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The Development of Immunoassays to Identify and Quantify Species Source of Gum Arabic

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Gum arabic from *Acacia senegal* is commonly used as an additive in foodstuffs. Adulteration of gum arabic by other gums is a potential problem for reasons of safety and quality. This study aimed to develop and evaluate the use of enzyme-linked immunosorbent assays (ELISAs) for the detection of potential adulterants of gum arabic. Indirect competitive ELISAs (IC-ELISAs) were developed using the monoclonal antibodies SY CC7 (*A. senegal*), SY HH3 (*Acacia seyal*), and SY J1A1 (*Combretum erythrophyllum*). All IC-ELISAs had a working range of 0.005−10 mg/mL. The antibodies used were tested using the IC-ELISAs for cross-reactivity with other *Acacia* species and other gums. The antibodies were very specific for their respective antigens. Significant cross-reactivity was found for SY CC7 (between *A. senegal* and *A. mellifera*e) and SY J1A1 (between *C. erythrophyllum* and *A. seyal*). The IC-ELISA was adapted further to test confectionery samples for the presence of gum arabic, which was successful, although recovery rates were reduced. Both IC- and plate trapped antigen ELISA (PTA-ELISA) formats were able to distinguish an adulterated sample of gum arabic when blended with either *A. seyal* or *C. erythrophyllum*. The PTA-ELISA was more sensitive for *A. seyal* than the IC-ELISA, but both were equally sensitive for *C. erythrophyllum*. The results suggest that the antibodies SY CC7, SY HH3, and SY J1A1 could be used in combination with each other for the detection of potential adulterants of *A. senegal* and the detection of gum arabic in foodstuffs.

KEYWORDS: Gum arabic; Acacia; Combretum; ELISA

INTRODUCTION

Gum arabic is commonly used in foods and beverages as a stabilizer, emulsifier, and thickener. It is obtained as a dry exudate from *Acacia senegal* trees, mainly from the Sudan (*1*). However, the most recent definition of gum arabic encompasses gums produced by *A. senegal* (L.) and *Acacia seyal* (family Leguminosae) (*2*). Adulteration of gum arabic with other *Acacia* and non-*Acacia* gums is a potential problem. Gum from *Combretum erythrophyllum* has not been approved for use in foodstuffs (*3*) and is a potential adulterant. European Union legislation states that only additives, which comply with the requirements set by the Scientific Committee for Food, may be used in foodstuffs. Gums from *C. erythrophyllum* and *A. seyal* exhibit substandard emulsification properties as compared to *A. senegal* (*4*). Therefore, a rapid and cost effective means of identifying the species source of gum would be advantageous.

A variety of methods capable of distinguishing different gums are available such as nuclear magnetic resonance spectroscopy, multiangle light scattering, and gel permeation chromatography, none of which can absolutely identify different gum species (*5*−*9*). Some of these methods have been combined to give a chemometric approach to identify species (*5*), although the approach is time consuming.

Immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), have the potential to be used for speciation and adulteration of gums (*10*). ELISAs are relatively simple to use, rapid, and cost effective. This technique utilizes antibodies that have the ability to detect specific epitopes on target analytes. Several antibodies have been raised against *Acacia* gums with varying degrees of specificity (*10*−*13*). ELISAs have been developed previously that have been able to quantify gums in samples of gum arabic or food (*11*−*14*).

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Table 1. Cross-Reactivity of the Monoclonal Antibodies with Various Gums[a]

