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New Method of DNA Isolation from Two Food Additives Suitable for Authentication in Polymerase Chain Reaction Assays

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Locust bean gum and guar gum are galactomannans used as additives (E 410 and E 412, respectively) in the food industry as stabilizing agents. Analytical discrimination between the two additives in gums and foods is now feasible by molecular techniques. However, only complex and time-consuming DNA isolation protocols are available to date. We have developed simple improved protocols to obtain enough DNA suitable for PCR amplification from a few milligrams of commercial E 410 and E 412 additives (containing more than 75% polysaccharides). The suspension of additives in water or 10 mM Tris-HCl, pH 8.5, efficiently recovers DNA suitable for authentication in PCR assays. However, the Tris method was much more efficient for the extraction of DNA from E 410 than for E 412 additives. Conversely, the water method was the most suitable for detecting DNA extracted from E 412 or from E 410/E 412 mixtures. Combined with the use of the two specific ribosomal primer pairs previously designed, our methods are well-suited for a fast and simple high-throughput sample treatment of commercial gums for molecular certification.

KEYWORDS: Guar; locust bean; gums; molecular certification

INTRODUCTION

Locust bean gum and guar gum are neutral polysaccharide products (galactomannans) extracted from the seed endosperms of two leguminous plants. Among other industrial uses, they are used as food additives (locust bean gum coded as E 410 and guar gum coded as E 412) as stabilizing agents in the food industry, in a wide array of products such as frozen desserts, cheese, meat, jams and jellies, milk products, soups, sauces, gravies, toppings, and syrups. Guar gum is far less expensive than locust bean gum, and cases of adulteration in commercial gums have been suspected to frequently occur, but they were difficult to demonstrate unequivocally.

The analytical discrimination between both gums is particularly problematic since their structure is basically the same, only differing in their galactose:mannose ratios, being 1:4 and 1:2 for locust bean gum and guar gum, respectively (*1*). Several chemical and physical methods have been used to identify and quantify these additives, based on the detection of the galactose:mannose ratio, including electrophoresis and gas−liquid chromatography (*2*). However, and depending on the origin and supplier of the samples, variations in the degree of substitution of the mannan chain have been described (*3*, *4*).

In the past decade, different authors have attempted to differentiate the two gums by DNA-based methods. However, some authors (*5*) failed in their purpose to extract DNA from locust bean gum and guar gum and concluded that both gums were not good substrates for DNA isolation due to their thickness and absorption characteristics. More recently, a complex and time-consuming DNA isolation protocol involving enzymatic treatments with β-mannanase and α-galactosidase, hexadecyltrimmethylammonium bromide (CTAB) extraction, and DNA column purification has been developed (*6*). Using this protocol, and based on PCR amplification of a cpDNA (chloroplast DNA) intergenic spacer, these authors have been able to differentiate between locust bean gum and guar gum.

The isolation of DNA from samples showing a high polysaccharide content is a technical challenge in molecular biology. During DNA isolation by routinary protocols (*7*, *8*), the DNA usually coprecipitates with the polysaccharide matrix after the alcohol precipitation step, forming highly viscous solutions that are difficult to handle and further process. In addition, PCR amplification of DNA from polysaccharide-rich environments is not an easy task, since polysaccharides are good inhibitors of the activities of most enzymes used in molecular biology, such as endonucleases, ligases, and polymerases (*9*). This is exactly the situation that one will find if a PCR method has to be applied to the detection of DNA in commercial preparations

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Table 1. Comparison of Efficiency in DNA and Polysaccharide Extraction Methods and Correlation with PCR Results

gum	extraction method	polysaccharide[a] (mg/mL)	DNA[b] (ng/mL)	DNA/polysaccharide	PCR	
					PA23/PA21	PG22/PG21
guar 100%	water	0.639 ± 0.020	46.9 ± 0.5	1/14 000	+	−
guar/locust bean 30%	water	0.523 ± 0.018	8.6 ± 0.2	1/60 000	+	+
guar/locust bean 10%	water	0.541 ± 0.022	10.8 ± 0.2	1/50 000	+	+
locust bean 100%	water	0.572 ± 0.025	14.2 ± 0.3	1/40 000	−	+
guar 100%	Tris	1.056 ± 0.034	77.3 ± 2.1	1/14 000	+	−
guar/locust bean 30%	Tris	0.361 ± 0.014	156.1 ± 4.2	1/2500	−	+
guar/locust bean 10%	Tris	0.494 ± 0.013	232.5 ± 6.4	1/2000	−	+
locust bean 100%	Tris	0.721 ± 0.020	509.7 ± 8.5	1/1500	−	+
guar 100%	acetonitrile	0.013 ± 0.003	4.8 ± 0.6	1/3000	+	−
guar/locust bean 30%	acetonitrile	0.019 ± 0.002	1.7 ± 0.04	1/11 000	−	−
guar/locust bean 10%	acetonitrile	0.030 ± 0.006	1.9 ± 0.04	1/16 000	−	−
locust bean 100%	acetonitrile	0.029 ± 0.007	1.7 ± 0.04	1/17 000	−	−
guar 100%	ethanol	ND[c]	3.2 ± 0.06	ND	−	−
guar/locust bean 30%	ethanol	ND	0.3 ± 0.01	ND	−	−
guar/locust bean 10%	ethanol	ND	0.6 ± 0.020	ND	−	+
locust bean 100%	ethanol	ND	0.9 ± 0.020	ND	−	+

