

previously described starting from a semi-defined lactose-rich medium via fermentation by *Rhanelia aquatilis*. Since lactose is the major solid component of whey, production of that high-added value exopolysaccharide may help alleviate the environmental problems raised by whey as the major by-product of the cheesemaking industry

The polysaccharide was produced using five different fermentation media: a synthetic medium, plain whey under aerobic conditions, whey permeate, whey with 2% NaCl, and plain whey under anaerobic conditions. Lactan was recovered, from each medium, by precipitation with 50% ethanol and then lyophilized. Each aliquot of polysaccharide was solubilized in deionized water and solutions with different polysaccharide concentrations, different pH values, and different ionic strengths were analyzed under steady shear flow using a controlled stress rheometer (Carri-Med 50) with cone-and-plate geometry.

All lactan solutions showed a shear-thinning behaviour, and increases in viscosity were observed for increasing concentrations of polysaccharide as expected. The polysaccharide produced from whey with 2% salt and plain whey fermented under anaerobic conditions showed lower viscosity than that obtained from the remaining media. Addition of salts (e.g. KCl and CaCl<sub>2</sub>) led to decreases of viscosity, which was virtually the same irrespective of salt concentration. Changes of pH (3–11) affected slightly the viscosity of the polysaccharide solutions, although higher viscosities were obtained at pH 7. The slight effect of pH and ionic strength can be somewhat implicated with the galacturonic acid residues present in the polymer chain. The polysaccharide samples possessed different protein contents, which may have influenced to some extent the rheological behaviour of the gel. The shear-thinning properties of the gum lead to potential applications in several food and non-food products.

### Regio- and Stereoselectivity Issues in Allylic Reactivity of Vinylogous Esters/Carbonates Bearing the 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl Auxiliary

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Carbohydrate-based dienes have been shown to exhibit good-to-excellent diastereofacial selectivity in their reactions with cyclic dienophiles and aza-dienophiles. Glucose-bound vinylogous systems have previously been synthesized and shown to undergo diastereoselective addition reactions. In the present study new cyclic and acrylic vinylogous esters/carbonates were synthesized and their diastereofacial reactivity studied. With bromoallylic derivatives the nucleophilic displacement of the bromine atom was performed with oxygen, nitrogen and sulphur nucleophiles affording, in some cases, allylic rearrangement products where a new stereogenic centre was developed with medium-to-good diastereoselectivity. A model of reactivity is advanced on the basis of the absolute stereochemistry established by X-ray analysis.

### Quantification of Mercury in Sugar by Cold Vapour Atomic Absorption Spectrometry

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Mercury is a heavy metal which levels in environment are of growing concern due to its biomagnification in certain live systems and consequent human dietary intake. Several studies have been performed to accomplish for the Hg contamination extent, almost all of them being performed in sediments, soils, water and fish. Nevertheless, some studies have also included the monitorization of mercury in agricultural and horticultural crops, namely crowns and leaves of sugar beet, and in the corresponding soils where they were cultivated, to correlate the element contents. Posterior determinations of mercury in crops were performed, which included sugar cane. Recently, additional studies have highlighted the correlation between mercury soil contamination and respective plant contents as is reported for rosemary and mushrooms. As a consequence, some countries have already proposed maximum acceptable levels for mercury in crops and the Joint FAO/WHO Experts Committee on Food Additives established a provisional tolerable weekly intake for total mercury of 0.3 mg of Hg/person.

As saccharose is a purified product from vegetal origin, it is important to control its mercury contamination and this communication presents a method for its quantification. The sample preparation consists of a wet digestion of the matrix with H<sub>2</sub>SO<sub>4</sub> and NHO<sub>3</sub> (1:2, v/v) in a closed PTFE container at controlled temperature (80°C) for 2 h; afterwards, saturated KMNO<sub>4</sub> solution is gradually added into the sample solution to complete the mineralization. The measurement of the element is achieved by cold vapour atomic absorption spectrometry after reduction of the oxidized mercury compounds with sodium borohydride solution.

The detection limit is 0.28  $\mu$ g/L and the linearity range in the optimized conditions is 0.28–20  $\mu$ g/L. The precision is 9.0% and 11.0% for the analytical and over-all procedure, respectively. The validation of the procedure was performed by using a reference material and by the standard additions method (2.5, 5.0 and 10.0  $\mu$ g/L), being the recoveries higher than 90%.

### Quality Evaluation of Portuguese Honeys

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Honey is the sweet viscous substance elaborated by the honey bee from the nectar of floral plants. It is produced in almost every country of the world and is a very important energy food, used as an ingredient in hundreds of manufactured foods, mainly in cereal based products, for sweetness, colour, flavour, caramelization and viscosity. Several types of honey are produced in Portugal. Sugars represent the largest portion of honey composition (95–99% of the honey solids). Fructose and glucose are the most abundant sugars in it, but others are usually mentioned, namely, saccharose, maltose, melibiose and trealose. Its composition depends highly on the types of flowers used by the bees as well as regional and climatic conditions.

