA damage both influence efficiency in PCR and DNA amplification depends on initial template concentration and reaction efficiency (*12*), extract quality was assessed using real-time quantitative PCR by measuring the production of an amplicon relative to a standard. Conventional PCR (*13*−*15*), competitive PCR (*16*), and real-time quantitative PCR (*15*, *17*) have been previously used for assessing template quality recovered from several matrices using different extraction methods. In this study, an endogenous gene, potato sucrose synthase (*sus*), was used as a target for this comparative assessment.

The potato products used for this study represented different points along various stages of the processing chain (**Figure 1**). For example, a fried, ready-to-eat snack made from whole potatoes (product A) represented a lightly processed food, whereas an extruded, cooked snack (product B) represented a more highly processed food (**Figure 1**). Potato starch, which is prepared using a process designed to separate the starch granules from other cellular material (*18*; **Figure 1**), was the most highly refined of all the products tested.

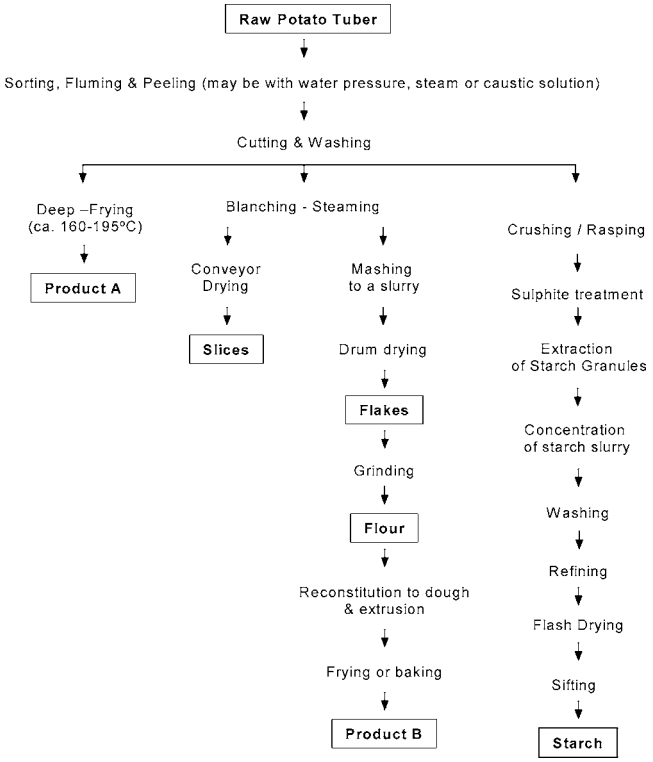

Figure 1. Summary of some general processes used to manufacture various potato products (*8*, *18*). Samples of the products shown in boxes were selected for this study.

MATERIALS AND METHODS

Sample Material. Whole Russet Burbank and Russet Norkotah field tubers were obtained from seed growers. Russet Burbank and Russet Norkotah tubers were also raised in a laboratory greenhouse. All processed potato products, dehydrated potato slices, potato flakes, potato flour, potato starch, and two ready-to-eat foods, products A and B, were purchased from local retailers. Product A was a shoestring-type fried snack made from whole potatoes, containing 40% fat by weight. Product B was a snack made from potato flour and contained 20% fat by weight. A general outline of the processes used to manufacture these products is shown in **Figure 1**.

Homogenization. Prior to extraction of DNA, some of the sample materials were homogenized. For all of the methods except the Mo-Bio, whole potatoes were thinly sliced, placed into tared plastic bags (Bioreba), and weighed. After the addition of the appropriate buffer (**Table 2**), the tissue was pounded and finely homogenized using a Homex (Bioreba). The homogenate was recovered, carefully weighed, and used for the subsequent DNA extractions according to the procedure referenced in **Table 2**. For the Mo-Bio method, 50 or 100 mg of tuber tissue was mixed with 2 mL of the extraction buffer and the grinding beads included in the kit, as indicated in **Table 2**. The tissue was ground using an adapted Vortex Genie for 10 min at high speed.

Table 2. Procedures for the Small-Scale Extraction of DNA from Various Potato Matrices

method	extraction/lysis buffer (EB)	matrix	vol of EB/ 100 mg of sample	lysis incubation conditions	final extract vol/ 100 mg of sample	procedure reference
Wizard	150 mM NaCl, 2 mM EDTA, 500 mM guanidine-HCl, 20 mM Tris-HCl (pH 7.5), 0.8 mg/mL proteinase K; water-soaking step omitted	tuber	0.35 mL	180 min at 57 °C	100 μL of DNase-free water	*19*
		products A and B; starch and slices	1.0 mL			
		flour and flakes	1.5 mL			
DNeasy	buffer AP1[a] with 1 mg/mL of RNase A[a]	tuber	0.40 mL	10 min at 65 °C	100 μL of buffer AE[a]	kit instructions
		flour	0.80 mL			
		flakes	1.2 mL			
		slices	0.75 mL			
		starch	0.40 mL			
Roche I	cellular lysis buffer[a] and proteinase K[a]	tuber	2.5 mL	60 min at 65 °C	100 μL of DNase-free water	kit instructions
		all processed products	2.5 mL			
Hi-Pure	binding buffer[a] with proteinase K[a.]	tuber	0.40 mL	10 min at 70 °C	100 μL of elution buffer[a]	kit instructions
		products A and B	1.0 mL			
		all others	1.5 mL			
CTAB[b]	2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0)	all	0.75 mL	30 min at 65 °C	100 μL of DNase-free water	*20*
Mo-Bio	bead solution[a] and solution P1[a]	tuber	2.0 mL and 60 μL	10 min at 65 °C, vortexed at full speed for 10 min	50 μL of solution P5[a]	kit instructions
Kingfisher	EB: same as Wizard (*18*); for other buffer formulations, see text	tuber	0.35 mL	180 min at 57 °C	80 μL of DNase-free water	this study, see text; program Genomic_DNA_mL_1 on Kingfisher instrument
		all processed products	1.0 mL			
Magnesil KF	EB, same as Wizard (*18*); lysis buffer[a] added to first supernatant to total vol of 1 mL	tuber	0.35 mL	180 min at 57 °C	100 μL of DNase-free water[a]	kit instructions; program PromegaGenomic on Kingfisher instrument
		all processed products	1.0 mL			
Nucleospin	buffer CF[a] with 0.4 mg/ mL proteinase K[a]	all processed products	0.75 mL	30 min at 65 °C	100 μL of elutionbuffer CE[a]	kit instructions
PM Food	buffer A[a] with 160 μg/mL RNase[a] followed by buffer B[a]	all processed products	A: 1.0 mL B: 0.25 mL	10 min at ambient temp	100 μL of DNase-free water	kit instructions; manual magnetic device used to effect separation

[a] Included with the kit. [b] Cetyltrimethylammonium bromide.

The dehydrated slices, products A and B were each ground to fine particles using a Waring model SS110 pulverizer on a conventional Waring blender base. The flour, flakes, and starch were used as they were supplied.

Small-Scale DNA Extractions. DNA was extracted from the sample materials using the methods and procedures outlined in **Table 2**. For the Kingfisher and Magnesil KF methods, samples were homogenized and incubated as described for the Wizard method (**Table 2**). Digests were cooled and centrifuged at 10000*g* for 10 min.

