Extraction of Trace Amount of Severely Degraded DNA

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DNA extraction from food is always problematic especially from highly processed samples which contain only trace amounts of severely degraded DNA fragments. In this work, to extract trace amounts of small DNA fragments of the traditional Chinese medicine (TCM) *colla corii asini* derived from highly processed *Equus asinus* skin, three strategies were compared for its authentication. With some optimizations, the modified QIAquick spin column method achieved higher DNA yield and purity in comparison with the "SDS/proteinase K" method and the "Wizard magnetic DNA purification system for food" method. Further studies showed that at least 0.4 g *colla corii asini* was needed to obtain enough DNA extracts for PCR-based detection by the method and only amplicons of less than 100 bp could be generated from the DNA extracts which confirmed the efficiency of the method in small DNA fragment extraction. The DNA obtained by this method was suitable to be used in PCR-based authentications.

Key words: DNA Extraction, Highly Processed Material, Severely Degraded DNA

Introduction

Colla corii asini, also named as donkey-hide gelatin, Ass-hide glue, E jiao or Lu Pi Jiao, is a kind of most valuable traditional Chinese medicine (TCM) and health care food that has a long history of over two thousand years. This kind of TCM is well kown in promoting hematopoiesis, arresting bleeding (Wu *et al.*, 2007; Li, 2003; The State Pharmacopoeia Committee of the P. R. China, 2005), and has a good performance in treatment of gynecologic diseases and some chronic diseases which was recorded in many important Chinese medical classics like Ben Cao Gang Mu (Li, 2003). Thus, it is commonly used for health care especially in China and some other East Asian countries.

As recorded in the Chinese Pharmacopoeia, *colla corii asini* should be made of dry skin derived from *Equus asinus* (The State Pharmacopoeia Committee of the P. R. China, 2005). However, adulterations made of or partly blended with skins or even bones from other related animals like *Bos taurus* or *Sus scrofa* were found in some batches of *colla corii asini* products which may contain little therapeutic effect and do harm seriously to people's health (Xu *et al.*, 2005; Qu *et al.*, 2006). Since the traditional morphological, physical and chemical inspection can't distinguish *colla corii asini* from the adulterants and substitutes effectively (Wang *et al.*, 2007), the establishment of modern biological authentication methods for *colla corii asini* becomes much critical in order to ascertain people's health.

With the rapid development of biotechnology, molecular authentication of traditional Chinese medicines seems to be quite popular because it is more effective, reliable, inexpensive and convenient (Shaw *et al.*, 2002; Zhang *et al.*, 2007). However, no such *colla corii asini* authentication methods have been reported yet, possibly because of the difficulty in obtaining enough DNA extracts from highly processed *colla corii asini* and other related solid glues.

DNA extraction from food matrix was always problematic (Piknova and Kuchta, 2006) mainly due to DNA degradation during the processing of food and the possible co-extraction of various kinds of chemicals such as polysaccharides and polyphenols (Holden *et al.*, 2003) that may severely inhibit further nucleic acid amplifications. Some studies focused on the effects of critical processing procedures upon DNA degradation in processed food have found that pH value and temperature

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are the two most effective parameters (Weiss *et al.*, 2007) for large scale gene degradation. A low pH value can induce depurination reactions and subsequent DNA damages (Kharazmi *et al.*, 2003; Bauer *et al.*, 2003), while high temperature may cause more seriously DNA degradation. For example, the plasmid pUC18 was reduced 10^3-fold after autoclaving at 121 °C for 30 min (Klein *et al.*, 1998), the endogenous gene of roundup-ready soybean was considerably degraded from about 836 bp to 162 bp during the sterilization procedure at 121 °C for only 30 s (Chen *et al.*, 2005). Moreover, under some extreme conditions, DNA can even be degraded to undetectable level (Gawienowski *et al.*, 1999).

