Immunological and functional properties of the exudate gum from northwestern Mexican mesquite (*Prosopis* spp.) in comparison with gum arabic

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Abstract

A comparison between the fine structural features of exudate gum from mesquite (*Prosopis* spp.) indigenous to NW Mexico and commercial gum arabic from *Acacia* spp. was achieved by means of immunological techniques. Their functional properties were compared from the ability to form oil-in-water emulsions and encapsulate cold press orange peel essential oil by spray drying. Fine comparison of the antigenic compounds in both materials against polyclonal rabbit antibodies, showed that the carbohydrate-rich components with slow mobility of mesquite gum are closely related to the faster ones of gum arabic. Also, close identity was observed for the components in the proteic fraction of both gums. Similar tannin concentrations were found in both materials ($\approx 0.43\%$) with only dark coloured samples bearing higher amounts ($\approx 1.9\%$). Gum arabic retained nearly 100% of the quantity of orange peel essential oil emulsified in water before spray drying, while mesquite gum did so for 90.6% of the citrus oil. From these results it is believed that mesquite gum might be a suitable replacement of gum arabic in arid regions of the world were Prosopis trees have widespread occurrence.

Keywords: Mesquite gum; Gum arabic; Immunological identity

1. Introduction

Mesquite (*Prosopis* spp.) is a N-fixing, leguminous plant of widespread occurrence in arid and semi-arid zones of the world. In the plains of the Sonoran Desert, Mexico, two varieties of *Prosopis* predominate, namely *Prosopis velutina* and *Prosopis glandulosa* var. Torreyana [1]. Two distinct kinds of polysaccharide mucilage materials have been identified in mesquite, namely a bark exudate gum [2,3], and a galactomannan fraction in the endosperm of the pod seeds [4,5]. Exudate polysaccharides are produced in *Prosopis* and

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many other trees and shrubs under conditions of drought and heat stress [6]. In the past, the exudate gum of mesquite was used in folk medicine by the native indians of Sonora and southern Arizona [1]. Currently, mesquite gum (locally called 'chucata') still has some domestic use, chiefly in folk medicine and it is commonly chewed as a sweet.

The mesquite gum primary structure is a highly branched heteropolymolecular complex polysaccharide, bearing a protein component, whose concentration varies with botanical origin (1.2–5.8%) [7,8]. The primary structure of the carbohydrate component of mesquite gum has been described as a core of β-D-galactose residues, comprising a $(1\rightarrow3)$-linked backbone with $(1\rightarrow6)$-linked branches, bearing L-arabinose (pyranose and furanose ring forms), L-rhamnsose, β-D-glucuronate and 4-*O*-Me-β-D-glucoronate as a single residue or oligosaccharide side chains [9–11]. A closely related primary structure has been identified in the polysaccharide fraction of gum arabic [12–14], including as well a protein component ($\approx$ 2.3%) [15]. A 'wattle blossom' model has been proposed to describe the tertiary structure of gum arabic [16–18], by virtue of which several polysaccharide domains of $M_w \approx 2 \times 10^5$ are held together by a peptidic chain. The hydrodynamic size of native mesquite gum molecule resembles that of the arabinogalactan (AG) major fraction of gum arabic [19].

Despite the closely related primary structure between mesquite gum and gum arabic, the two materials may be distinguished by antibodies specific to non-reducing carbohydrate chain termini [20]. Monoclonal anti-antibodies raised against the various components in arabinogalactan proteins (AGPs) show differences in the relative abundance of epitopes in different fractions [21]. The nature of the epitopes in AGPs is still largely unknown. Some of these point to specific carbohydrate termini at the surface of low and high molecular weight components [20,22,23] and some others to the amino acids present in the AGP fraction and in a third minor fraction (ca. 1% of the total) with a high protein content (ca. 50% w/w) referred to as a glycoprotein (GP), since very little, if any, affinity was found for the AG fraction [24].

The aims of this study were on the one hand to gain further understanding about the fine molecular nature of mesquite gum and compare it with that far more studied of gum arabic. On the other, to reinvestigate key analytical aspects which have restricted the use of mesquite gum in food, and to compare the functional properties of both gum materials on a typical industrial application in which gum arabic has a well established unique performance, namely in flavour encapsulation.

