Nature of the Pectin Constituent of Commercial Lemon Juice Cloud

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The cloud pectin content of two commercial lemon juice concentrates was 4.0 and 4.2% of the original cloud, respectively. The cloud pectin was solubilized, to varying degrees, in 6% citric acid, pH 7.0; in 10 M urea–6% citric acid, pH 2.5; by hydrolysis of the cloud protein with protease; and in 0.1% sodium oxalate, pH 4.5. These solubilities, for the most part, are irreversible. Between half and two-thirds of the cloud pectin appears to be soluble pectin that is physically entrapped within a matrix of cloud protein. A small amount of the cloud pectin is classified as protopectin. The remainder of the cloud pectin is mainly in the form of calcium pectate. It is highly unlikely that lemon cloud pectin exists as a complex with protein via an electrostatic interaction.

The turbidity of citrus juices is attributed to a fine suspension of particles known as cloud. Approximately half the total weight of the cloud is due to high molecular weight polymeric materials. These are protein, pectin, hemicellulose and cellulose (Sinclair, 1984, p 411). The remaining cloud is comprised of many low molecular weight materials such as hesperidin crystals and liposome vesicles. The nature of the protein constituent of commercial lemon juice cloud as well as a summary of research on the composition and properties of the cloud has recently been published (Klavons and Bennett, 1985).

Citrus juice cloud represents a dynamic system whose properties are best understood via a comprehensive study of all its components and their interactions with one another. We have undertaken a detailed investigation of the properties of lemon juice cloud pectin and present evidence of its interaction with cloud protein.

Pectin is a polymer rich in α-1,4-linked D-galacturonic acid but also contains neutral sugars such as L-arabinose, D-galactose, and L-rhamnose. A portion of the galacturonic acid present is esterified with methanol. This portion is known as the degree of esterification of the pectin and is expressed as a percentage of the total galacturonic acid content. Pectin is a ubiquitous polysaccharide in the albedo (mesocarp) and in the edible portion (endocarp) of citrus fruits and primarily serves a structural role.

EXPERIMENTAL SECTION

Sources of Juice Samples. Two commercial lemon juice concentrates were used to prepare single-strength samples, A and B. A is from a pasteurized, 45.9° Brix concentrate (Ventura Coastal Corp.), produced by high-temperature evaporation. It was stored frozen at −8 °C. B is from a pasteurized, 61.9° Brix concentrate (Sunkist), produced and stored as A.

Preparation of Single-Strength Juice Samples. Samples A and B were prepared by reconstitution of the concentrates to a final 8.2° Brix (an arbitrary value for single-strength lemon juice) with deionized water. They contained no visible particles (pulp), after reconstitution, and filtration through a 210-μm mesh resulted in no visible residue on the filter.

Isolation and Extraction of Cloud. The juice (10 mL) was centrifuged at 27000g for 15 min. This produced a supernatant whose optical density (OD) at 600 nm was less than 0.05 (about 1% of the original turbidity of the juice). The supernatant was decanted, and the cloud pellet was redispersed in 10 mL of deionized water by vortexing. The suspension was centrifuged as before and the supernatant decanted. This washing process was repeated once more. The washed cloud was then extracted for 1 h with 10 mL of methanol with occasional vortexing. This extraction removed liposomes and most low molecular weight organic constituents and also dehydrated the sample. The suspension was centrifuged as done previously and the supernatant decanted. The cloud (which was now white) was dried under a stream of nitrogen. The dried cloud was further extracted for at least 30 min with 0.5 mL of dimethyl sulfoxide (Me_2SO), followed by addition of 20 mL of isopropyl alcohol to the Me_2SO–cloud mixture. Precipitation of macromolecular components was allowed to occur for at least 1 h, the suspension was centrifuged, and the pellet was dried as done previously. The Me_2SO extraction was necessary to remove the flavanone glycoside hesperidin, which along with other small molecular weight organic constituents tends to interfere with pectin analysis. The washed and dried cloud samples were then used for subsequent analysis. All reagents used were of the highest purity obtainable.

Total Cloud Weight. Total cloud weight was obtained by freeze drying the cloud to constant weight.