To extract severely degraded DNA from highly processed food, the silica-based DNA extraction technology is usually used (Smith *et al.*, 2005). A silica matrix can efficiently bind and purify DNA in the presence of chaotropic salts (Davoren *et al.*, 2007), and it can recover relatively high amounts of fragmented nucleotides with few PCR inhibitors compared with the traditional DNA extraction methods. For example, the highly degraded DNA from potato starch was successfully extracted by the Wizard method and other silica-based methods where only an amplicon of 84 bp was generated (Smith *et al.*, 2005), and using an improved silica-binding procedure, severely degraded DNA was obtained from seeds heated at 200 °C for up to 8 h (Giles and Brown, 2008). However, there still existed some food matrices from which no PCR-amplifiable DNA or quite few could be obtained, like fermented soybean products (Mafra *et al.*, 2008), mays oil, chocolate cream (Di Pinto *et al.*, 2007). They might be subjected to severe processing or contain many PCR inhibitory substances. So, for DNA analysis of this kind of highly processed food, some focus should be made on developing a method especially for severely degraded DNA.

Colla corii asini and other related TCM solid glues are prepared from the skin or shell of animals through an initial decoction for about 10 h at nearly 120 °C, pH 5.0–6.5, and further evaporation at 120–130 °C for 15–20 h. Obviously, it is a kind of highly degraded product with trace amounts of severely degraded DNA fragments (less than 100 bp) and large amounts of degraded collagen peptides (possibly more than 80%) which has been proven an inhibitor for thermostable DNA polymerases (Scholz *et al.*, 1998). Therefore, it could be concluded that DNA extraction from *colla corii asini* is a big challenge, and an approach specifically designed for trace amounts of severely degraded DNA has to be established when molecular authentication of this severely processed TCM is carried out (Shaw *et al.*, 2002; Zhang *et al.*, 2007).

The objective of the present study was to develop an effective and reliable method to extract amplifiable DNA fragments of quite short length and quite trace amount from *colla corii asini* for its molecular authentication. Different DNA extraction methods were compared and a modified silica-based method was established and optimized to trap the trace amount of highly degraded DNA fragments of less than 100 bp from a large amount of degraded collagen peptides.

Material and Methods

Samples

Colla corii asini was produced by Shandong Donge E-jiao Group, Shandong Province, P. R. China.

DNA extraction

Before DNA preparation, all solid glue samples were subjected to surface-irradiation with UV light at each side for 2–3 h. Then, DNA extraction from *colla corii asini* was tried using 3 different methods: classical "SDS/proteinase K" method, "Wizard magnetic DNA purification system for food" method (Promega, Madison, MI, USA), and a modified QIAquick spin column method (Qiagen, Düsseldorf, Germany). Each of the methods relies upon different principles for DNA extraction and purification.

"SDS/proteinase K" method

The methodology is commonly used for DNA extraction (Sambrook and Russell, 2001) and was initially applied to this study. The experiment was conducted following the standard protocol. Briefly, after surface-irradiation by UV light, 1 g sample was ground to a powder and collected in a 60-ml centrifugal tube containing 1 ml extraction buffer (50 mM Tris-HCl [tris(hydroxymethyl) aminomethane hydrochloride], 50 mM EDTA, 0.5% SDS, pH 8.0). The tube was incubated at 50–60 °C for several minutes with occasionally shaking until the sample was dissolved complete-

ly. After 4–5 ml Tris (50 mM, pH 10.5) were added to adjust the final pH value to 8.0, the tube was rotated at 180 rpm and 37 ºC for 1 h. Consequently, 30–35 μl proteinase K (20 mg/ml) were added and the tube was rotated at 180 rpm and 56 ºC for 3 h. Then, the solution was further treated by phenol/chloroform extraction and alcohol precipitation. The extracted DNA was dissolved carefully in 100 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at –20 ºC until use.

"Wizard magnetic DNA purification system for food" method

The kit (Promega, Madison, MI, USA) for DNA purification from a variety of food samples including processed food is applied. Unlike the spin column-based system, the method utilizes magnetic silica-based particles to selectively bind DNA. The procedure was as follows. Briefly, 1 g sample was ground to a powder after UV irradiation of each side and was transferred into a 60-ml centrifugal tube containing 2.5 ml lysis buffer A and 25 μl RNase A from the kit. Then, the tube was mixed by vortexing with the assistance of a pipette tip. The sample was further treated following the recommended procedures. 100 μl DNA extract were then obtained and stored at –20 ºC until use.