inhibitor gum	IC_{50} (mg mL^{-1}) SYCC7	SYHH3	SYJ1A1
A. senegal	0.03	>10.00	6.68
A. senegal (spray-dried)	0.07		
A. seyal var. *seyal*	4.99	0.10	1.05
A. seyal var. *fistula*		0.10	2.72
Acacia drepanolobium	1.58	1.99	1.16
Acacia dudgeoni	5.00	6.12	2.53
Acacia laetae	>10.00	>10.00	0.86
A. melliferae	0.01	5.60	>10.00
A. nilotica var. *nilotica*	5.12	0.05	2.94
Acacia polyacantha	>10.00	>10.00	1.93
Acacia sieberiana var. *sieberiana*	>10.00	0.66	2.29
Acacia sieberiana var. *woodii*	>10.00	>10.00	2.98
Albizia arabica	5.78	4.10	
Albizia zygia	>10.00	>10.00	10.00
A. latifolia (Ghatti)	3.96	3.06	1.36
Astralgus gummifer (Tragacanth)	3.76	6.37	>10.00
C. erythrophyllum	5.49	>10.00	0.02
Dyera costulata (Pontianak)	>10.00	>10.00	>10.00
L. coromandelica (JEOL)	2.30	0.13	0.77
Sterculia setigaera var. *tartar*	5.36	>10.00	
Stericulia urens (Karaya)	>10.00	4.14	3.61
Styrax officinalis (Storax)	>10.00	>10.00	>10.00
Xanthorrea australis (Accroides)	>10.00	>10.00	6.84
Pseudomonas elodea (Gellan)	5.80	>10.00	>10.00
Xanthomonas campestris (Xanthan)	>10.00	>10.00	>10.00
Cyanopsis tetragonolobus (Guar)	>10.00	>10.00	>10.00
gelatin	>10.00	4.71	>10.00
pectin	>10.00	>10.00	5.35
starch	>10.00	>10.00	>10.00

[a] *A. senegal* IC-ELISA: The plates were coated with 50 μg/mL of *A. senegal*. Dilutions of the antibodies were as follows: SY CC7 antibody, 1/400; second antibody, 1/1000. *A. seyal* IC-ELISA: The plates were coated with 50 μg/mL of *A. seyal*. Dilutions of the antibodies were as follows: SY HH3 antibody, 1/4; second antibody, 1/1000. *C. erythrophyllum* IC-ELISA: The plates were coated with 50 μg/mL of *C. erythrophyllum*. Dilutions of the antibodies were as follows: SY J1A1 antibody, 1/8; second antibody, 1/1000. Note: empty cells were not tested.

This paper aims to determine the specificity of previously developed monoclonal antibodies, to identify possible cross-reacting food components, and to assess the ability of the ELISA to determine the species source of gum in mixtures of gum and in confectionery.

MATERIALS AND METHODS

Unless otherwise noted, all reagents were of analytical grade (Sigma Chemical Co. Ltd., Poole, United Kingdom). All of the gums used in this project for assay development and calibration were obtained from a collection of authenticated samples and are listed in **Table 1**.

The monoclonal antibodies (SY CC7, SY HH3, and SY J1A1) used in this study have been previously described (*12*, *13*). Optimization of the antibodies and plate-coating concentrations was achieved using standard checkerboard titrations. Hybridoma supernatants were used at all times.

Indirect Competitive (IC)-ELISA of Pure Gums. Following optimization of antibodies and plate coatings, an IC-ELISA was developed for each gum. Plates (Immulon 4HBX, Dynex Technologies Ltd., Worthing, West Sussex, United Kingdom) were coated with 100 μL/well gum [50 μg/mL in phosphate buffered saline, pH 7.2 (PBS)] at 4 °C overnight. The plates were then blocked with dried nonfat milk powder [3% (w/v) in PBS, 250 μL/well for 1 h, 25 °C]. The contents were removed, and the plates were washed with PBST [PBS + 0.05% (v/v) Tween 20]. The plates were then incubated with 25 μL/well of pure inhibitor gum in PBS plus 25 μL/well of anti-gum monoclonal antibody supernatant diluted in sample buffer [PBS + 0.005% (v/v) Tween 20], to give the optimum final concentration (SY CC7 at 1/400; SY HH3 at 1/4; and SY J1A1 at 1/8), for 1 h at 37 °C. The contents were removed, and the plates were washed with PBST. A secondary peroxidase-conjugated anti-mouse isotype specific antibody (Sigma A5278, goat anti-mouse IgG for SY CC7, and Sigma A8786, goat anti-mouse IgM for SY HH3 and SY J1A1) at 1/1000 dilution in 3% (w/v) dried nonfat milk powder in PBST was added to each well (50 μL) and incubated at 25 °C for 1 h. The plates were then washed as before, and the enzyme substrate, *n*,*n*,*n*,*n*-tetramethylbenzidine (TMB) (Vetoquinol Ltd., Bicester, United Kingdom), was added (100 μL/well) and incubated for 1 h at 25 °C in the dark. The reaction was stopped by the addition of 50 μL/well 1 M orthophosphoric acid, and the plates were read at 450 nm using a Dynex MRXII plate reader.

