



## Development of bioactive edible film from turmeric dye solvent extraction residue



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### ABSTRACT

We developed and characterized turmeric flour films using the turmeric dye solvent extraction residue. We evaluated how the heating temperature and pH affected film properties using a  $2^2$  central composite design. Multi-response analysis furnished the film formulation that offered larger resistance to break, as well as lower water solubility, WVP, and opacity. The heating temperature and pH affected the mechanical properties, solubility, moisture content, WVP, and opacity of the resulting film. High heating temperature promoted more interactions between the polymers present in the turmeric flour (starch, protein, lipid, and fiber), affording a more resistant polymeric structure with lower WVP, moisture content, and opacity. Higher pH values also favored a more mechanically resistant and dense matrix with lower water solubility and WVP. The optimized conditions were:  $T = 86.7\text{ }^\circ\text{C}$  and  $\text{pH} = 8.5$ . The films produced under these conditions displayed high mechanical strength (18 MPa), low solubility (36%), and low WVP ( $0.167\text{ g mm h}^{-1}\text{ m}^{-2}\text{ kPa}^{-1}$ ). However, because these films contained lignocellulosic fibers, they presented low elongation at break (1.8%), which elicited a non-continuous structure. HPLC and DPPH assays showed that the turmeric dye solvent extraction residue can be a promising source to develop films with antioxidant activity.

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### 1. Introduction

It is possible to make edible films from biopolymers such as polysaccharides and proteins extracted from plant or animal raw material. In general, these biopolymers constitute a poor barrier to water vapor; moreover, compared with synthetic packaging, they have unsatisfactory mechanical strength and elongation. To make protein and starch films mechanically stronger, some authors have incorporated fibers of different plant origins (hemp, jute, flax, bagasse, cotton, sisal, wheat fibers, etc.) into biocomposites, to reinforce agromaterials (Montaño-Leyva et al., 2013; Satyanarayana, Arizaga, & Wypych, 2009). The results are not always favorable: the polymers may be thermodynamically incompatible, culminating in phase separation (Grinberg & Tolstoguzov, 1997). To overcome this problem, researchers have prepared biodegradable films from natural mixtures (carbohydrates, proteins, lipids, and fibers) obtained in the flour form from raw materials of plant origin such as cereals, tubers, and rhizomes (Andrade-Mahecha, Tapia-Blácido, & Menegalli, 2012; Dias, Muller,

Larotonda, & Laurindo, 2010; Gontard, Guilbert, & Cuq, 1993; Tapia-Blácido, Mauri, Menegalli, Sobral, & Añón, 2007; Tapia-Blácido, Sobral, & Menegalli, 2005, 2011). A recent trend is to use industrial residue to obtain natural mixtures of biopolymers as flour, which may provide the packaging market with a competitive product.

*Curcuma longa* L. belongs to the family Zingiberaceae, commonly known as turmeric. It consists of a rhizomatous perennial herb with primary and secondary rhizomes; and its shape varies from spherical to slightly conical, hemispherical, or cylindrical (Jyothi, Moorthy, & Vimla, 2003). This herb is bright yellow because it contains Curcumin, a diphenolic compound, that exerts anti-inflammatory action and is a potential candidate to treat cystic fibrosis, Alzheimer's, malarial diseases, and cancer (Maheshwari, Singh, Gaddipati, & Srimal, 2006; Yallapu, Jaggi, & Chauhan, 2012). Turmeric also contains the curcuminoids demethoxycurcumin and bisdemethoxycurcumin (Joshi, Jain, & Sharma, 2009).

One can extract the turmeric oleoresin from dried rhizomes by Soxhlet extraction, sonication, and liquid–liquid extraction using acetone, methanol, ethanol, and isopropanol as organic solvents. It is also possible to obtain this resin by supercritical fluid extraction with  $\text{CO}_2$  (Braga, Leal, Carvalho, & Meireles, 2003; Braga, Moreschi, & Meireles, 2006; Euterpio, Cavaliere, Capriotti, & Crescenzi, 2011;

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Joshi et al., 2009). The turmeric oleoresin usually contains 30–45 g/100 g curcuminoids and 15–20 g/100 g volatile oil. Because this extraction generates a residue that consists predominantly of starch and fibers and may present residual levels of curcuminoids with antioxidant properties (Braga et al., 2003, 2006; Kuttigounder, Rao, & Bhattacharya, 2011), we propose that this residue be used to develop a biodegradable film. The resulting turmeric film could be an active packaging material the presence of curcuminoids confers the film an antioxidant character. Therefore, this study aimed to develop turmeric films from turmeric dye solvent extraction residue using the response surface methodology and multi-response analysis, to obtain the formulation that would afford the turmeric flour film with optimal mechanical properties and low water vapor permeability, and to evaluate the antioxidant property of these films.

## 2. Material and methods

### 2.1. Materials

The turmeric powder (*Curcuma longa* L) was furnished by the industry “Flores e Ervas” (Campinas, Brazil). The turmeric flour was obtained by milling and sieving (0.075 mm) the turmeric dye soxhlet extraction residue. The extraction followed the method proposed by Braga and Meireles (2007), using ethanol/isopropanol (1:1) (Synth - São Paulo, Brazil).

Sorbitol, used as plasticizer, was purchased from Sigma–Aldrich (São Paulo, Brazil).