[a] Polysaccharide content was determined by the phenol–sulfuric acid method. Data are the mean ± SD of at least three independent experiments. [b] DNA content was measured with the PicoGreen dsDNA quantitation kit of molecular probes. Values are the mean ± SD of at least three independent experiments. [c] ND, not determined.

of guar and locust bean gums, because by definition (*1*) they must contain at least 75% polysaccharides (galactomannans)

The routinary authentication analysis, using DNA markers, of locust bean gum and guar samples that would be used in the food industry demands a simple, fast, and inexpensive method for DNA isolation. We have developed simple and friendly protocols to obtain enough DNA, suitable for PCR amplification, from a few milligrams of commercial locust bean gum and guar gum. The purity of the gums could be verified by use of species-specific oligonucleotides, based on nuclear ribosomal sequences that bind exclusively to the DNA of *Ceratonia siliqua* and *Cyamopsis tetragonoloba*, the plant sources of locust bean gum and guar gum, respectively.

MATERIALS AND METHODS

Gum Samples. Commercially available locust bean gum and guar gums from several suppliers were kindly provided by Carob SA (Mallorca, Spain). Controlled mixtures of these gums (ranging from 2% to 30%) were performed to test the limits of detection of the adulterant guar gum in locust bean gum.

DNA Extraction. The gums were extracted by standard protocols based on detergents [hexadecyltrimmethylammonium bromide, CTAB (*7*), and sodium dodecyl sulfate, SDS (*8*)], ethanol (*9*), or commercially available DNA kits (Nucleon PhytoPure plant DNA extraction kit, Amersham Bio Sciences, Cerdanyola, Spain; Dneasy Plant kit, Qiagen, Barcelona, Spain). In addition, DNA extraction was performed as follows: 10 mg of gum was suspended in 10 mL of either water (water method), 10 mM Tris-HCl, pH 8.5 (Tris method), water/acetonitrile 7:3 (v/v; acetonitrile method), or 35% ethanol (ethanol method). Suspensions were vortexed for 5 min and centrifugated for 1 min at 15000*g*. Supernatant fluids were recovered for analysis except in the ethanol method, where the protocol (*9*) was modified and the final pellet was dissolved in 0.5 mL of water.

DNA and Polysaccharide Quantification. One hundred microliters of sample (the above supernatant fluids) was used for DNA quantification with the PicoGreen dsDNA quantitation kit (Molecular Probes Inc., Eugene, OR). Ten microliters of the sample was used for determination of total sugars by the phenol−sulfuric acid method (*10*).

Amplification Protocols. PCR amplification was carried out following the patented protocol previously described (*11*). Two pairs of primers, PG22 (5′-TGCCTGGGCGTCGCGCGTC-3′)/PG21 (5′-TCCAAAACAAGATGGAGTCG-3′) and PA23 (5′-TGCCTGGGTGTCACACACT-3′)/PA21 (5′-CGCAATCCCGGGTATGACA-3′), binding to, respectively, the nuclear ribosomal ITS 2 sequences of *Cyamopsis tetragonoloba* (GenBank Accession Number AJ245577) and *Ceratonia siliqua* (GenBank Accession Number AJ245576) were used. They were designed to amplify diagnostic fragments of the same length for each species. In addition, each species-specific PCR fragment contained different restriction endonuclease targets (e.g., *Hae*III, *Xho*I, *Alu*I) that allowed a further verification of the specificity of the PCR products. PCR reactions were performed in 50 μL, containing 1× *Taq* buffer, 50 μM each dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, 5 μL of supernatant, and 1.25 units of *Taq* polymerase (Pharmacia, Pfizer Inc., New York). Amplifications comprised 35 cycles of 90 s at 94 °C, 90 s at 56 °C, and 90 s at 72 °C. A final extension at 72 °C for 5 min was included. PCR products were analyzed on 2.0% agarose gels and stained with ethidium bromide.