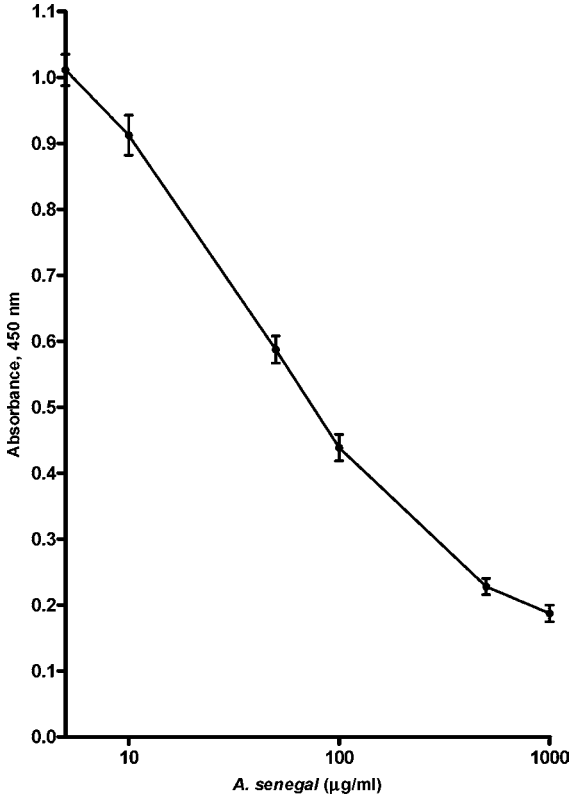

Figure 1. Typical standard curve for the *A. senegal* gum IC-ELISA. Plates were coated with 50 μg/mL of *A. senegal*. Dilutions of antibodies were as follows: SY CC7 antibody, 1/400; second antibody, 1/1000. Data are presented as the means ± standard errors, $n = 6$.

IC-ELISA of Gum in Confectionery. Spiked confectionery (fruit pastilles and jelly babies) was tested by IC-ELISA. Confectionery was spiked with gum at 10, 5, 1, and 0% (w/w). Confectionery was then dissolved in 2.5 mL of sample buffer (PBS + 0.005% v/v Tween 20). The IC-ELISA was performed as for pure gums.

IC-ELISA of Gum Blends. ELISAs were performed as for the IC-ELISA of pure gums except that blended gum samples were used as standards instead of pure gum and incubation of the substrate was reduced to 10 min. Optimal antibody dilutions for this assay were as follows: SY HH3 at 1/10 and SY J1A1 at 1/8.

Plate-Trapped Antigen (PTA)-ELISA of Gum Blends. A PTA-ELISA of blended gums was optimized using checkerboard titration and developed for the detection of substandard gums (*A. seyal* and *C. erythrophyllum*).

Various gum blends (50 μg/mL) were prepared in PBS and applied to plates (Immulon 4HBX), 50 μL/well gum, and incubated at 4 °C overnight. The plates were then blocked with 3% (w/v) dried nonfat milk powder in PBS, 250 μL/well for 1 h, at 25 °C. The plates were washed with PBST and then incubated with 50 μL/well of anti-adulterant gum antibody in sample buffer for 1 h at 37 °C. Optimal dilutions of antibodies for these assays were as follows: SY J1A1 at 1/2 and SY HH3 at 1/2. The plates were washed with PBST and then incubated with the appropriate peroxidase-conjugated anti-mouse isotype specific antibody in 3% (w/v) dried nonfat milk powder in