2. Experimental

2.1. Materials

Mesquite gum samples from *Prosopis velutina* tree specimens were collected between 29 June and 11 July 1995, at four different locations on plain areas of the Sonoran Desert (100 km around Hermosillo). The mean average mesquite gum yield per tree, found in the native form (i.e. without incisions), was 81.6 g ($n = 24$; 95% S.E. = 17.7 g). Gum arabic from Acacia trees was from Sigma (St Louis, MO). Sample vouchers were lodged at University of Arizona herbarium with the kind help of Dr Richard S. Felger. For the functional trials the following materials were used: a batch (8 kg) of commercial mesquite gum was purchased from a local retailer; a batch (5 kg) of corn acid-modified starch Amiogum 23® from American Maize Products, (Hammond, IN); a batch (1 l) of cold-pressed orange peel essential oil from Esencitricos S. de R.L. (Tlalnepantla, Edo. de Mex., Mexico).

2.2. Analytical procedures

Mesquite gum samples were dissolved in water, clarified by filtration (Whatman No.4 filter paper), and the resulting solutions were freeze-dried. Gum arabic was used as sold in powder form. Distilled water was used in all subsequent experiments and all chemicals were of analytical grade from Sigma. The samples were analysed for tannin [25] and protein contents [26].

2.2.1. Fractionation using thiophilic affinity chromatography

Matrix material was T-Gel (β-mercaptoethanol coupled to divynilsulphone-activated agarose) from Kem-En-Tec (Copenhagen, Denmark). Chromatography was done according to Lihme and Heegaard [27]. The matrix was placed on a 1×10 cm column and allowed to equilibrate in 1 M $(NH_4)_2SO_4$. 10 ml (10% w/w) of each gum (arabic or mesquite) in 1 M $(NH_4)_2SO_4$ were loaded to the column. After washing, the gum samples were eluted with 0.1 M NaCl. Total carbohydrate contents were analysed on the eluted fractions by the phenol-sulphuric test [28].

2.2.2. Immunization and purification of antibodies

Specific rabbit antibodies for commercial gum arabic and for mesquite gum collected during the spring season of 1995 were raised [29] and purified by chromatography on a Protein A column [30]. After purification, antibodies were called Anti-A for anti-arabic gum and Anti-M for anti-mesquite gum.

2.3. Electrophoretic methods

2.3.1. SDS-PAGE

SDS-polyacrylamide gel electrophoresis was performed on 10–20% gradient gels according to Laemmli [31].

2.3.2. Electroblotting and immunodetection

After SDS-PAGE fractions eluted from the column were electrotransferred to nitrocellulose membrane by semi-dry blotting. Membranes were blocked for 2 min with 0.05 M Tris, 0.15 M NaCl, 5 mM NaN_3, pH 7.2 plus 2% Tween 20 [32]. Two sets of strips were incubated for 2 h with anti-arabic and two sets with anti-mesquite gum antibodies in the same blocking solution added with 0.05% Tween 20. Once washed, the strips bound antibody was detected with horse radish peroxidase (HRP)-conjugated swine anti-rabbit antibodies for 1 h, washed and developed.

2.3.3. Crossed immunoelectrophoresis

Electrophoresis in 1% agarose gels was performed according to Heegaard and Bøg-Hansen [33]. Electrophoresis buffer was 0.1 M glycine, 26 mM Tris, pH 8.6. Samples (7 μl) of arabic or mesquite gums were run initially at 10 V/cm, 1:30 h in the first dimension gels. The second dimension was run at 2–3 V/cm overnight, gels contained 125 μl of Anti-A plus 125 μl of Anti-M gum rabbit antibodies. Plates size was 5×6.3 cm.

2.3.4. Affinity immunoelectrophoresis

It was done according to Bøg-Hansen et al. [34]. The gel in the first dimension contained 20 $\mu g/cm^2$ of lectins (concanavalin A, Con A or soybean agglutinin, SBA). Gums were applied as formerly for crossed immunoelectrophoresis in the first dimension and in the second dimension all the plates contained Anti-M antibodies.

2.4. Functional properties

2.4.1. Emulsions formulation and preparation

The emulsions base formulae had the following composition (% w/w): cold-press orange peel essential oil 5.0; Amiogum 23® 5.0; water 82.5, and either mesquite gum (dissolved and filtered through a cloth) or gum arabic 7.5. Emuslions were prepared by stirring the solutions on with an Ultraturrax T25 (Janke and Kunkel Gmbh, Staufen, Germany) fitted with a microshaft at speed setting of ca. 20 500 rpm for approximately 1 min.