Adulteration of honey is possible, so its quality must be controlled analytically with the aim of guaranteeing the genuinity and preserve consumer from commercial speculations. The present work was conducted to investigate the quality of 25 brands of Portuguese honeys commercially available on the market, in a total of 50 samples. Carbohydrate composition was determined by HPLC-RI to evaluate the monosaccharides, fructose and glucose, the disaccharides, sucrose, maltose, trehalose and melibiose and the trisaccharide melizitose. Sucrose content is important to determine heavy

sugar feeding of the bees or adulteration by direct addition of sucrose. The ratio fructose to glucose was also evaluated, because it is suggested that it can be used to typify honey samples from different origins and also indicates the crystallisation tendency.

Honey quality can also be affected by heating during extracting, liquefying or clarifying processes or by aging during storage with production of 5-hydroxymethylfurfuraldehyde. This compound was quantified by HPLC-UV. Some other physicochemical quality parameters were also carried out according to the Official Portuguese Methods (NP-1307, Port. no. 449/76) and Codex Alimentarius Commission (1968) in order to determine moisture, ash content, diastase activity, total acidity and water insoluble solids. The chemical characteristics of the samples investigated in this study generally agreed with the major national and international honey specifications.

### Preparation of Starch Esters

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Several hydrophobic starch fatty acid esters have been synthesised from both maize and potato starch which may have potential industrial application as surface coating materials. Maize and potato starch esters were prepared under heterogeneous reaction conditions, using the corresponding acid anhydride and pyridine via a nucleophilic acyl substitution reaction, in dimethylformamide. The starch esters synthesised varied in both degree of substitution (D.S.) and ester group configuration. Esterifying reagents varying in range from two (acetic anhydride) to six (hexanoic anhydride) carbon atoms were utilised. The preparation of maize starch acetate was also investigated as a function of time. Although up to forty-eight hours was utilized for the reaction time, it was found that the D.S. increased only slightly after three hours after the esterifying reagent had been added. Maize and potato starch hexanoate were also synthesised from hexanoyl chloride under heterogeneous reaction conditions using dimethylformamide (DMF) as the reaction medium. The starch esters were then characterised in terms of ester bond formation by Fourier transform infrared spectroscopy (FT-IR) and determination of D.S. by proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR). D.S. values varied between 0.5–2.5. Trends regarding starch source and ester group chain length were then evaluated, which indicate that generally maize starch produces higher D.S. value esters than potato starch, and that the longer the acid anhydride acyl chain the lower the D.S. value ester obtained. The solubility profiles of the starch ester derivatives have also been evaluated for a range of organic solvents.

### Preparation and Characterization of Hydrogels Based on Sucrose Modified with Glycidyl Methacrylate

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Recently, various hydrogels containing sugars have been developed. Due to its low price and high production (more than 100 million tons per year), sucrose would be particularly

important for this purpose. Although many authors referred the modification of this sugar with vinyl monomers by chemical methods, not much work has been done on the study of its application as monomers for hydrogels which could be of use as materials for drug delivery systems, bioimplants, contact lenses, etc.

In this work we wish to report some of the work that we have been doing to modify sucrose with glycidyl methacrylate and its application as a monomer for the synthesis of a copolymer containing hydroxyethyl methacrylate. In the first part of this project the introduction of a vinyl group in sucrose was carried out by reacting the disaccharide with glycidyl methacrylate. The product obtained was characterised by NMR and FTIR, showing the modification of the sugar. In the second part of the work, copolymers of hydroxyethyl methacrylate and the modified sucrose were prepared. The hydrogels were characterised physically and chemically by determining their water sorption capacity, their possibility of use as a drug delivery system and their structure by differential scanning calorimetry (DSC).

### Preparation and Characterization of Hydrogels Based on Sucrose Modified with Acryloyl Chloride

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In this work we wish to report some of the work that we have been doing to modify sucrose with acryloyl methacrylate and its application as a monomer for the synthesis of a copolymer containing acrylamide. In the first part of this project the introduction of a vinyl group in sucrose was carried out by reacting the disaccharide with acryloyl chloride. The product obtained was characterised by NMR and FTIR, showing the modification of the sugar. In the second part of the work, copolymers of acrylamide and the modified sucrose were prepared. The hydrogels were characterised physically and chemically by determining their water sorption capacity, their possibility of use as a drug delivery system and their structure by differential scanning calorimetry (DSC).

### Preliminary Studies of Baía Orange Peel Pectins

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One of the applications of pectins are based upon their ability to form gels. This ability is in turn dependent on the molecular properties of these polysaccharides namely, molecular weight, degree of esterification, terogeneity, degree of amidation, and the presence of acetyl groups at O-2 or O-3.

The yield and quality of pectins depends strongly on the