For the Kingfisher method, supernatants (200 μL for the tuber samples and up to 500 μL for the processed potato samples) were collected and dispensed into the first well of a Kingfisher sample strip. Binding buffer (20 mM bis-Tris, pH 6.7, 6 M guanidine hydrochloride, 1 mM EDTA) was added to a final volume of ~1 mL followed by the addition of 200 μL of paramagnetic silica particles (Promega). The second well contained 1 mL of washing buffer (10 mM Tris-HCl, pH 7.6, 80 mM potassium acetate, 0.04 mM EDTA in 55% ethanol v/v). The third and fourth wells each contained 1 mL of 80% ethanol (v/v), and the fifth well contained 100 μL of nuclease-free water. The sample strips were installed into the Kingfisher mL magnetic particle processor (ThermoLabsystems). The Magnesil KF extractions were performed in the same manner as the Kingfisher method, except that the magnetized silica beads and the binding, washing, and elution buffers were included in a kit purchased from Promega and were used according to the kit instructions (**Table 2**).

Large-Scale Extractions. DNA was extracted from 2 g of each of two potato starch subsamples using the DNeasy Maxi Plant extraction kit (Qiagen) and the Wizard method. The Qiagen-Maxi extraction was carried out according to kit instructions, and the DNA was eluted in a final volume of 500 μL. The Wizard extraction was performed as described in **Table 2** except that 2 g of sample was suspended in 5 mL of extraction buffer.

Electrophoresis of Genomic DNA. DNA extracts were incubated with 0.2 mg/mL RNase A for 30 min at 37 °C and analyzed on 0.8% agarose (Invitrogen) gels. The gels contained 0.5 μg/mL ethidium bromide and were run in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA). A *Hin*dIII/*Eco*R1 digest of λ DNA (Promega) was used as a molecular size standard. Digital images of the gels were viewed and captured using the GeneGenius BioImaging system (Syngene, Cambridge, U.K.).

DNA Quantification. DNA was quantified using the Picogreen reagent (Molecular Probes) according to the kit instructions. Quantified λ DNA ranging in concentration from 1.0 to 1000 ng/mL was used as a standard, and sample extracts were diluted to a minimum of $^1/_{100}$ and $^1/_{200}$ in 1 mM EDTA and 10 mM Tris-HCl (TE), pH 7.5. A $^1/_{200}$ dilution of Picogreen reagent in TE, pH 7.5 (100 μL), was mixed with 100 μL of diluted sample or standard and incubated in the dark for 3−5 min before the fluorescence was read. Assays were performed in opaque black microplates (Greiner), and fluorescence was measured with a model Flx800 microplate fluorometer (Biotek Instruments, Winooski, VT) using an excitatory wavelength of 485 nm at an emission wavelength of 528 nm.

Quantitative PCR for *sus*. The RotorGene 3000 real-time fluorescence thermocycler (Corbett Research, Sydney, Australia) was used for PCR. The primer sequences and cycling conditions used for the quantification of the 216 and 84 base pair (bp) fragments of *sus* are shown in **Table 3**. Primers for the 84 bp amplicon were designed using Clone Manager Suite (v. 7) and amplified a target between bases 2416 and 2499 of a previously published sequence for *sus* (*21*). All reactions were performed in a final volume of 25 μL using QuantiTect SYBR

Table 3. Primer Sequences and Reaction Conditions for Real-Time Quantitative PCR for the 216 and 84 bp Fragments of Potato Sucrose Synthase

	216 bp fragment	84 bp fragment
forward primer (name: 5′-sequence-3′)	Pss01n5′: tgacctggacaccacagttat	PSS5: ggttgcacttgctattcg
reverse primer (name: 5′-sequence-3′)	Pss01n3′: gtggatttcaggagttcttcga	PSS6: gacagctcctcaacaact
reference for primer sequences	*22*	this study
cycling conditions	15 min at 95 °C	15 min at 95 °C
	40 cycles of	40 cycles of
	30 s at 95 °C	30 s at 95 °C
	30 s at 60 °C	30 s at 59 °C
	30 s at 72 °C	30 s at 72 °C
	15 s at 75 °C (fluorescence acquisition)	15 s at 77 °C (fluorescence acquisition)
	final extension: 3 min at 72 °C	final extension: 3 min at 72 °C
postreaction melt conditions	ramp from 55 to 95 °C	ramp from 55 to 95 °C
	1 °C per step	1 °C per step
	5 s per step	5 s per step
electrophoretic analysis[a]	2.0% agarose (Invitrogen) run with	2.5% agarose (Invitrogen) run with
	50 bp (Invitrogen) size standard	25 bp (Invitrogen) size standard

[a] Gels were stained with ethidium bromide and images captured using the GeneGenius BioImaging System (Syngene). Electrophoresis was not performed for every sample.

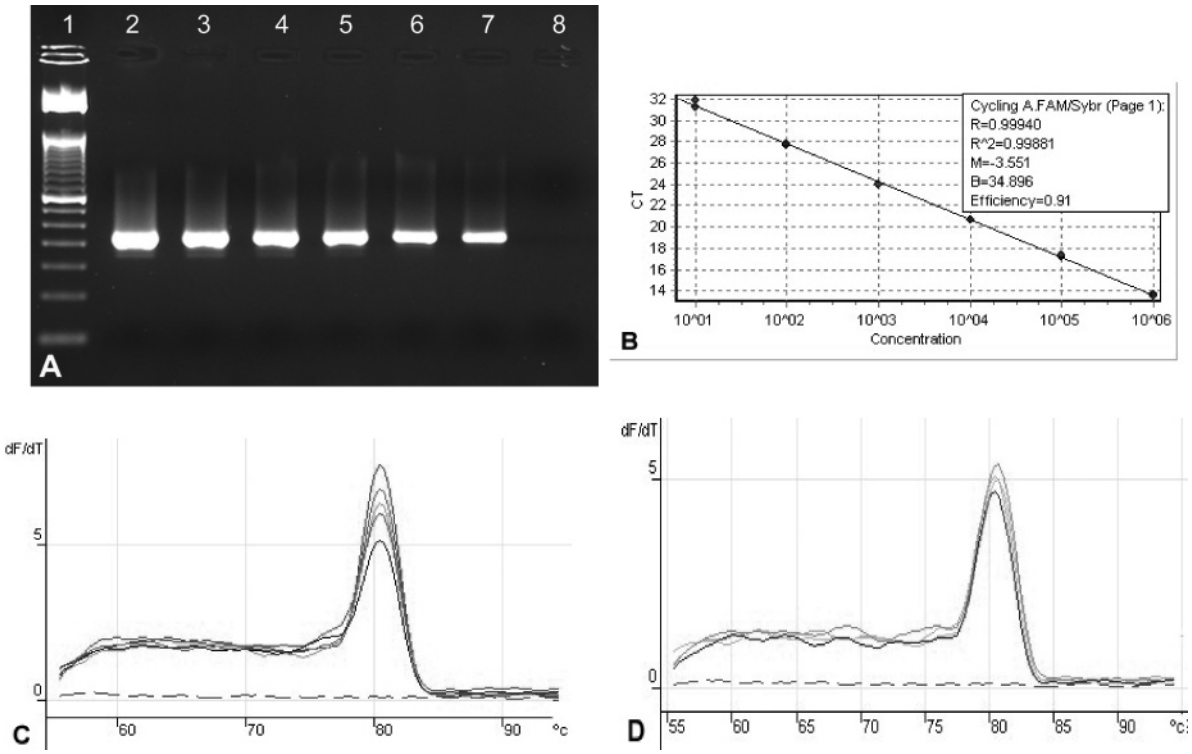

Figure 2. Specificity of real-time PCR for 216 bp fragment of *sus*. (**A**) Agarose gel electrophoresis of PCR products amplified using (lanes 2–7, respectively) 10^6, 10^5, 10^4, 10^3, 10^2, and 10 copies of *sus* standard as template; lane 1, 50 bp standard; lane 8, no template control. (**B**) Example of a standard curve generated in real-time PCR using duplicate reactions containing 10–10^6 copies of *sus* standard template per reaction. (**C**) Melt curves of amplicons using, in descending order from the highest d*F*/d*T*, 10^6, 10^5, 10^4, 10^3 10^2, and 10 copies of *sus* standard as reaction templates. The no-template control reaction is depicted with the dashed line. (**D**) Typical melt curve generated from samples used in this study demonstrates the specificity of the PCR reaction; melt curves for the reactions generated from template DNA purified from the four tuber samples using the DNeasy method are shown. The no-template control reaction is depicted with the dashed line.

