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Influence of the extraction process on the rheological and structural properties of agars

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ABSTRACT

Agars obtained by traditional hot-water (TWE) and microwave-assisted (MAE) extractions were compared in terms of their rheological and physicochemical properties and molecular self-association in solutions of low (0.05%, w/w) and high (1.5%, w/w) polymer concentrations. At low concentration, thin gelled layers were imaged by AFM. Slow or rapid cooling of the solutions influenced structure formation. In each case, TWE and MAE agar structures were different and apparently larger for MAE. At high concentration, progressive structural reinforcement was seen; while TWE agar showed a more open and irregular 3D network, MAE agar gel imaged by cryoSEM was denser and fairly uniform. The rheological (higher thermal stability and consistency) and mechanical (higher gel strength) behaviors of MAE agar seemed consistent with a positive effect of molecular mass and 3,6-anhydro-α-L-galactose content. MAE produced non-degraded agar comparable with commercial ones and if properly monitored, could be a promising alternative to TWE.

1. Introduction

In recent years, the application of non-conventional energy sources in the extraction of natural compounds has gained great interest. Microwave-assisted extraction (MAE), which promotes the simultaneous heating of the whole sample matrix by using microwave irradiation, has been successfully applied in the extraction of a wide variety of natural products (Chan, Yusoff, Ngoh, & Kung, 2011; Fishman, Chau, Cooke, & Hotchkiss, 2008; Perino-Issartier, Abert-Vian, & Chemat, 2011; Santana, Ferrera, & Rodriguez, 2005). The use of specialized microwave systems as opposed to the classical domestic devices many times used in the past by researchers, allowed the proper control of vital operational parameters such as pressure and temperature, thus becoming of great interest for the industries. MAE advantages include drastic reduction of extraction times, higher recoveries, lower energy and solvent consumptions and reduced byproduct formation (Belanger & Paré, 2006; Leonelli & Mason, 2010; Srogi, 2006). Very recently, our group applied, for the first time, the MAE technique to the isolation of agar (Sousa, Alves, Morais, Delerue-Matos, & Gonçalves, 2010; Sousa et al., 2012), a gelling polysaccharide of great commercial value (Selby & Whistler, 1993). This previous research showed that agars with better gelling properties could be extracted when using MAE instead of the conventional extraction route (*i.e.* traditional hot-water extraction, TWE; Villanueva, Sousa, Gonçalves, Nilsson, & Hilliou, 2010).

Agar is ideally based on the neutral polysaccharide agarose, built up from 3-linked β-D-galactose and 4-linked 3,6-anhydro-α-L-galactose (3,6-AG) repeating units and responsible for the polymer's gelling character. Commonly, the anhydride bridge is absent and several substitution groups are observed throughout agar's backbone. This non-gelling polysaccharide is globally named agaropectin and comprises the charged fraction of the polymer (Matsuhashi, 1990). Agar gelation is believed to result from a two steps mechanism: a conformational transition (coil-to-helix) upon cooling an agar aqueous solution where the molecules are homogeneously distributed and interhelical aggregation, at temperatures below the polymer gelation point (Arnott et al., 1974; Clark & Ross-Murphy, 1987; Labropoulos, Niesz, Danforth, & Kevrekidis, 2002a; Nordqvist & Vilgis, 2011). When sufficient polymer concentration is reached, a 3D network (*i.e.* macroscopic gel) is formed while insufficient concentration will result in local aggregates, networks and/or individual molecules (Morris, Kirby, & Gunning, 2010). While agarose forms rigid polymeric networks, the sulfate ester groups present in the agaropectin fraction of agar cause kinks in the perfect alignment of the helices, thus leading to the formation of less compact structures (Labropoulos et al., 2002a). Several research papers have aimed at investigating the behavior of gel-forming polysaccharides in aqueous media by atomic force microscopy (AFM;

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e.g. Ikeda, Morris, & Nishinari, 2001; Noda et al., 2008; Roesch, Cox, Compton, Happek, & Corredig, 2004). Only two AFM studies of agarose have been reported (Maaloum, Pernodet, & Tinland, 1998; Pernodet, Maaloum, & Tinland, 1997) neither focusing on dilute solutions. However, cryogenic scanning electron microscopy (cryoSEM; *e.g.* Nordqvist & Vilgis, 2011; Tuvikene et al., 2008) and rheological (Labropoulos et al., 2002a; Labropoulos, Niesz, Danforth, & Kevrekidis, 2002b; Mohammed, Hember, Richardson, & Morris, 1998) studies of agarose gels seem well documented in the literature. Also, to the best of our knowledge, no imaging or rheological studies have been published comparing agars obtained by TWE and MAE.

The aim of the present work was to compare agars extracted using conventional (thermal heat; TWE) and non-conventional (microwaves; MAE) energy sources. The viscoelastic behavior of both polysaccharides was monitored through rheological measurements and relevant physicochemical properties typically associated with agar quality were determined. A commercial agar, extracted with thermal heat, was also characterized to better infer the quality of the extracted samples. AFM and cryoSEM techniques were used to image the molecular associations of MAE and TWE agars in solutions of, respectively, low and high polymer concentrations. Wild *Gracilaria vermiculophylla* from Ria de Aveiro, northwestern Portugal, was used as raw-material and optimal conditions described in previous reports were applied (Sousa et al., 2010; Villanueva et al., 2010).