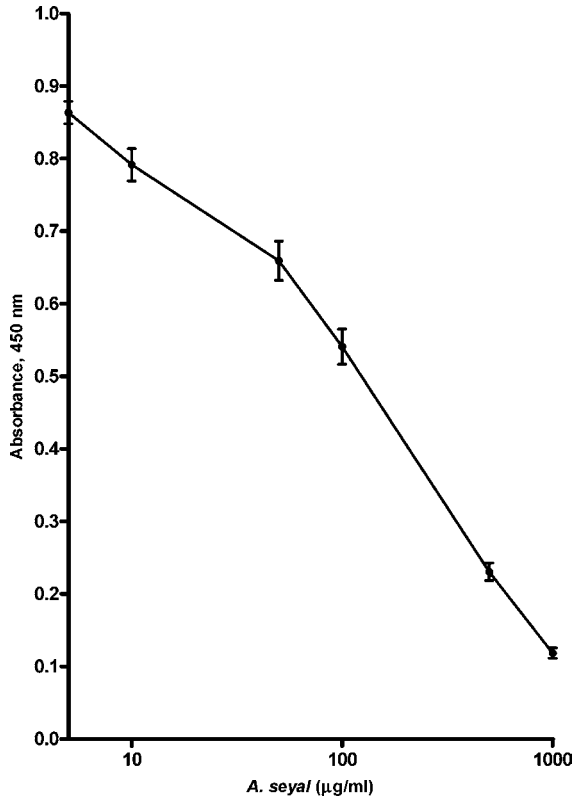

Figure 2. Typical standard curve for the *A. seyal* gum IC-ELISA. Plates were coated with 50 μg/mL of *A. seyal*. Dilutions of antibodies were as follows: SY HH3 antibody, 1/4; second antibody, 1/1000. Data are presented as the means ± standard errors, $n = 6$.

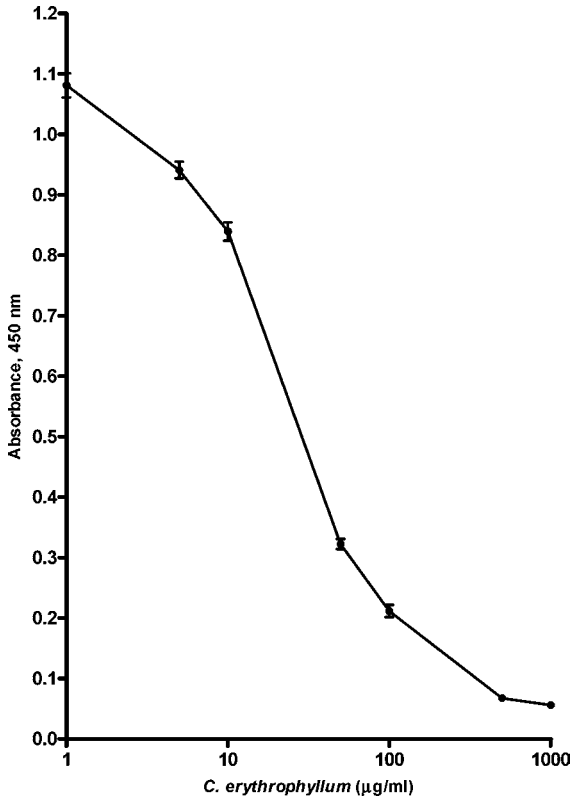

Figure 3. Typical standard curve for the *C. erythrophyllum* gum IC-ELISA. Plates were coated with 50 μg/mL of *C. erythrophyllum*. Dilutions of antibodies were as follows: SY J1A1 antibody, 1/8; second antibody, 1/1000. Data are presented as the means ± standard errors, $n = 6$.

PBST, 50 μL/well at 25 °C for 1 h. The plates then were washed as before, and TMB was added (100 μL/well) and then incubated for 10 min at 25 °C. The reaction was stopped by the addition of 50 μL/well 1 M orthophosphoric acid, and the plates were read as previously described.

Statistical Analysis. All data are reported as means ± standard errors, unless otherwise noted. The difference between sample means was evaluated through use of the Dunnett's multiple comparison test, post hoc.

RESULTS

A typical standard curve generated for *A. senegal*, *A. seyal*, and *C. erythrophyllum* gum using the IC-ELISA method can be seen in **Figures 1−3**, respectively. The limit of detection for these ELISAs was 5 μg/mL ($p < 0.05$ when compared to 0 μg/mL). Intraassay coefficient of variation (%CV) values for each ELISA were typically <10 (range 3−8%), <15 (range 4−14%), and <10% (range 3−6%), respectively. Interassay %CV values for each ELISA were <10%.

The specificities of the monoclonal antibodies toward a range of polysaccharides were determined and expressed as the concentration that exhibited 50% inhibition of gum binding (IC_{50}) using the IC-ELISAs. The specificities of the monoclonal antibodies used in this study were confirmed for *A. senegal* (SY CC7, $IC_{50} = 0.03$ mg/mL), *A. seyal* (SY HH3, $IC_{50} = 0.10$ mg/mL), and *C. erythrophyllum* (SY J1A1, $IC_{50} = 0.02$ mg/mL) (**Table 1**).