Green master mix (Qiagen) with a template volume of 2.5 μL, and each primer was added to a final concentration of 0.5 μM. The fluorescence signal was captured during each cycle (**Table 3**) using the default FAM/SYBR channel (M_{Ex} at 470 nm, M_{Em} at 510 nm).

A quantified standard for each target was prepared by amplifying either the 216 or 84 bp fragment of *sus* from a potato (cv. Russet Burbank) DNA extract as described using the method above. Both amplicons were purified from an agarose gel using the QIAquick Gel Purification Kit (Qiagen). The purified amplicons were quantified using $A_{260/280}$ spectrophotometry with an Eppendorf Biophotometer (Brinkmann Instruments). The purified amplicon preparations were diluted into 0.1 mM EDTA and 1 mM Tris-HCl, pH 8.0, to 10^6 copies/2.5 μL, decreasing 10-fold to 10 copies/2.5 μL. These preparations were stored in sterile screw-cap vials at 4 °C and used to construct standard curves for each PCR run performed. Concentrations of *sus* in the sample extracts were determined relative to the standard curve generated in each run.

Statistical Analyses. Total DNA yield (nanograms of DNA per milligram of sample), total amplifiable DNA (copies of *sus* per milligram of sample), and DNA quality (copies of *sus* per nanogram of DNA) from tubers were analyzed using two-way ANOVA with Bonferonni multiple-comparison post-tests. Total DNA yield (nanograms of DNA per milligram of sample) and total amplifiable DNA (copies of *sus* per milligram of sample) for each of the processed matrices also were analyzed with two-way ANOVA. Bonferonni multiple-comparison post-tests were used to compare the total DNA

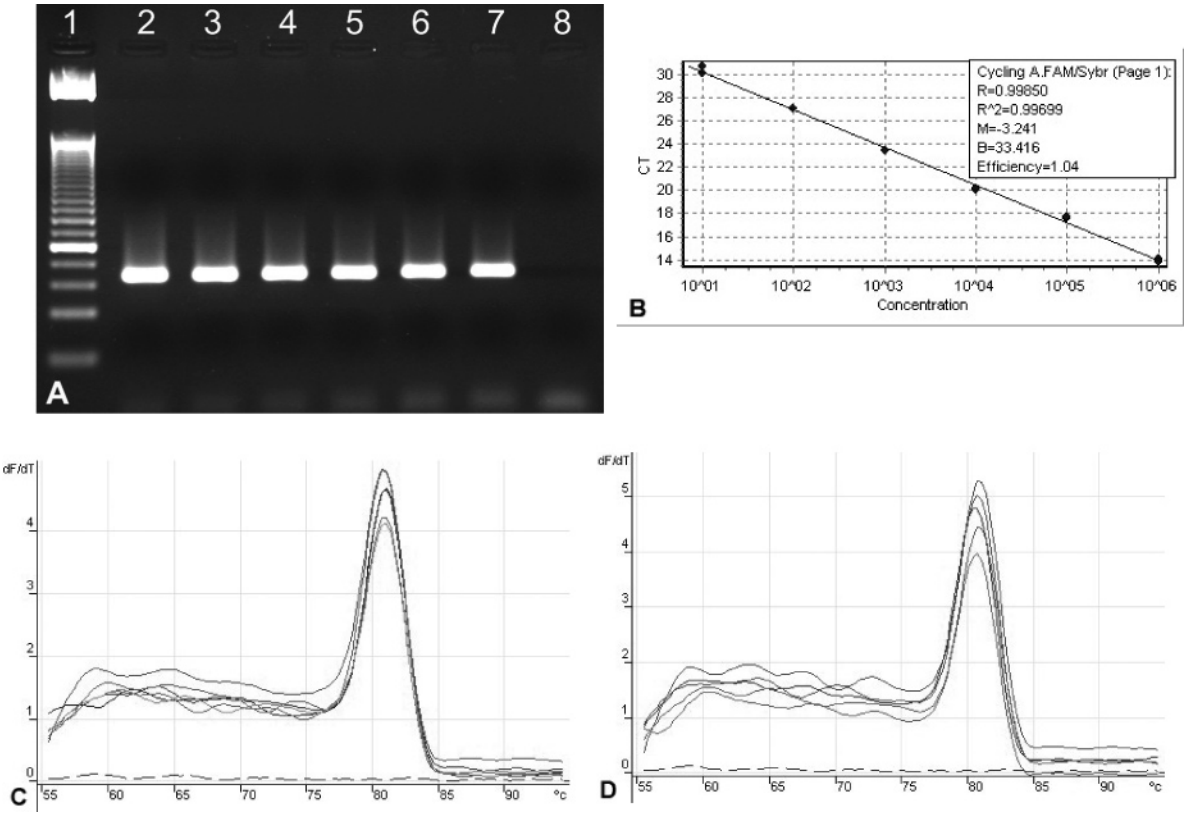

Figure 3. Specificity of real-time PCR for 84 bp fragment of *sus*. (**A**) Agarose gel electrophoresis of PCR products amplified using (lanes 2–7, respectively) 10^6, 10^5, 10^4, 10^3, 10^2, and 10 copies of *sus* standard as template; lane 1, 25 bp standard; lane 8, no template control. (**B**) Example of a standard curve generated in real-time PCR using duplicate reactions containing 10–10^6 copies of *sus* standard template per reaction. (**C**) Melt curves of amplicons using, in descending order from the highest d*F*/d*T*, 10^4, 10^3, 10^6, 10^5, 10^2, and 10 copies of *sus* standard as reaction templates. The no-template control reaction is depicted with the dashed line. (**D**) Typical melt curve generated from samples used in this study demonstrates the specificity of the PCR reaction; melt curves for the reactions generated from template DNA purified from product B using, in descending order from the highest d*F*/d*T*, the Wizard, Nucleospin, DNeasy, CTAB, and Roche l methods are shown. The no-template control reaction is depicted with the dashed line.

and total amplifiable DNA that could be recovered from each of the processed matrices. The effect of the extraction methods on the above parameters was also determined separately for each matrix with Dunnet's multiple-comparison Test. For scaled-up potato starch extracts, an unpaired *t* test was used, as only two extraction methods were used. All statistical analyses were performed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Ten-fold serial dilutions of the purified 216 bp and the 84 bp *sus* standard generally yielded standard curves with linear correlation coefficients (R^2) ranging between 0.996 and 0.999. Typical examples are shown in **Figures 2** and **3**, respectively. For the 216 bp target, reaction efficiencies of the standard dilution series spanning 6 orders of magnitude ranged between 91 and 99% over 17 runs. For the 84 bp target, reaction efficiencies ranged between 90 and 104% over 3 runs. Both reactions were specific, as a single band typically corresponded to a single peak in the melting curve at 80.5 °C for the 216 bp target and at 81 °C for the 84 bp target (**Figures 2** and **3**, respectively). Interassay coefficients of variation for amplifiable *sus* in single extracts typically ranged between 7 and 18%.