2. Materials and methods

2.1. Algae collection and sample preparation

G. vermiculophylla was collected in Ria de Aveiro, northwestern Portugal (40°38″N, 8°43″W), during January 2011. The location and month of collection as well as the sample preparation were similar to those described in a previous work (Villanueva et al., 2010).

2.2. Agar extraction

Agar traditional hot-water (TWE) and microwave-assisted (MAE) extractions were performed according to previously optimized procedures (Villanueva et al., 2010; Sousa et al., 2010). A MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA) apparatus with a maximum power output and a batch system of fourteen sealed Teflon® vessels was used for agar MAE process. The optimal conditions used in the TWE method were: 2 h at 85 °C under conventional heating, 200 mL of solvent and without agitation (Villanueva et al., 2010) while the optimized MAE conditions were: 5 min at 90 °C under microwave heating, 20 mL of solvent, with maximum stirring (Sousa et al., 2010). The extracts were purified and their water content estimated as described in Sousa et al. (2012). A commercial agar sample from Sigma–Aldrich (A-7002) was used as reference.

2.3. Preparation of agar solutions with low concentration

Dilute agar aqueous solutions of 0.05% (w/w) (*ca.* 500 μg/mL) concentration were prepared for AFM studies following two different procedures. The first one consisted in dispersing the appropriate polysaccharide amount in distilled water under vigorous stirring. The dispersions were then heated at ~96 °C for one hour and the resulting solutions were left to slowly cool to room temperature ('*slow cooling*' method). The other adopted procedure consisted in diluting with cold distilled water the concentrated solutions (1.5% (w/w); see Section 2.4) while hot (*i.e.* random coil state), to a final concentration of 500 μg/mL ('*fast cooling*' method). The pH of the solutions fell in the range ~6.4–6.7.

2.4. Preparation of agar solutions and gels with high concentration

Concentrated agar aqueous solutions were prepared by dispersing the appropriate amount of polysaccharide in distilled water under vigorous stirring and heating at ~96 °C for one hour. Approximately fifteen grams of the hot 1.5% (w/w) (*ca.* 1.5% g/mL) solutions were transferred to a cylindrical container with 30 mm diameter and properly covered to avoid water evaporation. The agar gels were left to set at room temperature and to equilibrate for 20 h. The gel depths were approximately 21–22 mm. The remaining hot agar solutions were used in the oscillatory rheological measurements.

2.5. Atomic force microscopy (AFM) imaging

AFM studies were performed in air at room temperature using a PicoLe Atomic Force Microscope (Molecular Imaging, USA) operating in dynamic tip deflection mode (Acoustic Alternating Current mode, AAC). Aliquots (25 μL) of each low concentration agar solution, prepared as described in Section 2.3, were deposited onto freshly cleaved discs of mica (Muscovite V-4, 15 mm diameter), allowed to dry in air for about 20 min, washed thoroughly with Millipore water, dried under a stream of nitrogen, and imaged by AFM in the tapping mode. Silicon cantilever (ACT-50, AppNano, USA) with a tip (pyramidal shape) height in the range of 14–16 μm, radius of curvature (ROC) lower than 10 nm, spring constant of 25–75 N/m, and a typical resonance frequency in the range 200–400 kHz was used for these purposes. Several areas of the mica surface were scanned in topography, amplitude, and phase modes with a resolution of 512 × 512 pixels and are representative of 5 μm × 5 μm regions over different locations on the studied mica surfaces. AFM images were corrected for bow/tilt by a second-order flattening using the PicoView™ 1.8.2 software (Agilent Technologies). The free Gwyddion 2.22 software was used to obtain the AFM parameters such as average root-mean-square surface roughness (R_{ms}) and the average heights (H_{av}) of the structures observed in Figs. 1 and 2, as well as the height profiles represented underneath the same images.

2.6. Penetration tests of agar gels

The gel strength (GS; g cm^{-2}), defined as the stress required for breaking the gel surface, was obtained from the force-deformation data measured in penetration tests, using a texture analyzer (Stable Micro Systems model TA-XT2, Surrey, England). Tests were performed at room temperature using a cylindrical plunger with 10 mm diameter attached to the equipment, operating at a penetration rate of 0.2 mm s^{-1}. Three replicates were performed for each agar gel.

2.7. Oscillatory rheological measurements

Dynamic rheological measurements were performed in a stress-controlled rheometer (ARG2, TA Instruments, USA) following a procedure described elsewhere (Sousa et al., 2010) with some improvements. A cone-and-plate geometry (4 cm diameter, 2° angle and a 54 μm gap) was used in all the determinations. The hot agar 1.5% (w/w) solutions (prepared in Section 2.4) were kept above 80 °C until the beginning of the experiment. The samples were covered with a layer of paraffin oil to prevent evaporation after having been placed into the measuring device, pre-heated at 80 °C. After an equilibration time of 10 minutes, the cooling step for gel formation