Food Hydrocolloids 41 (2014) 10–18

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Characterization of molecular structural changes in pectin during juice cloud destabilization in frozen concentrated orange juice

Ashley L. Galant, Wilbur W. Widmer, Gary A. Luzio, Randall G. Cameron*

Citrus and Other Subtropical Products Research Unit, US Horticultural Research Laboratory, USDA-ARS, 2001 South Rock Road, Ft. Pierce, FL 34945, USA

ARTICLE INFO

Article history:
Received 25 September 2013
Accepted 12 March 2014
Available online 20 March 2014

Keywords:
Pectin
Juice cloud
Orange juice
Pectin methylesterase
Cloud loss
Citrus sinensis

ABSTRACT

Pectin comprises one of the major components of cloud material in citrus juices. Juice cloud is a complex mixture of polysaccharides, proteins and lower molecular weight compounds that are responsible for the turbid appearance of citrus juices. The stability of juice cloud depends on a number of factors, including pectin degree of methylation (DM) and the availability of sufficiently-sized, charged demethylated blocks, but detailed information on the precise relationship between cloud state and pectin architecture is limited. To address this gap, we have systematically treated commercial frozen concentrated orange juice (FCOJ) with pectin methylesterases extracted from orange pulp cells to mimic the aggregation of pectin that naturally occurs in the presence of calcium in unpasteurized or under pasteurized juice. We then assessed the pectin structural and functional properties, including juice optical density (OD) and soluble sugar composition, DM, degree of blockiness (DB), degree of absolute blockiness (DB_{abs}) and rheological capacity from juice in various states of cloud loss. A strong positive correlation ($[r] = 0.916$) was observed between juice OD and DM, while a strong negative correlation ($[r] = -0.914$) was observed between OD and DB_{abs}. Most critically, a reduction of average DM from 74.7% to 68.3% in the course of cloud loss resulted in the formation of rheologically active pectin.

Published by Elsevier Ltd.

1. Introduction

In orange juice, cloud is responsible for imparting desirable texture, color, and flavor, as well as consumer-expected turbidity. Cloud is a component of juice distinct from pulp; while pulp tends to settle in juice, cloud forms a suspension of particles ranging from 0.4 to 5 μm in size (Klavons, Bennett, & Vannier, 1994). Smaller particles, typically those below 2 μm, form more stable suspensions than large particles, and if particle sizes become too large, cloud precipitation, known generally in the literature as cloud loss, can occur (Corredig, Kerr, & Wicker, 2001).

Orange juice cloud is comprised primarily of pectin, protein, cellulose, hemicellulose, some lipids, and small quantities of entangled bioflavonoids. Of these components pectin and protein are the most abundant, accounting for approximately one third and one half of the insoluble material respectively (Baker & Bruemmer, 1969; Braddock, 1999; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007; Scott, Kew, & Veldhuis, 1965). Citrus pectin is a complex molecule: it contains a homogalacturonan (HG) backbone of α-(1-4)-linked galacturonic acid (GalA) residues – some portion of which may be methylesterified – interspersed with regions containing repeating rhamnogalacturonan dimers (RGI) (Coenen, Bakx, Verhoef, Schols, & Voragen, 2007; Yapo, Lerouge, Thibault, & Ralet, 2007). The rhamnose moieties of RGI regions may be decorated with variably-sized arabinans or galactans (Doco, Williams, Vidal, & Pellerin, 1997). The degree to which pectin HG is methylated (degree of methylation, DM), as well as the pattern in which methylation occurs, plays a dominant role in determining the functional behavior of the molecule (Cameron, Luzio, Goodner, & Williams, 2008; Tanhatan-Nasseri, Crepeau, Thibault, & Ralet, 2011). Blocks of six or more contiguous demethylated GalA residues are required for formation of ionic "sandwiches" of pectin molecules and calcium ions (Liners, Thibault, & Cutsem, 1992; Luzio & Cameron, 2008); commonly referred to as the egg-box model (Powell, Morris, Gidley, & Rees, 1982). These demethylated blocks are formed through the activity of pectin methylesterases (PMEs; EC 3.1.1.11), four of which can be found naturally in citrus juice (Cameron, Baker, & Grohmann, 1998). When pectin networks form, the suspended material present in the citrus juice separates from the liquid phase, leaving behind a yellowish, unappetizing serum and the resulting precipitate (Ackerley, Corredig, & Wicker, 2002; Rouse, 1949). This precipitation represents the end-state of juice