2.4.2. Spray drying

The freshly prepared emulsions were fed into a pilot plant spray drier unit (APV Anhydro A/S, Søborg, Denmark) at a rate of 0.03 l/min. Atomization was achieved on a Bosch atomizer (Mod. 1210) powered at ≈ 3 kW. Temperatures at the inlet of the drying cabinet and outlet of the recovery tower were 195 and 80°C, respectively. The yield of recovered powder was $87.3 \pm 4.4\%$ and the powders had an average moisture content of $4.9 \pm 0.8\%$. The amount of citrus oil retained by the gum-starch carrier matrix during spray drying was determined by steam distillation of a powder sample (ca. 5 g) previously dissolved in water. The distilled oil was separated from the water with a mixture of chloroform:methanol (1:1) in a separation funnel. The solvent was distilled in a Goldfish

unit for ca. 2 h and the remaining of it evaporated on a convection oven. The quantity of oil obtained was referred to the original sample weight (allowing for moisture content). The percentage of oil recovered (i.e. encapsulated) was obtained as the ratio between the quantity of the oil recovered after steam distillation-extraction and the theoretical quantity expected (i.e. 28.6% w/w).

3. Results and discussion

3.1. Gum analysis

The protein content found in gum arabic from Sigma ($2.2 \pm 0.3\%$) tied in with the mean reported values for authenticated *Acacia senegal* samples ($2.2 \pm 0.2\%$) [15]. In turn, the protein content found in *Prosopis velutina* gum ($3.5 \pm 0.4\%$) was greater than that previously reported [7,8], being closer to the values for *Prosopis glandulosa*. A positive tannin test was detected in both gums. Tannin analysis mean value for mesquite gum samples from the inspected sites was $0.46 \pm 0.04\%$, while in gum arabic the measured content was $0.49 \pm 0.04\%$. Tannin levels in lightly coloured mesquite gum tears collected from the same tree in 1995 and 1996 were almost identical ($\approx 0.43\%$). A sample of dark coloured nodules (also from the same tree), collected in 1996, had much higher tannin content ($1.9 \pm 0.06\%$), than did the corresponding light tears ($0.43 \pm 0.03\%$). The tannin content on such analysed dark tear was similar to previously reported values for high-tannin commercial mesquite gum of Mexican origin [35]. The presence of high levels of tannins only in dark coloured tears, but not on light ones (i.e. with tannin levels comparable to gum arabic), could offer promising hope for mesquite gum eventual clearance for use in food, insofar as dark gum could easily be eliminated during sorting out by hand. Gum exudate from *Acacia senegal* and 'closely related species' included under the current definition of 'gum arabic of commerce' are the only materials which have been awarded 'ADI not specified' status for use in food [36]. By contrast, incorporation of mesquite gum in food has been indicated to be a potential hazard [35]. The leading argument used so far to raise suspicions about mesquite gum safety, has been the demonstrated presence of tannins and high Mn contents in gum samples from Prosopis species and other wild *Acacia* species associated with mutagenic effects claimed for tannic acid in the presence of Mn [35,37]. Direct intake of mesquite gum among people of Sonora has not so far been associated to any known toxic effects. Mesquite gum from controlled species and free from dark nodules might be a suitable replacement to gum arabic, in zones were Prosopis grows widely, such as NW Mexico.

3.2. Fractionation of gums on the T-Gel

Plant exudate gums are highly branched complex polysaccharides bearing 1.2–5.8% of protein [7,8]. Miskiel and Pazur [20] produced antibodies against terminal carbohydrate units of arabic and mesquite gums, we wanted to test if the protein moieties of the gums would also participate in immunological response. We have chose T-Gel for fractionation of both gums because it binds almost any kind of protein at high lyotropic salt concentration [27]; so it was believed that it would be effective in separating the different molecular fractions according to their relative protein content. Hardly any information on the nature of protein fractions of mesquite gum has been published.

When both gum solutions in 1 M $(NH_4)_2SO_4$ were loaded on to the T-Gel column, more than 98% of the total material was found in the unbound fraction (I). Fraction II which eluted from the column with 0.1M NaCl, represented only 0.7 and 1.6% of the total components of arabic and mesquite gums, respectively. Gum arabic bound fraction (II_A) was composed of 52% of protein and 48% of carbohydrate. Material from mesquite gum bound to the column (II_M) contained 36% of protein and 64% of carbohydrate. Randall et al. [17] fractionated gum arabic using hydrophobic affinity chromatography on phenyl-Sepharose and obtained three fractions. Although adsorption of proteins by T-Gel is salt dependent, their characteristics are not the same than those based on hydrophobic interaction. Thiophilic interaction is the result of the co-operative action of the sulphur

in the form of a thioether and the adjacent sulphone group to bind specific types of proteins [38]. Apparently, our II_A is similar than that obtained by Randall et al. [17] (fraction 3A) and to fraction GP reported by Williams [24], which accounts for ca. 50% of the total protein content.