High cross-reactivity was seen between *Acacia melliferae* and the SYCC7 antibody ($IC_{50} = 0.01$ mg/mL) and *Acacia nilotica* and *Lannea coromandelica* gum with SY HH3 ($IC_{50} = 0.05$ and 0.13 mg/mL, respectively). *L. coromandelica* gum also cross-reacted to a lesser extent with both of the other antibodies used. SY J1A1 cross-reacted with all *Acacia* species tested (except *A. senegal* and *A. melliferae*) and also with *Anogeissus latifolia* and *L. coromandelica* (IC_{50} values ranged from 0.77 to 2.98 mg/mL).

Table 2. Summary of ELISA Performance in Confectionery[a]

	A. senegal		*A. seyal*		*C. erythrophyllum*	
	jelly babies	fruit pastilles	jelly babies	fruit pastilles	jelly babies	fruit pastilles
recovery (%)	71	92	103	65	72	78
CV in working range (%)	<5	<10	<10	<10	<5	<10
limit of detection (mg/mL)	7.5	7.5	10.0	10.0	5.0	5.0

[a] *A. senegal* IC-ELISA: The plates were coated with 50 μg/mL of *A. senegal*. Dilutions of the antibodies were as follows: SY CC7 antibody, 1/400; second antibody, 1/1000. *A. seyal* IC-ELISA: The plates were coated with 50 μg/mL of *A. seyal*. Dilutions of the antibodies were as follows: SY HH3 antibody, 1/4; second antibody, 1/1000. *C. erythrophyllum* IC-ELISA: The plates were coated with 50 μg/mL of *C. erythrophyllum*. Dilutions of the antibodies were as follows: SY J1A1 antibody, 1/8; second antibody, 1/1000.

IC-ELISAs were able to detect the appropriate gum in both jelly babies and fruit pastilles. The recoveries varied between the different ELISAs but were always greater than 65%. The assay precision remained high with %CV values <10% in all cases. There was a loss in sensitivity in all cases such that the limits of detection in the confectionery were as follows: SY CC7, 0.75% w/w (7.5 μg/mL); SY HH3, 0.1% w/w (10 μg/mL); and SY J1A1, 0.5% w/w (5 μg/mL) (**Table 2**) ($p < 0.05$ when compared to 0% matrix).

The antibodies were tested further by using blended gum samples. Both PTA- and IC-ELISA formats were able to detect the presence of *A. seyal* or *C. erythrophyllum* gum when blended with *A. senegal*. The PTA-ELISA was able to detect *A. seyal*

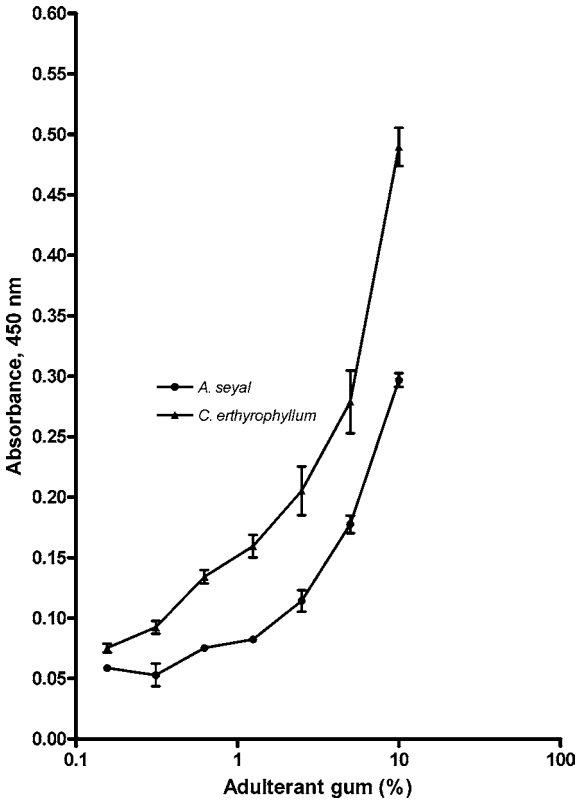

Figure 4. Typical standard curves of the PTA-ELISA blended gum assays. Plates were coated with various gum blends. Dilutions of antibodies were as follows: SYHH3 or SYJ1A1 antibodies, 1/2; second antibody, 1/1000. Data are presented as the means ± standard errors, $n = 6$.