* Corresponding author. Tel.: +1 772 462 5856; fax: +1 772 462 5986.
E-mail address: Randall.Cameron@ars.usda.gov (R.G. Cameron).

http://dx.doi.org/10.1016/j.foodhyd.2014.03.013
0268-005X/Published by Elsevier Ltd.

clarification however the initial stages of cloud loss are not readily visible to the unaided eye. For instance, Ellerbee and Wicker (2011) and Croak and Corredig (2006) reported a shift in cloud particle size in juice containing native PME activity from 2–50 μm to 2–100 μm without a corresponding change in % transmittance. Likewise, Croak and Corredig (2006) reported that the apparent diameter of juice cloud particles began to increase almost immediately following addition of PME, but remained below the 2 μm stability threshold for all treatments during the 30 min observation period. The longer the factors responsible for cloud loss are allowed to persist in juice, the more readily apparent the effects become. Cameron et al. (1998) observed an increase in settling pulp corresponding to a reduction in juice cloud absorbance within three days following addition of citrus PMEs isozymes to FCOJ samples; within ten days the juice was completely clarified. Krop (1974) likewise observed clarification of reconstituted juice treated with PME within two days, and the corresponding release of approximately 80% of the saponifiable GalA methyl groups after a total of 12 days. From the above referenced studies on the phenomenon of juice cloud loss it is obvious that calcium is naturally present in sufficient concentration in commercial orange juices to allow for the cross-linking of sufficiently sensitized pectin, though the minimal structural modifications and their relationship to calcium concentration have not been determined. The United States Department of Agriculture reports a value of 11 mg calcium per 100 g chilled orange juice (includes from concentrate) and 140 mg per 100 g for calcium fortified orange juice (U.S. Department of Agriculture, 2013).

From an industry perspective, issues of juice cloud and cloud loss present unique challenges and difficulties. Because cloud loss eliminates juice marketability and thus has a negative economic impact on juice producers and associated downstream industries, it is important that the critical structural details of cloud and its incorporated pectin be fully assessed.

From the antecedently illustrated studies and many others a macroscopic image of juice cloud and cloud stability has been documented; comparatively less is known however about the nanostructural changes pectin undergoes during cloud loss. Previously, we characterized the composition, macromolecular properties, and architecture of pectin from *Citrus sinensis* FCOJ (Galant, Luzio, Widmer, & Cameron, 2014). We now report on the changes to pectin molecular structure, including DM, DB, and DB_{abs} for pectin isolated from the same FCOJ at various stages of cloud loss.

2. Materials and methods

2.1. Materials

Frozen concentrated orange juice was provided by Nestlé Professional Vitality (Tampa, FL). *C. sinensis* cultivar 'Pineapple' pulp cells were a generous gift from Don Gillette and Louis-Dreyfus Citrus Inc. Polygalacturonases (EPG-M1, EPG-M2) were purchased from Megazyme (Wicklow, Ireland). Pectinase (P2166) and proteinase K were purchased from Sigma Aldrich (St. Louis, MO), while Rapidase ADEX-P was purchased from DSM Food Specialties (Delft, The Netherlands). Celluclast was purchased from Novozymes (Hellerup, Denmark).