Potato Tuber Extraction. Most of the extraction methods recovered large molecular weight fragments of DNA from all four tuber types (**Figure 4**). DNA recovered from the field Russet Burbank with the Wizard method appeared to be slightly more degraded than the other extracts (**Figure 4A**), and no Mo-Bio-extracted DNA could be seen on the gels (**Figure 4**).

Method, tuber type, and method−tuber type interaction all exerted highly significant effects on the amount of DNA (nanograms of DNA per milligram of sample) and the overall amplifiable DNA (copies of *sus* per milligram of sample) recovered from the potato tubers (**Table 4**). Method effects generated the highest *F* values for these parameters, and therefore most of the variation observed in the data can be attributed to the effects of the extraction method used on DNA yield and amplifiable *sus*.

The cetyltrimethylammonium bromide (CTAB) method was the most efficient extraction method for all of the tuber types ($t > 3.7$, $p < 0.01$) except for the Russet Norkotah greenhouse tubers, in which the Roche l and CTAB methods did not yield significantly different levels of DNA ($t = 2.6$, $p > 0.05$), (**Figure 5A**). CTAB is particularly effective in extracting DNA from materials that contain high levels of polysaccharides (*10*). No quantifiable DNA was recovered using the Mo-Bio method (**Figure 5A**). This indicated that bead-grinding using a vortex mixer was not very effective for lysing the tuber cells.

The highest mean levels of amplifiable *sus* in each were recovered using the CTAB method (**Figure 5B**); however, this was statistically significant only for the Russet Burbank greenhouse tubers ($t > 3.7$, $p < 0.01$). This can likely be attributed to the high overall yield of DNA recovered from Russet Burbank greenhouse tubers with this method (**Figure 5A**). For the other tuber types, the differences in the levels of amplifiable *sus* per milligram of sample material recovered using

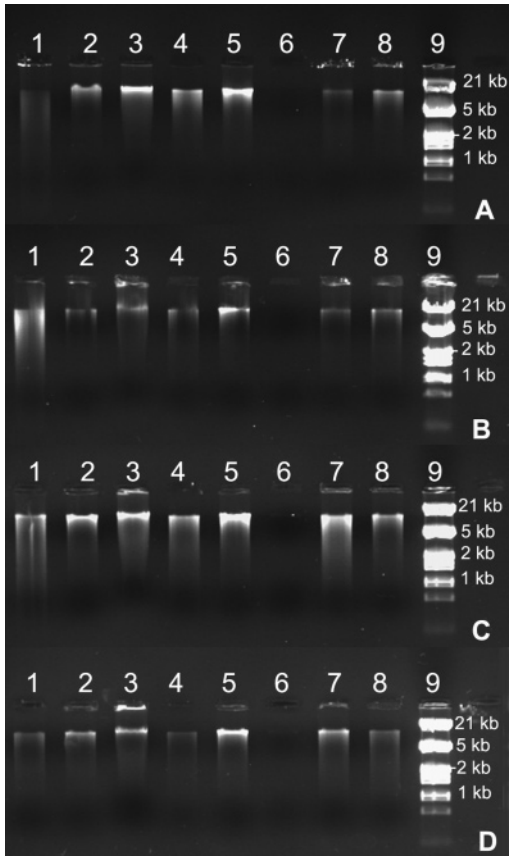

Figure 4. Agarose gel of genomic DNA prepared from (**A**) Russet Burbank field tubers, (**B**) Russet Burbank greenhouse tubers, (**C**) Russet Norkotah field tubers, and (**D**) Russet Norkotah greenhouse tubers according to the following methods: lane 1, Wizard; lane 2, DNeasy; lane 3, Roche l; lane 4, Roche Hi-Pure; lane 5, CTAB; lane 6, Mo-Bio; lane 7, Kingfisher; lane 8, Magnesil KF (lane 9, molecular size standard).

the CTAB and Kingfisher methods were not significant ($t < 2.1$, $p > 0.10$). In the Russet Norkotah greenhouse tubers, there was no significant difference in the amplifiable *sus* per milligram of sample in the extracts prepared using the CTAB, DNeasy, Kingfisher, or Magnesil methods ($t < 1.1$, $p > 0.10$). This suggested that the quality of DNA recovered by the CTAB method, particularly in Russet Norkotah greenhouse tubers, was inferior to that recovered using the other methods.

Amplifiable *sus* per unit of DNA concentration was used to estimate the overall quality of the DNA extracted from the tuber samples (copies of *sus* per nanogram of DNA). Both extraction method and tuber type had significant effects on copies of *sus* per nanogram of DNA (**Table 4**). There was, however, no significant interaction between tuber matrix and extraction method (**Table 4**), suggesting that the levels of amplifiable *sus* per nanogram of DNA recovered from the different tuber types did not vary differently with any given extraction method used.

The Kingfisher method recovered significantly higher ($t(3) > 3.3$, $p < 0.01$) amplifiable *sus* per nanogram of DNA than any other method in all of the tuber types except the Russet Burbank field tuber (**Figure 5C**), suggesting that, overall, this method yielded the best quality template. This may be due to either a lesser degree of copurification of inhibitors or a lower degree of damage to the template DNA during extraction. In the case of the Russet Burbank field sample, there was no significant difference between the DNA quality recovered by the Kingfisher and Magnesil methods ($t = 0.88$, $p > 0.10$). Of all the commercial kit methods, the Magnesil KF method produced the most consistent, highest quality template, whereas the DNeasy also performed well (**Figure 5C**).

Processed Potato Products. DNA fragments extracted from product A (**Figure 6A**) appeared to be <5 kb in size, smaller than the DNA extracted from the tubers (**Figure 4**). Less DNA between 1 and 5 kb was evident in the potato slice extracts (**Figure 6B**) than in product A (**Figure 6A**). Visible DNA fragments in most of the potato flakes and flour extracts were <1 kb in size (**Figure 6C**,**D**), although the Roche l method appeared to have recovered some larger size DNA from the flakes (**Figure 6C**). None of the methods produced visible DNA extracted from product B (**Figure 6E**) or starch (data not shown).

Not surprisingly, matrix effects on total DNA yield and *sus* copy number generated very large *F* values (**Table 4**). To better determine how well the extraction methods performed for each matrix, data for each of the matrices were analyzed separately.

Product A contained significantly more recoverable DNA than the other food products (e.g., Wizard method, $t > 43.5$, $p < 0.001$); therefore, these data are presented in different figures to allow appropriate scaling of the *Y*-axes (**Figures 7** and **8**, respectively). The Wizard method was the most efficient method ($q > 3.47$, $p < 0.05$) for extracting DNA from product A, but only slightly more so than the Roche l and Kingfisher methods (**Figure 7A**). The CTAB method, which was the most efficient method for DNA extraction from tuber material, yielded only about half the DNA recovered using the Wizard, and Kingfisher methods (**Figure 7A**). Although both the Roche l and CTAB methods are based on selective precipitation, almost twice as much DNA was recovered using the Roche l kit.