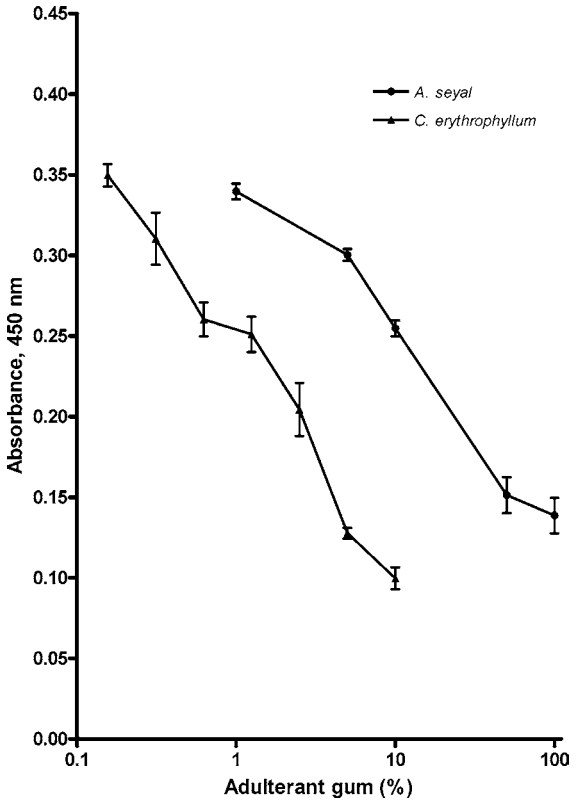

Figure 5. Typical standard curves of the IC-ELISA blended gum assays. Plates were coated with 50 μg cm^{-3} of *A. seyal* or *C. erythrophyllum*. Dilutions of antibodies were as follows: SYHH3 antibody, 1/10, or SYJ1A1 antibody, 1/8; second antibody, 1/1000. Data are presented as the means ± standard errors, $n = 3$.

(0.6% w/w) and *C. erythrophyllum* (0.2% w/w) (**Figure 4**). IC-ELISA could also detect *A. seyal* and *C. erythrophyllum* at 1.5% (w/w) and 0.2% (w/w), respectively (**Figure 5**). Higher binding affinity between antigen and antibody was seen in the PTA-ELISA format (IC_{50} = 3.4% w/w) than in the IC-ELISA (IC_{50} = 36% w/w) for *A. seyal*. The binding affinity was the same for *C. erythrophyllum* in both ELISA formats (IC_{50} = 3.4% w/w). The %CV values for both PTA- and IC-ELISAs were typically <15%.

DISCUSSION AND CONCLUSIONS

ELISAs were developed using the monoclonal antibodies described previously for the gums *A. senegal*, *A. seyal*, and *C. erythrophyllum* (*12*, *13*). The ELISAs were very sensitive and were able to identify and quantify the three gums mentioned. The sensitivity of each of the ELISAs is consistent with that previously reported (*12*, *13*). When the ELISAs were applied to a variety of other gums, a small number of cross-reactive species were determined. SY CC7 was identified as being very specific for *A. senegal* and *A. melliferae* suggesting a common epitope that is recognized by this antibody. SY HH3 had a high affinity for *A. seyal* but also cross-reacted strongly with *L. coromandelica*. SY HH3 recognizes carbohydrate epitopes (*13*), and *L. coromandelica* is known to have a higher ratio of arabinose and galactose when compared with *A. seyal*, suggesting that the epitope consists of arabinose and galactose sugars (*13*, *15*). SY J1A1 did cross-react to some extent with many of the other gums but did not cross-react with *A. senegal*. This indicated that SY J1A1 would not only be beneficial for detection of *C. erythrophyllum* adulteration of *A. senegal* but could also be useful in epitope mapping studies to determine molecular structure.

As adulteration of *A. senegal* with *C. erythrophyllum* is thought to be a problem (*16*) and *A. seyal* has substandard emulsification properties (*4*), ELISAs developed to determine adulterated *A. senegal* samples would be advantageous. The ELISAs reported here are able to detect adulteration of *A. senegal* gum with *A. seyal* (0.6% w/w) or *C. erythrophyllum* (0.2% w/w). Therefore, despite the fact that there is cross-reactivity with a number of other gums, these three antibodies have the potential for use in determining the species of gum samples. When the ELISAs were applied to confectionery, the precision profile remained good but there was a loss in sensitivity. However, the data demonstrate that the assays can be applied to foods.

The data presented in this paper confirm the potential for immunoassays in the detection and quantification of gums both in gums from primary producers and in processed products such as foods. There is also clear potential for the use of these antibodies in determining structural relationships between different species (*10*).

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