SDS-PAGE of the fraction II for both gums revealed that in addition to carbohydrate species of high molecular weight, there are at least three protein components under 35 kDa and a similar component at 66 kDa (Fig. 1). The fraction II_A from gum arabic contained a 21 kDa principal protein, two thin bands around 31 and 35 kDa, and a faint band of 17 kDa. II_M from mesquite presented the same 17 and 31 kDa bands, plus a principal component of 23 kDa and a faint band of 29 kDa. Blotting and immunodetection of II fractions showed that proteins were also involved in antibodies production. Anti-arabic gum antibodies recognised components of 21 and 35 kDa,

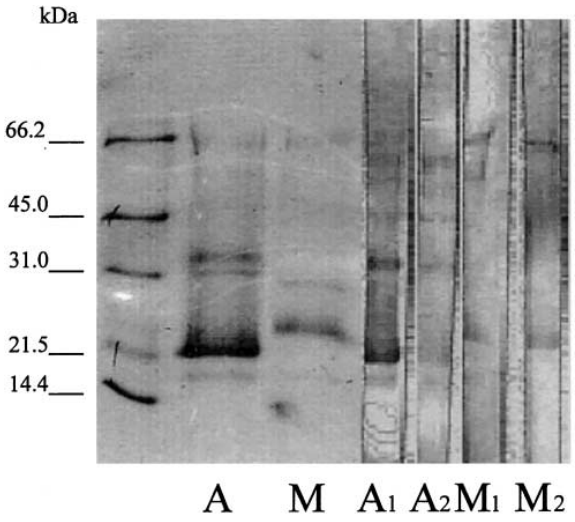

Fig. 1. SDS-PAGE patterns of II fractions obtained by chromatography on T-Gel, electroblotting and immunodetection. The first lane corresponds to molecular weight marker, lane A: gum arabic fraction II_A, and lane M: mesquite gum fraction II_M in gel stained with Coomassie brilliant blue. Strips are immunoblotts of fraction II_A incubated with Anti-A (lane A_1) and Anti-M (lane A_2) antibodies, and immunoblotts of fraction II_M incubated with Anti-A (lane M_1) and Anti-M (lane M_2). Antibodies were prepared in rabbits and immunodetection was done by swine anti-rabbit antibodies conjugated to HRP.

while anti-mesquite antibodies did to the 23- and 66-kDa ones. Moreover, some cross reactivity was found between both types of antibodies (anti-arabic and anti-mesquite) against fraction II of each other gum. This experiment show that the polypeptide structure of the gum complex is exposed in solution producing antibodies in immunised animals. Thus, in addition to the carbohydrate nature of antigens in both arabic and mesquite gums, as probed by Miskiel and Pazur [20], protein components are also antigenic in spite of their low concentration as has been suggested by Williams and co-workers [24].

3.3. Crossed immunoelectrophoresis

Most of the compounds in gums were found to be acidic at pH 8.6 and hence, have a net mobility towards the anode. The second dimension gel contained antibodies which generated precipitates of characteristic height, stainability and shape. As it is illustrated in Fig. 2, plate a, whole mesquite gum presents four precipitation peaks, showing that four different populations of antigens produced antibodies by immunisation. In tandem immunoelectrophoresis (plate b), whole gum arabic produced a new peak (labelled 5) fused with peak 1 from mesquite gum, showing immunological identity between both antigens. Peak 5 comes from gum arabic as it is seen in plate c. Running time for the first dimension was longer than usual in order to clearly demonstrate the continuity between peaks 1 and 5. This experiment in addition to the described identity between two antigens, shows that antigens in mesquite gum are highly heterogeneous with respect to charge and possibly size. Part of the anti-arabic gum antibodies reacted with mesquite gum and part of the anti-mesquite antibodies reacted with arabic gum, which is in agreement with previous findings [20]. Recently, however, a detailed epitope characterisation [39] of a monoclonal antibody (CCRC-M7) which recognises mesquite gum but not gum arabic epitopes has been described with $(1\rightarrow6)$-β-D-galactosides longer or equal than three residues being the most effective competitors for the binding site.