RESULTS AND DISCUSSION

Polysaccharide precipitation was evident when locust bean gum and guar gums were extracted with CTAB, SDS, ethanol, and some of the most versatile DNA kits, resulting in sticky and viscous precipitates that were not adequate for DNA quantification and PCR amplification. However, simpler methods yielded more consistent results. The four assayed methods (water, Tris, acetonitrile, and ethanol) had different efficiencies in extracting the DNA from guar gum or locust bean gum. From the results shown in **Table 1** we conclude that success in DNA extraction does not always correlate with the efficiency of polysaccharide solubilization, as can be deduced for the DNA/polysaccharide ratios obtained for each solubilization method. In all cases the amount of DNA isolated was too low to be visualized by agarose gel electrophoresis. Clearly, the Tris method was the most efficient method, followed by water, acetonitrile, and ethanol methods. However, the Tris method was much more efficient for the extraction of DNA from locust bean gum than for that of the guar gum. This can be deduced from the amount of DNA extracted for pure gums (100% guar or 100% locust bean) by the Tris method but also from the ratios of DNA extracted by each method from each gum. Thus, for locust bean gum, the ratio of DNA extracted by Tris/water methods is 35.8, whereas the same ratio for guar gum is only 1.6. These results suggest that most of the DNA extracted from gum mixtures by the Tris solvent corresponds to locust bean DNA rather than guar DNA. This experimental evidence explains the negative results obtained when the DNA extracted from the gum mixtures by the Tris method was PCR-amplified with the primers PG22/PG21 specific for guar gum. By contrast, our results demonstrate that although the water method extracts less DNA than the Tris method for each gum and mixture

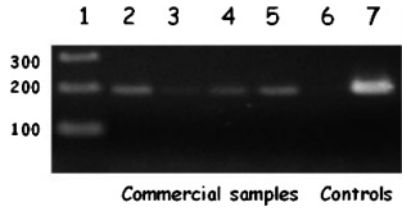

Figure 1. Specific detection of guar DNA in commercial samples labeled as E 410. DNA from samples of theoretically pure locust bean gum was purified as described in the text and amplified by use of primers PG22/PG21 specific for guar gum. PCR products were resolved on a 2% agarose gel and stained with ethidium bromide. Negative control (pure locust bean gum) and positive control (pure guar gum) are shown in lanes 6 and 7, respectively. DNA markers (in base pairs) are shown in lane 1.

assayed (**Table 1**), it is the most suitable for detecting guar DNA extracted from guar gum or locust bean/guar gum mixtures with the guar-specific primers PG22/PG21.

Laboratory preparations of locust bean gum containing different known amounts of guar gum, extracted by the water method and assayed by PCR with the PG22/PG21 primers, allowed us to detect as low as 2% contaminant guar gum in the spiking experiments. In all cases, we obtained positive amplifications using the specific locust bean primers PA23/PA21. Also, PCR results with PG22/PG21 primers from different guar gum producers were consistent, suggesting that although different manufacturers may produce gums with different amounts of DNA, depending on their fabrication processes or other unknown reasons, the qualitative detection of guar by this method is robust.

We obtained samples of locust bean gum from different manufacturers and selected for study those that showed viscosities above the usual values obtained with locust bean gum preparations (approximately 100 mPa·s at room temperature). We suspected that these abnormal viscosities (more than 250 cps at room temperature) could be due to the undeclared presence of guar gum in the locust bean gum preparations. As shown in **Figure 1**, all the four declared pure locust bean gum preparations with high viscosities produced positive amplifications by PCR with the PG22/PG21 primers, indicating that those preparations contained guar gum. We confirmed the specificity of our method by restriction analysis of the amplified products with *Xho*I, which showed the single restriction site and electrophoretic profile identical to those observed with amplicons from pure guar gum (*11*).

No inhibition of DNA amplifications was detected with the assayed protocols. This agrees with other results reporting that neutral polysaccharides were not inhibitory of *Taq* polymerase activity when added to PCR reactions (*12*, *13*). In addition, the highly dilute DNA extracts recovered with our method should have also diluted the potential effect of polysaccharide inhibitions (*14*).

In summary, two of the protocols assayed (the water and Tris methods) have been able to isolate in less than 10 min of lab work from a few milligrams of commercial gums enough genomic DNA to be used in more than 500 PCR reactions. These protocols are slightly more efficient than those previously developed, reporting a 5% detection limit of guar gum in locust bean gum (*6*), and could be used in an inexpensive single-step method from minute quantities of sample. Combined with the use of the two specific ribosomal primer pairs previously designed, our methods are well-suited for a fast and simple high-throughput sample treatment of commercial gums for molecular certification.

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