### 2.2. Turmeric flour chemical analyses

The turmeric flour moisture, crude protein, and ash contents were analyzed according to standard AOAC methods (AOAC, 1997). The starch amylose content was determined using the colorimetric method of Juliano (1971). The lipids content was calculated by the method of Bligh and Dyer (1959). The cellulose, hemicellulose, and lignin content were determined according to the methodology described by Gouveia, Do Nascimento, & Soto-Maior (2009). The material was submitted to hydrolysis with H<sub>2</sub>SO<sub>4</sub> at 720 mL/L, to quantify carbohydrates, organic acid, furfural, and hydroxymethylfurfural by HPLC. The concentrations of cellulose and hemicellulose were calculated using the following conversion factors: cellulose (0.90 × glucose mass, 0.95 × cellobiose mass, 1.20 × HMF mass, 3.09 × formic acid mass); hemicellulose (0.88 × xylose mass, 0.88 × arabinose mass, 0.72 × acetic acid mass, 1.37 × furfural mass). Insoluble lignin was determined by the Klason method, and soluble lignin was determined by measuring the absorbance at 280 nm. All the analyses were performed in triplicate.

### 2.3. Film preparation

The turmeric flour films were prepared by casting. Initially, a 5 g/100 g suspension was prepared in deionized water and homogenized for 30 min in a magnetic stirrer (IKA MAC<sup>®</sup> C-HS7-Marconi, Piracicaba - Brazil). The pH of the solution was adjusted to 6.59, 7.0, 8.0, 9.0, or 9.41 using HCl 1 mol/L and NaOH 1 mol/L solution. Then, the suspension was heated at 78, 80, 85, 90, or 92 °C for 4 h while applying homogenization cycles at 12,000 rpm for 2 min at every hour using an ultra-turrax homogenizer (Ultracleaner 1400, Unique, Indaiatuba, Brazil). The plasticizer (30 g sorbitol/100 g flour) was added, and the mixture was heated for 20 min. In a preliminary test was determined that as the optimal concentration of sorbitol for turmeric flour film production with low solubility and high mechanical resistance. Then, the solution was sonicated

for 20 min, to remove bubbles. Subsequently, the solution was poured onto acrylic plates maintaining a weight of 0.15 g m<sup>-2</sup> and dried for 7 h in an oven with forced circulation (MA Q314M, Quimis, Piracicaba-Brazil), at 35 °C. Prior to characterization, all the films were preconditioned for at least 48 h in desiccators containing a saturated NaBr solution (58% RH).

### 2.4. Films properties

The mechanical tests were performed using a texture analyzer TA TX Plus (TA Instrument, England). The tensile strength (TS) and elongation at break (*E*) were obtained according to the ASTM D882-95 method (ASTM, 1995), taking an average of five determinations in each case. Sample films were cut into 2.54 cm wide strips with a length of at least 10 cm. The initial grip separation and the crosshead speed were set at 80 mm and 1.0 mm s<sup>-1</sup>, respectively. Young's modulus (YM) was calculated as the inclination of the initial linear portion of the stress versus strain curve using the software Texture Expert V.1.22 (SMS). The opacity was determined according to the Hunterlab method (1997), using a portable colorimeter MiniScan XE (Hunterlab). The solubility in water was calculated as the percentage of dry matter of the solubilized film after immersion for 24 h in water at 25 ± 2 °C (Gontard, Guilbert, & Cuq, 1992) as described by Tapia-Blácido et al. (2011). The moisture content in the films was also determined by drying the materials in an oven at 105 °C until constant weight (~24 h). The water vapor permeability (WVP) test was performed using a modified E96-95 ASTM Standard method (ASTM, 1995) at 25 ± 2 °C. Film samples were sealed over the circular opening of a permeation cell containing silica gel, and the cells were then placed in desiccators containing distilled water. After the samples had reached steady-state conditions (~20 h), the cell was weighed every 1 h, for 9 h, using an analytical scale. The WVP was calculated as  $WVP = w \cdot x / t \cdot A \Delta P$ , where *x* was the average thickness of the films, *A* was the permeation area (0.00196 m<sup>2</sup>),  $\Delta P$  was the difference between the partial pressure of the atmosphere over silica gel and over pure water (3.168 kPa, at 25 °C), and the term *w/t* was calculated by linear regression using data of weight gain as a function of time.

Solubility, WVP, moisture content, and opacity were analyzed in triplicate.

### 2.5. Scanning electron microscopy (SEM)

Film samples were maintained in a desiccator with silica gel for seven days and then fractured with liquid nitrogen, to investigate the samples cross-section. The samples were placed in aluminum holders and coated with gold by sputtering (Sputter Coater, model SCD050). A Microscope Scanning Electron model ZEISS EVO-50 under an accelerating voltage of 20 kV was used to analyze samples.

### 2.6. Optimal turmeric flour films antioxidant activity

The turmeric flour films antioxidant property was determined using two techniques: DPPH and HPLC. The curcuminoids present in the film were extracted using 2 mL of methanol for every 100 mg of the film, and the mixture was incubated for 3 h at room temperature. Next, 500 μL of the mixture supernatant (film/methanol) was added to 2 mL of the methanolic DPPH solution (0.06 mmol/L), and the resulting mixture was stirred for 30 min, at room temperature, and protected from light. The remaining DPPH was determined by absorbance at 517 nm using an HP Hewlett Packard 8453 spectrophotometer coupled to a microcomputer HP Vectra XA 51166. The control consisted of 500 μL of DPPH solution

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