No quantifiable DNA could be recovered from product A using either the Hi-Pure or PM Food (Promega Magnetic for Food) methods. This may have been due to the composition of the respective lysis buffers, as both tended to gel when mixed with this matrix. This likely limited DNA release from the material and prevented efficient separation of soluble nucleic acid and insoluble cellular debris in the centrifugation step.

Table 4. Analysis Summary of the Effect of Various DNA Extraction Methods in Different Tuber Types and Processed Potato Food Products (Matrix) on DNA Yield and Quality

		F (degrees of freedom); *p* value		
matrix	effect	ng of DNA/mg	copies of *sus*[a]/mg	copies of *sus*/ng of DNA
tubers	method	111.8 (6, 28); <0.0001	46.40 (7, 32); <0.0001	76.88 (6, 28); <0.0001
	matrix	95.05 (3, 28); <0.0001	26.09 (3, 32); <0.0001	10.70 (3, 28); <0.0001
	interaction	10.26 (18, 28); <0.0001	2.581 (21, 32); 0.008	1.59 (18, 28); 0.1332
foods[b]	method	478 (8, 45); <0.0001	110 (8, 45); <0.0001	not analyzed
	matrix	1607 (4, 45); <0.0001	1116 (4, 45); <0.0001	
	interaction	159.2 (32, 45); <0.0001	86.3 (32, 45); <0.0001	

[a] As measured using 216 bp target only; therefore, the food analyses do not include the 84 bp product data for product B. [b] Excluding starch data.

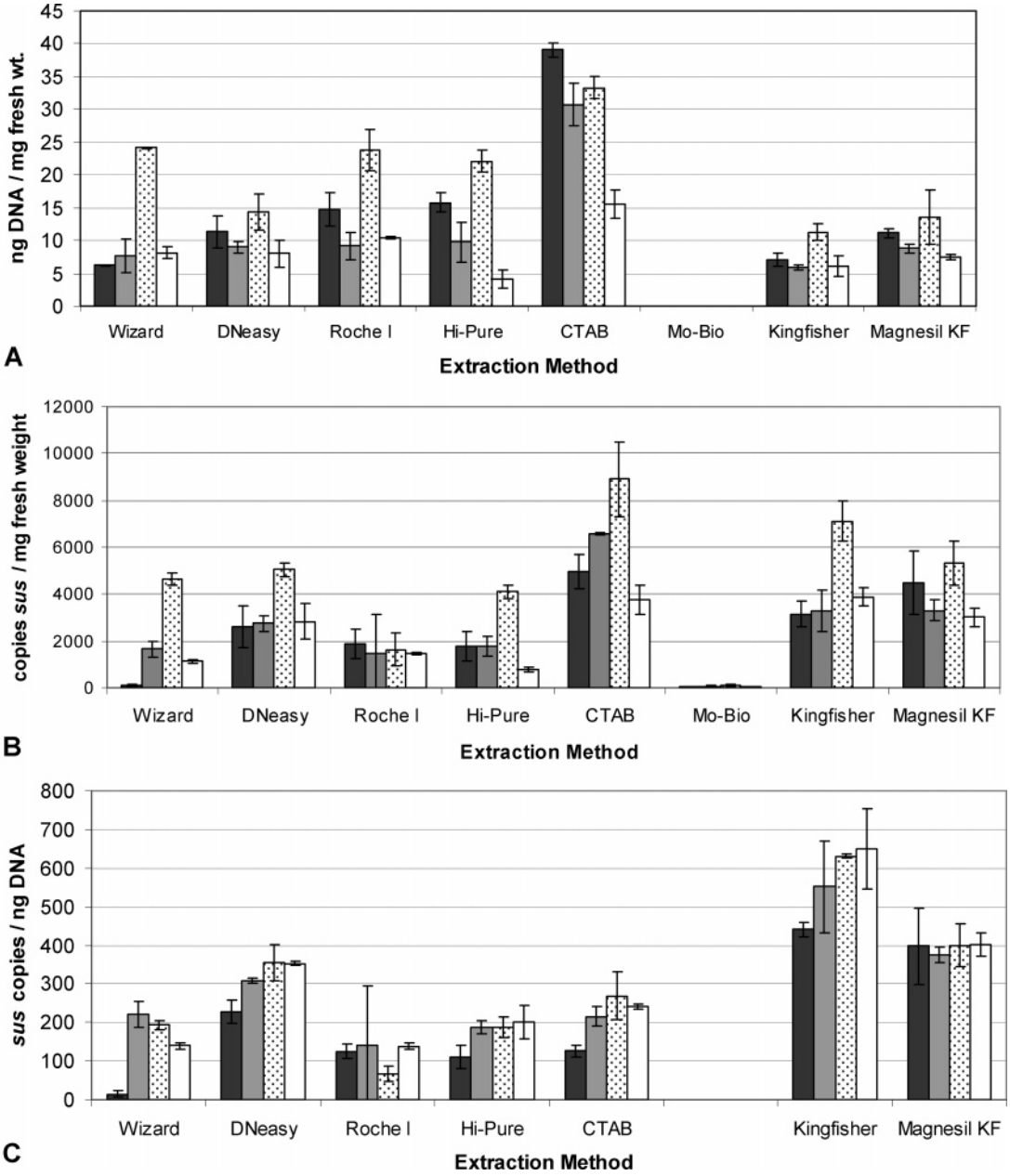

Figure 5. Concentrations of DNA (**A**) and relative amplifiable 216 bp *sus* target (**B**, **C**) in extracts of potato tubers prepared using various methods: (black bars) Russet Burbank field-grown tuber; (gray bars) Russet Burbank greenhouse-grown tuber; (dotted bars) Russet Norkotah field-grown tuber; (white bars) Russet Norkotah greenhouse-grown tuber. Error bars represent the standard deviation around the mean values derived from at least two observations on each of two replicate extracts.

The Roche 1, Kingfisher, and Magnesil KF methods recovered the highest levels of amplifiable *sus* from product A, between 1500 and 2000 *sus* copies per milligram of sample ($q < 2.65$, $p > 0.05$) (**Figure 7B**). Of all the methods, Roche 1 was the most expensive, whereas the Kingfisher was the most economical (**Table 1**) and one of the simplest to use.

The Wizard method recovered low levels of amplifiable *sus* in proportion to the amount of DNA recovered (**Figure 7C**). In contrast, the Magnesil KF method recovered ~3 times more amplifiable *sus* in proportion to recovered DNA than the other methods ($q > 15.2$, $p < 0.001$) (**Figure 7C**), suggesting that the Magnesil KF delivered a much higher template quality. Reasons for the high apparent template quality delivered by the Magnesil KF method were not clear. This method was primarily designed to extract high-quality template DNA from whole, anticoagulated blood (*23*), and therefore may be well suited to the extraction of DNA from food matrices containing high concentrations of fat and protein as well as a variety of chemical additives that may have an inhibitory effect in PCR.