Modified silica-based method

The QIAquick spin column (Qiagen) is used to catch DNA fragments of quite short length based on the silica membrane spin technology. Some modifications were made on this method for highly degraded DNA fragments from *colla corii asini*, including a sample pretreatment and a further DNA extraction process. After the UV irradiation procedure, 1 g sample was ground to a powder and transferred into a 60-ml centrifugal tube with 1 ml DNA extraction buffer (10 mM Tris-HCl, 25 mM EDTA, 100 mM NaCl, 0.5% SDS, pH 8.0) and 20 μl proteinase K (20 mg/ml). After incubating them for 0, 1, 2 h at 56 ºC with gently mixing several times, 10 ml PN buffer (Qiagen) were added to the sample and mixed completely. Then, 600 μl mixtures were added to the QIAquick column (Qiagen) and were centrifuged for 1 min at 10,000 × *g*. After discarding the flow-through appropriately, the step was repeated several times until all the mixture was centrifuged through the same QIAquick column. Then, the column was washed 1, 3, 5 times with PE buffer (Qiagen), and dried by evaporation for 5 min at room temperature. Thereafter, DNA was eluted with 100 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by centrifuging at 12,000 × *g* for 2 min and stored at –20 ºC until use.

In order to remove the oil and fats which constitute a considerable part of solid glues and might contribute to PCR inhibition, 4 ml hexane were added to the proteinase K-treated solution and mixed gently. The sample was centrifuged at 3000 × *g* for 5 min and the lower phase was carefully pipetted to another clean 60-ml centrifugal tube. Then 10 ml PN buffer (Qiagen) were added, and the next steps were just following the above mentioned procedures. Different modifications of QIAquick spin column methods named as A to G are listed in Table I.

DNA quantification

Quantification of all the DNA extracts by UV absorption at 260 nm was done on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Each sample was tested 3 times. The A_{260}/A_{280} ratio was also recorded for analysis of the purity of the extracted DNA.

Table I. Modifications of QIAquick spin column methods.

Modification	Proteinase K incubation time [h]	Number of PE buffer elution steps	Hexane[a]
A	0	3	No
B	1	3	No
C	2	3	No
D	1	1	No
E	1	5	No
F	1	3	Yes

[a] Hexane was added to remove the oil and fats from the samples.

DNA amplification

The oligonucleotide primer Ass-up/down designed from equine short interspersed element (SINE) ERE-1 (Accession No. D26565) was used to evaluate the performance of the three DNA extraction methods for *colla corii asini*.

To investigate the degradation degree of DNA extracted from *colla corii asini*, a set of oligonucleotide primers (Ass2-up/down, Ass3-up/down, Ass4-up/down) that produce amplicons of different sizes were designed based on equine SINE ERE-1.

All the primers were synthesized by Invitrogen, and are listed in Table II.

For PCR analysis of the DNA extracted from *colla corii asini* with the three DNA extraction methods, a total reaction volumn of 25 μl was mixed with 12.5 μl Premix Ex-taq™ Hot Start Version (Takara, Otsu, Japan), 0.4 μM reverse and forward primer (Ass-up/down), and 5 μl extracted DNA sample. The amplification process included an initial denaturation at 94 ºC for 4 min; 40 cycles of 30 s at 94 ºC, 30 s at annealing temperature, 30 s at 72 ºC; and a terminal extention of 7 min at 72 ºC.

The PCR amplification for degradation degree analysis of the DNA extracted from *colla corii asini* was performed using 4 primer pairs (Ass-up/down, Ass2-up/down, Ass3-up/down, Ass4-up/down). Each primer pair was used in a reaction mixture of 25 μl containing 12.5 μl Premix Ex-taq™ Hot Start Version, 0.4 μM reverse and forward primer, and 5 μl DNA extract (diluted to a final concentration of 10 ng/μl). The subsequent PCR amplification included an initial denaturation at 94 ºC for 4 min; 40 cycles of 30 s at 94 ºC, 30 s at 60 ºC, 30 s at 72 ºC; and a terminal extention of 7 min at 72 ºC.

All the PCR amplifications were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were visualized by 2–3% TAE agarose gel electrophoresis with GelRed™ Nuleic Acid Gel Stain (Biotium, Hayward, CA, USA) under UV light.