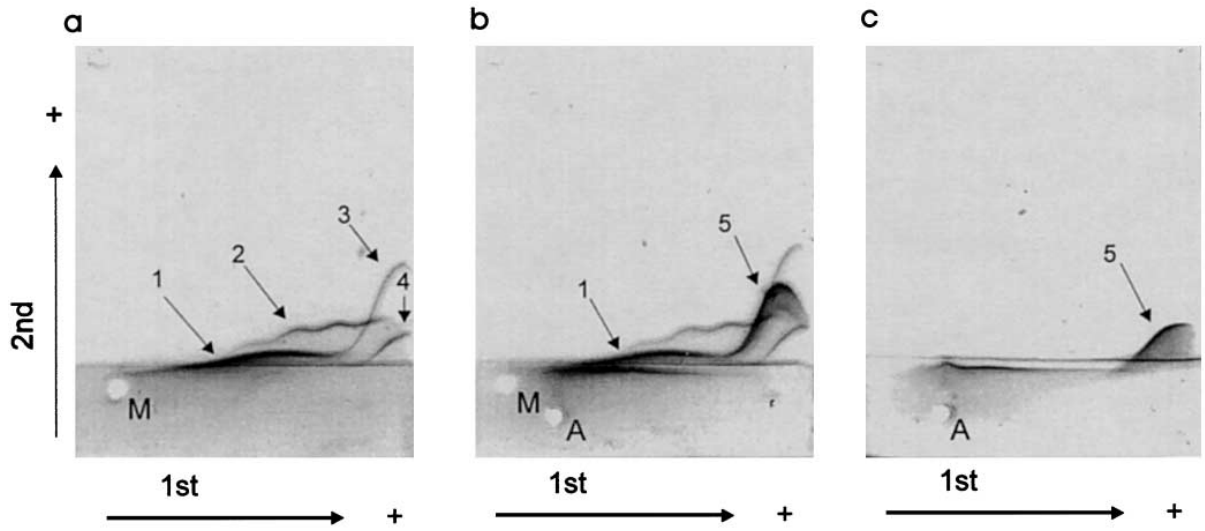

Fig. 2. Crossed immunoelectrophoresis. Samples of 7 μl of arabic (A) or mesquite (M) gums were separated by electrophoresis in 1% agarose gels in the first dimension. All the plates contained 125 μl of Anti-A + 125 μl of Anti-M antibodies mixed with the agarose gel in the second dimension.

3.4. Crossed affinity electrophoresis

In order to investigate the composition of the antigens, lectins with well established specific affinity for different carbohydrate structures were mixed with agarose in the first dimension of gels (Fig. 3 plates b and c). Plate a in Fig. 3 is a control presenting tandem immunoelectrophoresis of both gums with anti-mesquite gum antibodies in the second dimension gel. Con A, a lectin which interacts with glucosyl or mannosyl residues [40], strongly interacted with the component of slower mobility in mesquite gum (plate b, precipitate 1). Con A interacted with such component 1 in the first dimension producing a heavy precipitate as a rocket unable to migrate in to the second dimension. The effect of Con A on the corresponding precipitation peaks of gum arabic, was not evident. SBA, a lectin which recognises galactosyl residues [40] interacted in a very similar way than did Con A, although its overall affinity for the slowest mesquite gum component was weaker (plate c, precipitate 1). This finding could perhaps be associated to differences in the geometry of branching and/or in the carbohydrate residues exposed. It appears that discrete surface structures (epitopes) are identical between the slowest component in mesquite gum and the fast mobility component of arabic gum. Probably this is due to the presence of the β-D-glucoronosyl-(1 $\rightarrow$ 6)-D-galactose or 4-Me-β-D-glucoronosyl-(1 $\rightarrow$ 6)-D-galactose units at the non-reducing ends of arabic and mesquite gums, respectively, as it was postulated by Miskiel and Pazur [20]. This suggestion is in good agreement with recent light scattering evidence [19], which has shown that the hydrodynamic ratio of whole mesquite gum ($\approx$ 8.5 nm) is close to the size of the AG fraction in gum arabic ($\approx$ 9.2 nm).