The Wizard method recovered significantly higher DNA yields from the dehydrated potato slices than any of the other methods ($q > 10.4$, $p < 0.01$) except the Kingfisher method ($q = 1.83$, $p > 0.05$) (**Figure 8A**). With the flakes, flour, and product B, the Wizard method recovered significantly higher yields of DNA than any other method (flour, $q > 10.4$, $p < 0.01$; flakes, $q > 9.7$, $p < 0.01$; product B, $q > 5.8$, $p < 0.01$) (**Figure 8A**). All of the methods except the Hi-Pure and PM Food recovered some DNA from the dehydrated potato slices, but the Wizard, Roche l, Kingfisher, and Magnesil KF methods were the only methods that yielded quantifiable DNA from all of these matrices (**Figure 8A**). Levels of total DNA from the slices using the Wizard method were higher than those in the

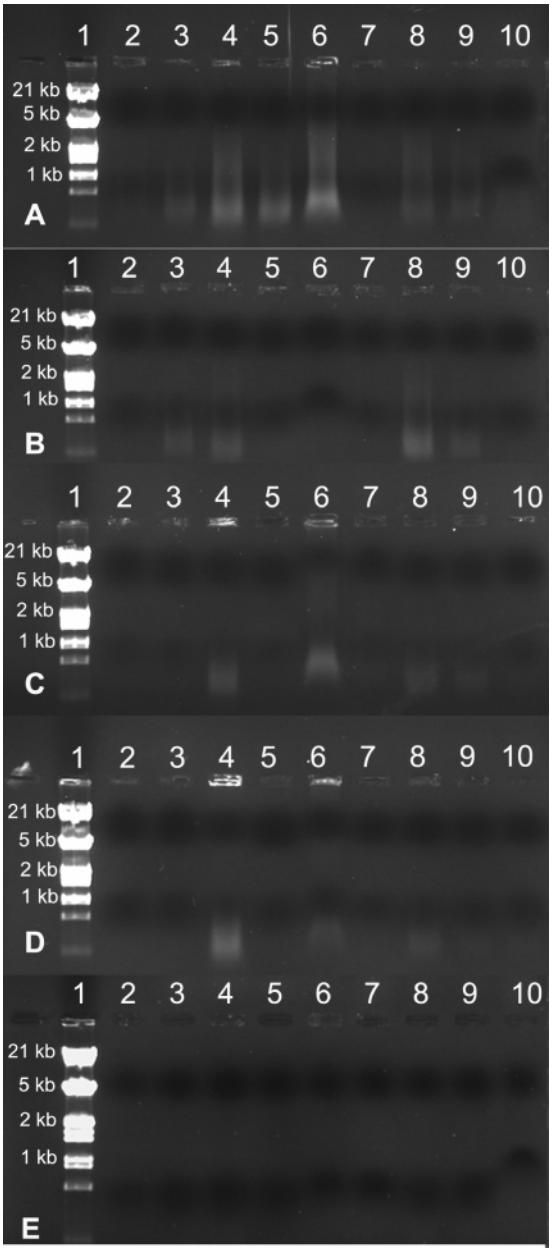

Figure 6. Agarose gels showing total DNA extracted from (**A**) product A, (**B**) dehydrated potato slices, (**C**) potato flakes, (**D**) potato flour, and (**E**) product B. Lane 1 contains a molecular size standard. The DNA was extracted in each of the above matrices using the following methods: lane 2, Nucleospin; lane 3, DNeasy; lane 4, Wizard; lane 5, CTAB; lane 6, Roche l; lane 7, Hi-Pure; lane 8, Kingfisher; lane 9, Magnesil KF; lane 10, PM Food.

flour ($t = 13.16$, $p < 0.001$), but not significantly different from those recovered from the flakes ($t = 1.80$, $p > 0.05$). Significantly more DNA was recovered from the flour compared to product B ($t = 5.39$, $p < 0.001$).

All of the methods recovered some amplifiable *sus* from the slices, flour, and flakes, although the PM Food method recovered only traces (**Figure 8B**). Again, the lysis buffer used with this kit tended to gel when mixed with these matrices. The Wizard method recovered the highest levels of *sus* from these matrices (slices, $q > 8.1$, $p < 0.01$; flour, $q > 4.1$, $p < 0.05$; flakes, $q > 5.3$, $p < 0.01$) (**Figure 8B**). Although the Roche l method appeared to recover the largest DNA fragments from the flakes (**Figure 6C**), low levels of amplifiable DNA were measured in this extract (**Figure 8B**). This indicated the likely presence of PCR inhibitors.

The levels of amplifiable 216 bp DNA recovered with the Wizard method were signficantly higher in slice extracts ($t > 13.16$, $p < 0.001$). The flour extracts contained slightly higher amplifiable DNA than the flake extracts ($t = 3.46$, $p < 0.05$), and flake extracts contained significantly higher levels than product B ($t = 10.88$, $p < 0.001$) (**Figure 8B**).

The amount of the 216 bp fragment that could be amplified from the product B extracts was very low (**Figure 8B**) and could not be detected in the potato starch extracts at all (data not shown). Much higher levels of an 84 bp fragment of *sus* could be detected in product B extracts (**Figure 9**), suggesting that the DNA that could be recovered from product B was highly degraded. The highest levels of 84 bp *sus* fragment were obtained in extracts prepared with the Wizard method, although there was considerable variability between duplicate extracts (**Figure 9**). This level was significantly higher than that recovered with any other method ($q > 6.1$, $p < 0.01$). Only traces of the 84 bp *sus* target could be detected in potato starch extracts prepared using the Nucleospin, DNeasy, Wizard, and CTAB methods, whereas none could be detected in the extracts prepared using the remaining methods (**Figure 9**).

Levels of amplifiable *sus* were approximately correlated with DNA yield for all of the matrices except product A (**Figure 8**; **Table 5**). The high r values calculated for the regression of copies of *sus* per milligram of sample versus nanograms of DNA per milligram of sample for the remaining matrices (**Table 5**) suggested that most of the variation observed in the levels of amplifiable *sus* in the slices, flakes, flour, and Product B using the different extraction methods was primarily due to the respective differences in the DNA yield (**Figure 8A**). The Wizard method did not necessarily recover DNA with lower concentrations of impurities and inhibitors. Any impurities present, however, did not decrease PCR efficiency sufficiently to effect a large decrease in the overall copies of *sus* per milligram of sample in the Wizard extracts compared to that measured in the other extracts containing lower template concentrations. The Wizard extracts of the flakes, for example, contained relatively low levels of amplifiable DNA (**Figure 8B**) in proportion to total DNA (**Figure 8A**), but still contained significantly higher levels of amplifiable DNA compared to the flake extracts prepared using the other methods (**Figure 8B**).

For the scaled up extraction of potato starch, only the Wizard, DNeasy Maxi, CTAB, and Roche l methods were considered, as these methods were easily amenable to scaling. The small-scale extraction experiments, however, had indicated that the DNeasy and Wizard methods could be expected to perform better than the CTAB and Roche l methods for the extraction of DNA from potato starch. Although no DNA could be quantified in any of the extracts (data not shown), both methods recovered DNA that could be amplified in PCR using the 84 bp target for *sus*. The Wizard method yielded the most amplifiable target, ~3 times more than that recovered using the Qiagen DNeasy maxi kit (**Figure 10**). This difference was statistically significant ($t = 4.35$, $p = 0.049$).