Extraction limit analysis

DNA samples extracted from 0.2 g, 0.4 g, 0.6 g, 0.8 g, 1 g *colla corii asini* by the modified QIAquick spin column method B were subsequently analyzed by PCR using the primer pair Ass-up/down. The PCR amplification was carried out in a volumn of 25 μl containing 12.5 μl Premix Ex-taq™ Hot Start Version, 0.4 μM reverse and forward primer, and 5 μl DNA extracts. The amplification reaction was composed of an initial denaturation at 94 ºC for 4 min; 40 cycles of 30 s at 94 ºC, 30 s at 60 ºC, 30 s at 72 ºC; and a final extention of 7 min at 72 ºC.

Results

Comparison of different methods for severely degraded DNA extraction from colla corii asini

The three methods for DNA extraction from *colla corii asini* were compared for the first time. A large difference among these methods was observed after DNA analysis by UV spectrophotometry (Table III) and PCR-based detection (Fig. 1), while none of these extracts was visible in the gel by direct DNA electrophoresis even using a more sensitive fluorescent stain such as GelRed™ instead of EB (data not shown). Therefore, it was believed that *colla corii asini* only contains trace amounts of small DNA fragments. The PCR primer pair Ass-up/down was designed from the short interspersed elements (SINEs) which are a class of highly repetitive nucleotides,

Table II. Primers for SINE-based PCR assays.

Name	Primer (5′-3′)	Amplicon size [bp]	T_m [ºC]	Genbank accession No.	SINE
Ass	Sense: CGGACATGGCACTGCTCAT Antisense: TATATTCTTCGTTGTGGGTCCTTCT	81	60	D26565	ERE-1
Ass2	Sense: CAGTGTTTCGTTGGTTCG Antisense: TCTAGTTGTGGCATGTGGGAC	89	60	D26565	ERE-1
Ass3	Sense: TGGCCGAGTGGTTAAGTTCG Antisense: CAGCCTGGTTTTGATGAGCA	102	60	D26565	ERE-1
Ass4	Sense: GGCCGAGTGGTTAAGTTCGC Antisense: ATATTCTTCGTTGTGGATTCTTCT	150	60	D26565	ERE-1

Table III. Quantity and A_{260}/A_{280} ratio of DNA extracted from *colla corii asini* by different methods.

Extraction methods	DNA recovered [ng/μl]	A_{260}/A_{280} ratio
"SDS/proteinase K" methods	ND[a]	ND
"Wizard purification" methods	8.79 ± 5.1	1.07 ± 0.08
Modified QIAquick spin column methods (A)	26.5 ± 8.6	1.24 ± 0.03
Modified QIAquick spin column methods (B)	29.2 ± 3.0	1.31 ± 0.05
Modified QIAquick spin column methods (C)	25.9 ± 2.5	1.33 ± 0.07
Modified QIAquick spin column methods (D)	28.8 ± 11.0	1.18 ± 0.05
Modified QIAquick spin column methods (E)	13.2 ± 2.2	1.26 ± 0.10
Modified QIAquick spin column methods (F)	9.4 ± 1.7	1.33 ± 0.05

[a] ND, not detected.

possibly up to 10^6 copies in a genome (Shedlock *et al.*, 2004), and can be used for species-specific detections (Table II) (Walker *et al.*, 2004). Equine SINE ERE-1 was found to be widely spread in equine genomes, specifically with a high copy number, and therefore it could be used to verify the presence of equine DNAs (Sakagami *et al.*, 1994).

The conventional "SDS/proteinase K" method was initially tried for severely degraded DNA extraction from *colla corii asini* since it is commonly used in DNA purification. However, the DNA extract from alcohol precipitation was just in a cloudy solution with lots of co-precipitated peptides and was not suitable for the quantitative measurement by UV absorption spectrophotometry and the subsequent PCR-based detection. It mostly contributed to the lack of amplifiable DNA fragments and the existence of many PCR inhibitors. In order to improve the method to trap the trace amount of highly degraded DNA from a large amount of degraded collagens, some modifications of the method such as repeating the phenol extraction step, elongating the initial sample digestion and the final DNA precipitation time, and increasing the enzyme amount were also tried, but the DNA extracts remained cloudy and unsuitable for PCR-based analysis. Furthermore, it was also found that a clear DNA extract was obtained only when the phenol extraction step was repeated more than 10 times. However, it did not generate any PCR amplicon with a quite small DNA concentration (data not shown). This could derive from too many phenol/chloroform extraction steps causing much loss of DNA fragments. The step had to be limited since DNAs of *colla corii asini* were quite few. Therefore, the "SDS/proteinase K" method was not suitable for highly degraded DNA extraction with trace amount.