3.5. Functional behaviour

Flavour encapsulation is one of the chief industrial applications of gum arabic [41]. Fig. 4 shows the amount of citrus oil retained by mesquite gum and gum arabic-based carrier matrices. It is evident that gum arabic had a greater capacity to retain oil during spray drying, with nearly 100% of the expected oil fraction being recovered in the powder. While mesquite gum matrix also retained most of the citrus oil (90.6%), its efficiency was somewhat lower than that of gum arabic. In

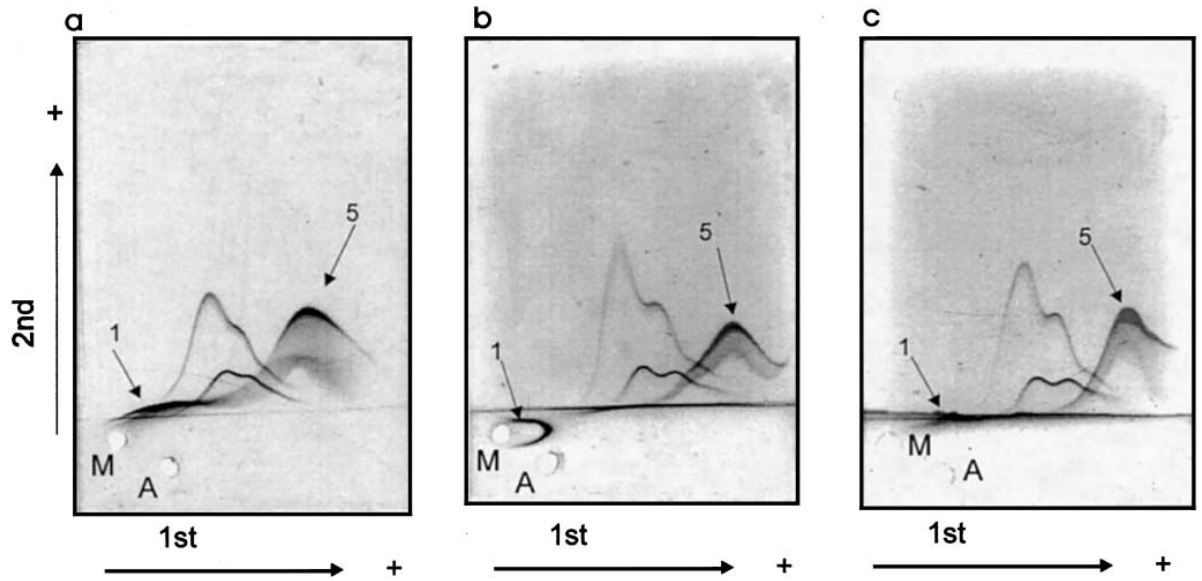

Fig. 3. Crossed immunoelectrophoresis of arabic (A) or mesquite (M) gums with lectin-containing gels in the first dimension. All the plates contained anti-M antibodies in the second dimension; plate a is a control (without lectin), plate b contained ConA and plate c soybean agglutinin in the first dimension.

recent studies [42], the emulsifying behaviour of the different fractions of gum arabic has been investigated at iso-nitrogen levels. The authors have shown that in general the emulsion stability increases with molecular weight and protein content, with the best results found for the fraction containing 8.7% of the protein (8% of the whole gum, i.e. the AGP fraction), with very poor emulsions formed by the protein-rich minor fraction (GP). Whole mesquite gum has also been shown to be able to form and stabilise o/w emulsions [43,44]. Fine differences in terms of the low molecular weight protein components could account for the lower ability of mesquite gum in comparison with gum arabic to retain citrus oil during atomisation and/or formation of o/w emulsions found here. Such hypopthesis is currently being tested experimentally in our laboratory.

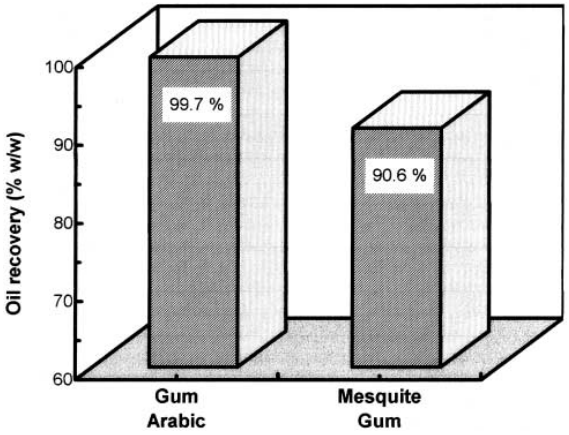

Fig. 4. Retention of orange peel essential oil by spray dried gum arabic and mesquite gum based carrier matrixes. The composition of the base emulsions was (in % w/w): orange oil 5.0, mesquite or arabic gum 7.5, Amiogum 23 (modified corn starch) 5.0 (spray drying load = 28.6%, total solids = 17.5%). Inlet and outlet temperatures on the spray drying unit were 195 and 80°C.

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