DISCUSSION

The copy number values determined for *sus* were not absolute but were determined relative to the standard curves. The standard target sequence was diluted into buffer, whereas the sample target sequences were in a biological matrix in a background of nontarget DNA. Therefore, it cannot be assumed that the reaction in standards and samples proceeded with equal ef-

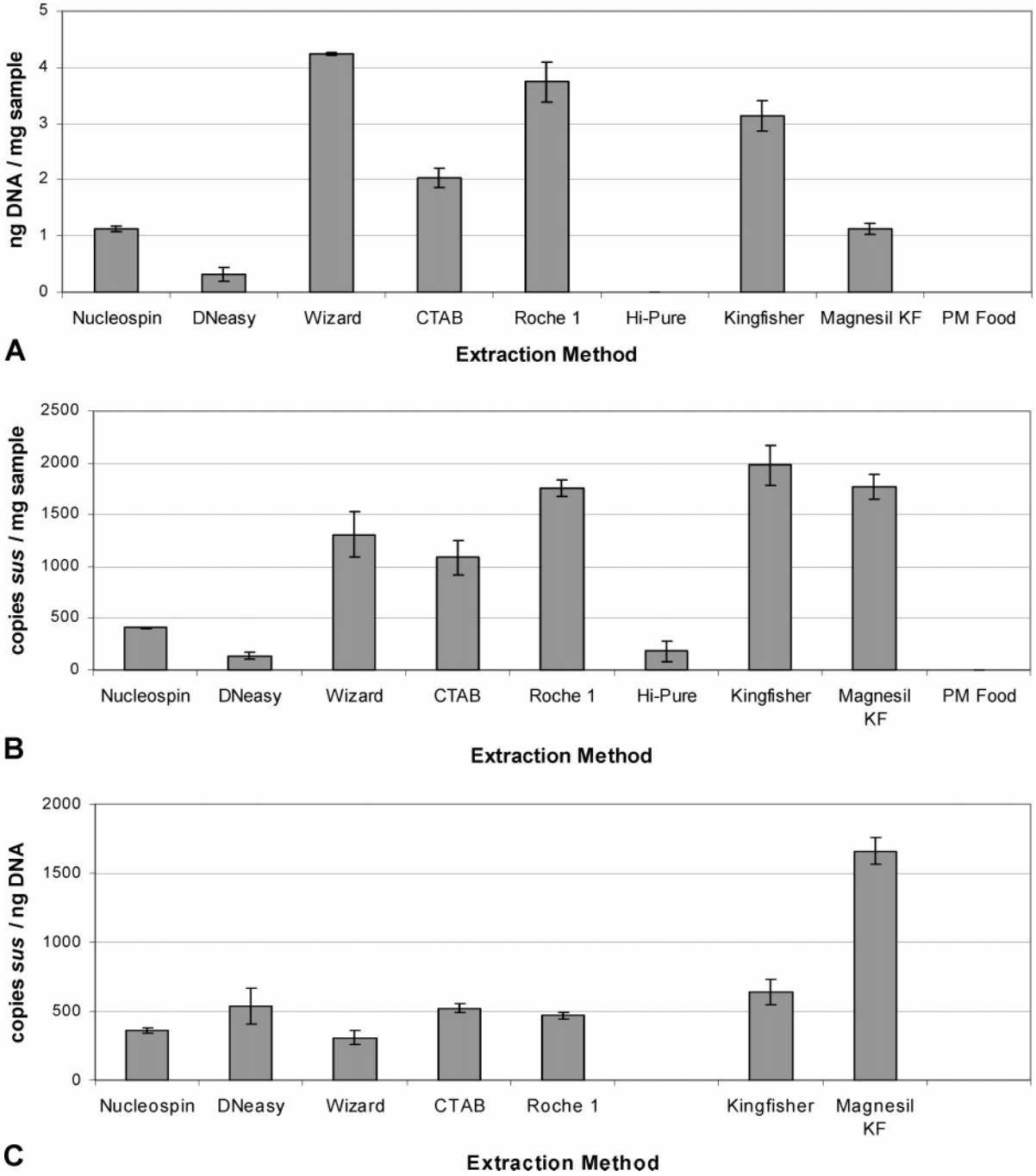

Figure 7. Concentrations of DNA (**A**) and relative amplifiable 216 bp *sus* target (**B**, **C**) in extracts of product A prepared using various methods. Error bars represent the standard deviation around the mean values derived from at least two observations on each of two replicate extracts.

ficiency. Amplifiable copy number values determined with this method may have underestimated the absolute number of *sus* copies. Comparison of amplifiable copy numbers of a single-copy endogenous gene among several extracts of the same sample remains, however, a useful means of comparing extraction methods. The amplifiable copy number determined in this fashion is influenced not only by the quantity of DNA recovered in the extract but also by the quality of the template DNA.

The type of matrix used in the extraction had a highly significant effect on the amount of total and amplifiable DNA recoverable from raw potato tubers and potato-derived food products. For the tubers, cultivar, storage, or growing conditions may have been factors, but no inferences were made because of the small numbers of individual tubers used. For the food products, these differences depended largely on the extent to which the tuber tissue had been processed. The trend was as follows, starting with the matrix that yielded the most total and amplifiable DNA: raw tuber tissue > whole potato products (i.e., product A > dehydrated potato slices) > flour and flakes > product B> starch. The respective size range of DNA fragments recovered from each matrix also tended to follow the same basic trend.

This observation was consistent with other studies which have indicated that both the quality and quantity of DNA recovered from food commodities tend to decrease with the extent to which a commodity is processed. Klein et al. (*16*) demonstrated the potential for DNA degradation at each step of the beet sugar refining process. DNA recovered from polenta, cracker, taco, and tofu samples had degraded substantially when compared to the DNA recovered from corn and soy flours (*15*). Further-