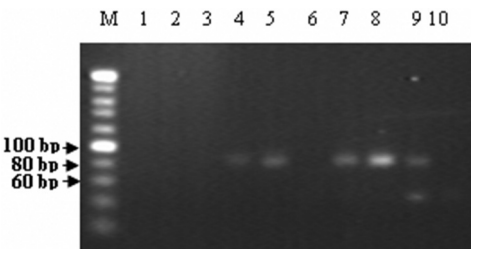

Fig. 1. PCR-based detection of equine SINE ERE-1 with DNA extracted from *colla corii asini* by three methods. Lanes 1–3, the "SDS/proteinase K" method; lanes 4–6, the "Wizard magnetic DNA purification system for food" method; lanes 7–9, the modified QIAquick spin column method B; lane 10, blank; lane M, 20-bp ladder DNA marker. The primer pair was Ass-up/down.

To maximize the DNA recovery and minimize the co-extraction of collagen and other contaminations, the "Wizard magnetic DNA purification system for food" method was then tried. First, the method can be used for DNA extraction from highly processed food based on the magnetic silica particles that can efficiently bind to DNA in the presence of chaotropic salts. The method is widely used to obtain clean DNA samples. Second, as recorded in the technical bulletin of the kit, the method has a capacity to purify DNA fragments of at least 100 bp from a variety of food samples. So it may be suitable for DNA extraction from *colla corii asini* rich in collagen but not much in highly degraded DNA fragments. Just as predicted, the method performed better than the "SDS/proteinase K" method in DNA extraction from *colla corii asini*. The solution of the DNA extract was not cloudy, and the concentration was 8–9 ng/μl measured by UV spectrophotometry (Table III).

However, the A_{260}/A_{280} ratio of the DNA extract was low. This possibly resulted from the existence of some contaminants. Furthermore, only two of the three replicates generated the expected but faint amplicons with one replicate failed, indicating that the method can not be used practically because of low yield (Table III) and unrepeatable and unstable PCR detection (Fig. 1, lanes 4–6).

As a result, the method had to be improved for severely degraded DNA extraction. First, a pretreatment of *colla corii asini* was carried out with proteinase K added so as to thoroughly digest the collagen peptides. Collagenase could replace the proteinase K leading to the same result as with proteinase K (data not shown). Proteinase K was effective enough to digest the highly denatured triple helix collagens. Second, since the DNA remainders in *colla corii asini* might be extremely short, the QIAquick spin column was selected for further DNA purification relying on the fact that the QIAquick spin column has the capacity to efficiently trap DNA fragments as short as oligonucleotides of more than 17 nucleotides.

Since the QIAquick spin column was originally designed for cleanup of radioactive-, biotin-, or DIG-labeled DNA fragments and oligonucleotides of more than 17 nucleotides from enzymatic reactions, not especially for DNA extraction from food matrix or medicinal materials, its capacity to trap short and severely degraded DNA would be reduced by various contaminants. Therefore, some modifications were needed to make the method more suitable for severely degraded DNA extraction from *colla corii asini* and other TCM-related solid glues. First, as noted before, the sample was pretreated by proteinase K digestion at 56 ºC with the incubation time of 1 h or 2 h to degrade the denatured collagen peptides, while, in contrast, an extraction procedure with no proteinase K digestion step was also performed. Second, the PE buffer elution step was repeated several times to inspect the contamination removal. Finally, hexane was also added to prevent the possible PCR inhibition derived from oil and fats which constitute a considerable part in *colla corii asini*. And then, the modified QIAquick spin column methods A to G for severely degraded DNA extraction from *colla corii asini* were established and tried in triplicate (Table I).

Beyond our expectation, all of the six modified methods can extract DNA of relatively high concentration when applied to *colla corii asini*, and all of the replicates can generate clear PCR amplicons with the same size as the positive control (Fig. 2). Adding proteinase K (method A) in the pretreatment for 1 or 2 h did not affect the yield seriously but affected the quality of the sample since the proteinase K-digested DNA extract (method B, method C) showed a relatively high A_{260}/A_{280} ratio. A single elution step with PE buffer for only once lowered the sample quality (method D) while the treatment for 5 times lowered the yield (method E). Making use of hexane for oil and fats removal generated a DNA extract with relatively high quality but low quantity (method F). As a result, in order to obtain more DNA samples with low co-extraction contaminants, the modified QIAquick spin column method B was selected for further investigations. The method also generated much clearer amplicons than others in its three replicates (Fig. 2, lanes 4–6). The below mentioned modified QIAquick spin column method was just referred to as method B.