2.2. Methods

2.2.1. PME purification

Four liters of orange pulp cells were diluted into two volumes of Buffer A (20 mM Tris, pH 8.0; 50 mM NaCl) and mixed for 1 h at 4 °C (Savary et al., 2010). The resulting slurry was filtered through four layers of miracloth, and the solids were centrifuged at 12,000 × g, 4 °C for 30 m. The supernatant from both the filtration and centrifugation were discarded after confirmation that it did not contain PME activity (see below). The wash, filtration, and centrifugation were repeated a second time, and again the solids were retained. Next, the pulp cell solids were diluted into 2 volumes of Buffer B (20 mM Tris, pH 8.0; 0.5 M NaCl), and stirred overnight at 4 °C to extract the PME. The slurry was filtered through 4 layers of miracloth and then centrifuged at 12,000 × g, 4 °C for 30 m. The solids were discarded once it was confirmed that most of the PME activity had migrated to the supernatant fraction. An AkroPak 500 (Pall, Port Washington, NY) in line with a Tangential Flow Filtration system (Pall, Port Washington, NY) containing a 30 kDa MWCO cartridge was pre-equilibrated with Buffer B, and used to concentrate the supernatant to ½ volume (approximately 2 L in this case). The permeate was discarded once the absence of PME activity was confirmed. The retentate was then diluted into 2.5 volumes of Buffer C (20 mM Tris, pH 7.5; 0.2 M NaCl). Diethylaminoethanol (DEAE, 320 g) was equilibrated with Buffer C, decanted, and added to the retentate; the resulting slurry was allowed to stir overnight at 4 °C. The next morning, the slurry was decanted into a sintered glass funnel, and the flow-through was collected using gravity filtration. The DEAE was washed twice with Buffer C, and the flow-through was pooled. Tangential flow filtration, after pre-incubation with Buffer C, was used to concentrate the flow-through down to 1.25 L, and the PME activity in the retentate was determined quantitatively by titrating 25 μL of retentate solution against 0.02 M LiOH in the presence of 2% 94% DM pectin. PME activity or the absence thereof was verified qualitatively throughout the purification by adding 30 μL of the fraction to be tested to 100 μL of indicator (0.05% Bromothymol Blue, pH 8.0; 0.2 M NaCl; 0.1% 94% DM pectin) and observing the color change after 1 min.

2.2.2. FCOJ demethylation

To the juice concentrate, three volumes of water, as well as lithium azide (LiN_3) to 0.02% (w/v) and potassium meta-bisulfate to 0.43% (w/v) were first added. The solution was brought to 30 °C in a stirring water-jacketed vessel (Wilmad-LabGlass, Vineland, NJ) and 3104 Units of *C. sinensis* 'Pineapple' PME activity was added per 0.5 L of pre-dilution juice concentrate. 10 mL aliquots of juice were collected periodically (every 2 h initially and then every h until an OD in the targeted range was reached) and the degree of cloud loss was determined by centrifuging the solution for 10 min at 360 × g in a centrifuge (International Equipment Company, Universal Model) with a swinging bucket rotor, and measuring the OD of the resulting supernatant at 660 nm on a UV-2401 PC spectrophotometer (Shimadzu) using distilled water as a blank. Once the desired OD was reached, the pH of the juice was adjusted to 1.8 with nitric acid, and the temperature was adjusted to 70 °C for a period of 3 h. At the conclusion of the incubation, the pH of the juice was adjusted to 2.2 with potassium hydroxide, and the solution was drained into 3 volumes of chilled isopropyl alcohol (IPA).

A second, smaller aliquot of FCOJ was prepared in the same manner for simultaneous demethylation and rheological analysis.

2.2.3. Pectin extraction

The procedure utilized for pectin extraction is outlined in Fig. 1. After at least 16 h at 4 °C, the juice in IPA was centrifuged for 30 min at 12,000 × g, 4 °C. The resulting supernatant was discarded, while the pellets were resuspended in two volumes of water. Proteinase K, 0.3 μg/mL, was added to the suspension, and it was allowed to stir overnight at 37 °C. After the incubation, the suspension was centrifuged for 30 min at 12,000 × g, 20 °C, and the supernatant (soluble fraction) and pellet (insoluble fraction) were separated. The supernatant was dialyzed (Spectra/Por MWCO: 6000–8000 Da) against two volumes of distilled water containing 0.02%