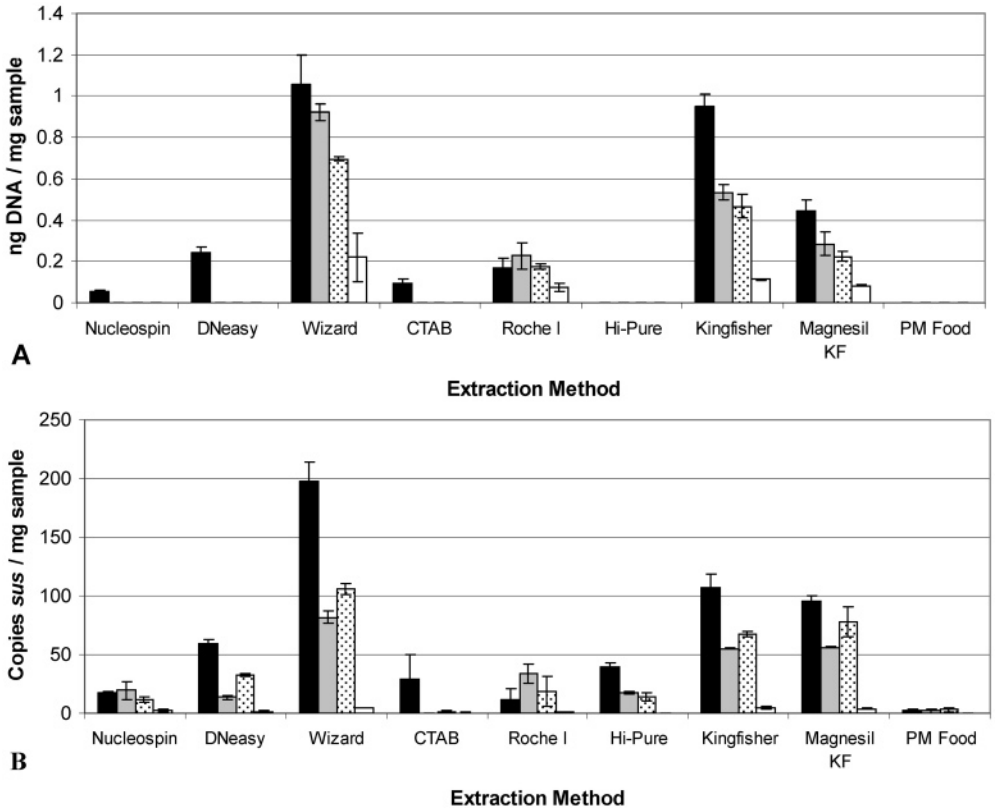

Figure 8. Concentrations of DNA (**A**) and relative amplifiable 216 bp *sus* target (**B**) in extracts of several potato-derived food products prepared using various methods: (black bars) dehydrated potato slices; (gray bars) potato flakes; (dotted bars) potato flour; (white bars) product B. Error bars represent the standard deviation around the mean values derived from at least two observations for each of two replicate extracts.

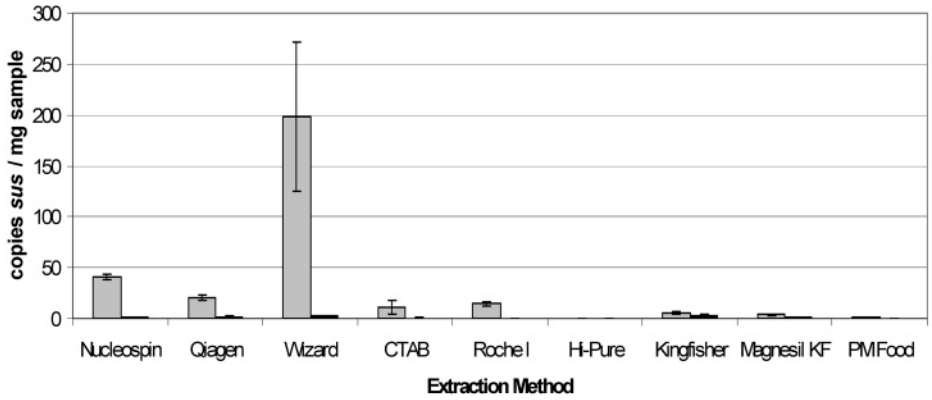

Figure 9. Concentrations of relative amplifiable 84 bp *sus* in extracts of product B (gray bars) and potato starch (black bars) prepared using various methods. Error bars represent the standard deviation around the mean values derived from at least two observations on each of two replicate extracts.

Table 5. Linear Regression Analysis of Copies of *sus*/mg as a Function of DNA Yield (Nanograms per Milligram) in Various Potato-Derived Food Products

sample	*r* (*n*); *p* value
product A	0.536 (14); 0.048
dehydrated slices	0.941 (14); <0.0001
flour	0.902 (8); 0.002
flakes	0.928 (8); 0.0009
product B	0.937 (8); 0.0006

more, the amount of total DNA that could be recovered from the more highly processed products was also remarkably less (*15*). The same trend was also observed when the fate of corn DNA was followed through the alkaline cooking process used to produce masa flour, tortillas, and corn chips (*17*).

Although extraction method had highly significant effects on DNA yield and quality, no single extraction method was ideal for all potato-derived matrices. Also, the fact that significant interaction was observed between method and matrix, for both tubers and processed samples, indicated that the extraction methods affected total DNA yield and yield of amplifiable DNA differently in each of the matrices. The composition of the matrix, for example, likely affected how well a particular lysis buffer was able to release the DNA. For each matrix, one to three of the extraction methods tested performed significantly better than the others.

Both yield and quality of the extracted DNA affected the amount of *sus* detected in tuber extracts. The CTAB method most consistently yielded the highest mean levels of DNA from the tubers, but did not recover the highest template quality.

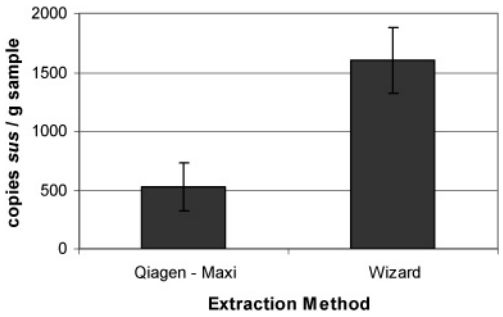

Figure 10. Concentration of relative amplifiable 84 bp *sus* target in potato starch extracts prepared using two scaled up methods. Error bars represent the standard deviation around the mean values derived from at least two observations for each of two replicate extracts.

Depending on the required analysis, however, this is not always an problem, as extracted DNA can be diluted if the yield is high enough, effectively reducing the concentration of copurified PCR inhibitors. The CTAB method was the most economical with regard to materials; however, it was also time-consuming and labor intensive, requiring several tube-to-tube transfers. Such transfers may increase the risk of sample cross-contamination in a diagnostic environment. A combination of CTAB lysis and centrifugation followed by a simple silica-based cleanup may provide good yields and superior template quality from raw tuber material.

Total DNA yield appeared to be the most important factor influencing the amount of amplifiable *sus* detected in extracts of the dehydrated slices, flour, and flakes. The Wizard method was therefore the best method for the extraction of DNA from these matrices. The Wizard method also recovered the highest levels of amplifiable DNA from the most highly processed products, product B and starch. It has been suggested that Wizard resin binds to smaller molecular weight nucleic acid fragments more efficiently than the silica used by some other manufacturers (*13*). This may explain why the Wizard method was the most effective in extracting DNA from the more highly processed products, which tended to yield smaller fragments of DNA. The Wizard method was also easily scalable, which, for a difficult matrix such as potato starch, was an important advantage.

In analytical molecular biology, nucleic acid extraction often becomes a tradeoff between yield and purity. Methods yielding high concentrations of analyte often contain impurities that may compromise the analysis, whereas methods producing a highly purified analyte often do so at the expense of yield (*13*), effectively reducing the sensitivity of the overall analysis. Ultimately, any extraction method must suit its intended end purpose. For example, a method that provides a suitable template for qualitative PCR with a given commodity may not provide sufficient template quality for amplified fragment length polymorphism (AFLP) analysis (*24*) or sufficient quantity to detect low levels of an unwanted ingredient derived from that commodity in a food matrix (*25*).

This study has demonstrated that the quality and quantity of DNA recovered from potato tubers and potato-derived food ingredients vary with each type of matrix as well as with the extraction method used. Differences in yield and template quality affected the total amplifiable DNA recovered from the tubers and a lightly processed product, whereas yield (template concentration) was the most important factor for PCR amplification in the more highly processed products. In the case of the potato tubers, the CTAB method would be preferred if an economical, high-yielding method were required, whereas the Kingfisher method would be suitable for extracting higher quality DNA using a semiautomated procedure. The amount of total and amplifiable DNA that could be recovered from a potato-derived matrix decreased with the extent to which product had been processed; therefore, a method optimized for high yield of small fragments, such as the Wizard method, would likely be the most effective for highly processed potato-derived matrices.

ABBREVIATIONS USED

DNA, deoxyribonucleic acid; PCR, Polymerase Chain Reaction; AFLP, amplified fragment length polymorphism; CTAB, cetyltrimethylammonium bromide.

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