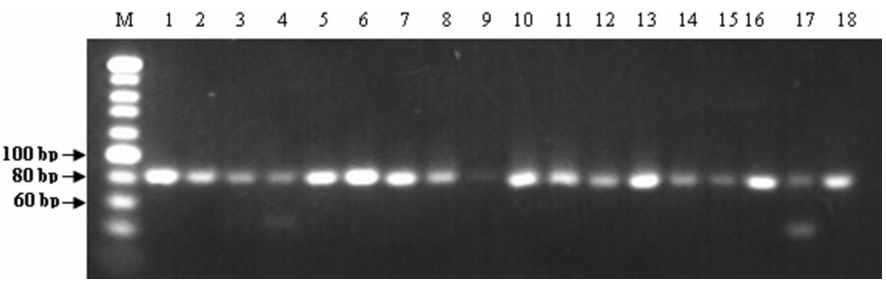

Fig. 2. PCR-based detection of equine SINE ERE-1 with DNA extracted from *colla corii asini* by six modified QIAquick spin column methods. Lanes 1–3, modified method A; lanes 4–6, modified method B; lanes 7–9, modified method C; lanes 10–12, modified method D; lanes 13–15, modified method E; lanes 16–18, modified method F; lane M, 20-bp ladder DNA marker. The primer pair was Ass-up/down.

Compared with the conventional "SDS/proteinase K" method and the "Wizard magnetic DNA purification system for food" method, the modified QIAquick spin column method was more effective and repeatable for highly degraded DNA extraction with trace amount from *colla corii asini.*

Degradation level of DNA extract from colla corii asini

After the DNAs were extracted from *colla corii asini* and subjected to PCR applications successfully by the modified QIAquick spin column method, the degradation level of the DNA extract was investigated with a PCR assay aimed at different amplicon sizes. The 83-bp and 89-bp amplicons were amplified from the DNA extract successfully, and the PCR products were sequenced and aligned with the predicted sequences; good identities were determined (data not shown). However, the 124-bp and 150-bp amplicons could not be obtained at all (Fig. 3). This implied that the DNA remains were mostly less than 100 bp in length and the DNA extract from *colla corii asini* was highly degraded. The results also indicated that the modified QIAquick spin column method can efficiently extract trace amounts of severely degraded DNA with a length of less than 100 bp.

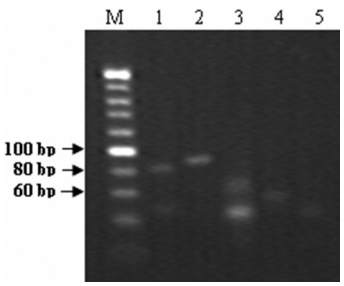

Fig. 3. PCR-based detection of equine SINE ERE-1 aiming at different amplicon sizes with DNA extracted from *colla corii asini*. Lane 1, primer pair Ass-up/down (81 bp); lane 2, primer pair Ass2-up/down (89 bp); lane 3, primer pair Ass3-up/down (102 bp); lane 4, primer pair Ass4-up/down (150 bp); lane 5, blank; lane M, 20-bp ladder DNA marker.

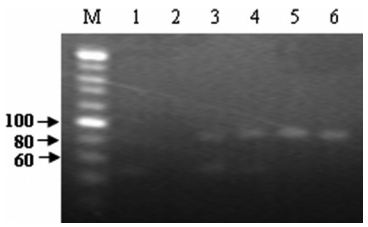

Fig. 4. PCR-based detection of equine SINE ERE-1 with DNA extracted from different amounts of *colla corii asini* by the modified QIAquick spin column method B. Lane 1, blank; lane 2, 0.2 g; lane 3, 0.4 g; lane 4, 0.6 g; lane 5, 0.8 g; lane 6, 1 g; lane M, 20-bp ladder DNA marker.

Extraction limit of the modified QIAquick spin column method

After investigating the degradation level of DNA extracts from *colla corii asini*, the extraction limit of the modified QIAquick spin column method was also determined by PCR analysis of DNAs extracted from different amounts of *colla corii asini*. It was found that a clear amplicon, as predicted, could be obtained from DNA samples extracted from 0.4 g, 0.6 g, 0.8 g and 1 g *colla corii asini* while DNA extracts from 0.2 g *colla corii asini* gave no amplicon (Fig. 4). The results indicated that at least 0.4 g *colla corii asini* is needed for DNA extraction when subjected to PCR-based detections.

Discussion

In the present work, a simple technique for detecting trace amounts of highly degraded DNA fragments was established successfully. In the silica-based column method, since DNA fragments bound tightly to silica particles in the presence of chaotropic salts, the food sample was initially preincubated with proteinase K and then directly subjected to a QIAquick column for DNA extraction. A previous study showed that different DNA extraction methods should be used to match DNA extraction from different food matrices (Mafra *et al.*, 2008; Di Pinto *et al.*, 2007). So, when applying the specifically designed method to DNA extraction from different severely processed food samples, some modifications of the method are very essential.

Colla corii asini is most valuable in the TCM markets for its good performance and long history. However, the existence of adulterants and substitutes has seriously hindered its development since no reliable and efficient authentication methods are available till now. Then, to establish workable methods for *colla corii asini* authentication becomes much more crucial. Our

plan was to develop such methods based on molecular authentication principles because of their good efficiency (Zhang *et al.*, 2007). Here, in this study, the step-by-step methods for DNA extraction from *colla corii asini* have been initially established which is suitable for further PCR-based detections.

A successful extraction of sufficient amplifiable DNA from *colla corii asini* depends on both the full DNA recovery and the removal of PCR inhibitor co-extractions. In our study, the QIAquick spin column method with some modifications was applied for DNA extraction. The pretreatment by proteinase K digestion did not affect the yield and PCR application seriously because large amounts of collagen were dissolved in chaotropic salts and further separated in the DNA absorption steps to avoid their co-extraction. The elution step with PE buffer was applied for the removal of some contaminants that might be co-extracted in the silica membrane. The step could not be repeated many times for DNA extraction from *colla corii asini* since overelution might lead to loss of some nucleotides absorbed with the contaminants. For the same reason, when hexane was used in the protocol, oil and fats were removed as well as some absorbed nucleotides (Poms *et al.*, 2001). Therefore, use of hexane should be limited except that the sample contains quite large amounts of oil and fats, like pig-hide glue.

The degradation level analysis of the DNA extracts from *colla corii asini* confirmed that DNA from *colla corii asini* was severely degraded into small fragments less than 100 bp in length. That is why the traditional DNA extraction method and the commonly used silica-based method failed to extract DNA from it. Our simple, modified, silica-based method, however, is able to catch the small DNA fragments from *colla corii asini*.

The QIAquick spin column has a capacity to load only 750 μl solution. For a large starting volume of the extraction solution, multiple loading operations of the QIAquick spin column have to be conducted. This might increase the opportunity of contamination. In fact, for DNA extraction from *colla corii asini* and other related solid glues, more than 10 loadings were needed so that many efforts should be made to control the contamination. In this work, our DNA extraction was operated in a clean environment with strict manipulation protocols just as an ancient DNA study in order to make the trace amount of small DNA extract suitable and reliable for further PCR-based detections.

The QIAquick spin column was also successfully applied for ancient DNA extraction directly from the proteinase K digest, where a 211-bp fragment was amplified in a human bone sample with an age of over 5000 years (Yang *et al.*, 1998). In our study, with some modifications, the QIAquick spin column method was capable to extract trace amounts of severely degraded DNA from highly processed TCM treated at 120–130 °C for 10–20 h, and only amplicons derived from high repetitive elements less than 100 bp in length could be generated.

The method was also very simple: preincubation with proteinase K, application to the QIAquick silica-based column, washing the column, and finally elution of the DNA extract. The method, therefore, has a big potential for high-throughput DNA analysis for *colla corii asini* authentication (Sassa, 2008).

In summary, in the present study, an effective and reliable method for DNA extraction from *colla corii asini* was established. DNA extracts from highly processed TCM were suitable for further PCR-based molecular authentications. Furthermore, the method also provides a new way for DNA extraction from other highly processed food with few severely degraded DNAs less than 100 bp in length.

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