Solubilization of Cloud Pectin at pH 7.0 and Subsequent Reversal to Initial Juice Conditions. Samples of washed and dried cloud were treated with 6% citric acid, pH 7.0 (buffered with KOH), overnight at 4 °C, with stirring. The fraction of the cloud that became soluble under these conditions was removed from the still insoluble fraction by centrifugation at 27000g for 15 min. The remaining insoluble fraction was washed with 10 mL of distilled water and centrifuged as done previously. This washing process was repeated once more. The insoluble fraction was then dehydrated by extracting with 10 mL of methanol and subsequently dried under a stream of nitrogen. The pH 7.0 solubilized fraction was dialyzed extensively vs. 6% citric acid, pH 2.5 (the natural environment of lemon juice), at 4 °C in Spectrapor membrane tubing of molecular weight cutoff 2000, obtained from Spectrum Medical Industries, Los Angeles, CA. The reprecipitated material was isolated via centrifugation, washed twice, dehydrated, and dried as done previously.

Solubilization of Cloud Pectin in 10 M Urea–6% Citric Acid, pH 2.5, and Subsequent Reversal to Initial Juice Conditions. Samples of washed and dried cloud were treated with 10 M urea–6% citric acid, pH 2.5 (hereafter referred to as urea–citrate), overnight at 4 °C, with stirring. The soluble and insoluble fractions were separated via centrifugation (at 20 °C, to avoid precipitation of the urea). The insoluble fraction was treated as done previously. Urea was removed from the urea–citrate solubilized fraction by dialysis vs. 6% citric acid, pH 2.5,

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Table I. Total Cloud and Pectin Weights[a]

sample	A	B
total cloud weight	245 ± 12	231 ± 8
total cloud pectin	9.81 ± 0.85	9.78 ± 0.90
total cloud pectin soluble in 6% citric acid, pH 7.0	4.98 ± 0.91	5.16 ± 1.00
soluble cloud pectin in 6% citric acid, pH 7.0, that reprecipitates upon dialysis vs. 6% citric acid, pH 2.5	0.20 ± 0.02	0.14 ± 0.02
total cloud pectin soluble in 10 M urea–6% citric acid, pH 2.5	7.64 ± 0.90	9.06 ± 1.17
soluble cloud pectin in 10 M urea–6% citric acid, pH 2.5, that reprecipitates upon dialysis vs. 6% citric acid, pH 2.5	1.61 ± 0.19	0.00 ± 0.00
total cloud pectin solubilized upon treatment of cloud with protease at pH 4.5	5.73 ± 0.91	6.55 ± 1.57
total cloud pectin solubilized upon treatment with protease at pH 7.0, 6% citric acid	6.97 ± 1.06	7.12 ± 1.09
total cloud pectin solubilized upon treatment with 0.1% sodium oxalate, pH 4.5	2.04 ± 1.32	2.76 ± 0.99

[a] Values given in milligrams/100 mL of single-strength lemon juice, 8.2° Brix, ± SEM (P = 0.05).

as done previously. The reprecipitated material was isolated via centrifugation and treated as done previously.

Solubilization of Cloud Pectin via Hydrolysis of the Cloud Protein with Protease. Samples of washed cloud were treated with 5 mL of Pronase (calcium free, pectinase free; Calbiochem-Behring Corp., La Jolla, CA), 2 mg/mL in 0.6% citric acid, pH 4.5 or 7.0, overnight followed by an additional 2–3 mg of solid Pronase for 1–2 h. The soluble and insoluble fractions were separated via centrifugation. The soluble fraction was discarded, as it remained contaminated with Pronase and autolyzed fragments thereof which make determination of pectin difficult. The insoluble fraction was treated as done previously.

Solubilization of Cloud Pectin in 0.1% Sodium Oxalate, pH 4.5. Samples of washed and dried cloud were treated with 10 mL of 0.1% sodium oxalate, pH 4.5, overnight at 4 °C, with stirring. The soluble and insoluble fractions were separated via centrifugation. The soluble fraction was discarded. The insoluble fraction was treated as done previously.

Assay Methods. Cloud precipitates were solubilized in appropriate volumes of 0.05 M potassium hydroxide. Pectin was determined by the method of McComb and McCready (1952), using galacturonic acid as a standard. The degree of esterification was determined by the method of Klavons and Bennett (1986).

RESULTS AND DISCUSSION

Research on the nature of the pectin constituent of commercial lemon juice cloud contributes to an understanding of the water-insoluble pectin fraction obtainable from citrus fruits. In order to study the properties of the cloud pectin we have attempted to solubilize it under a variety of conditions and to determine whether these solubilizations can be reversed, to yield the original insoluble pectin. The results of pectin solubilization at pH 7.0, in urea–citrate, following protease treatment, and in sodium oxalate are summarized in Table I. The degree of methylation was determined on total cloud pectin and on the insoluble pectin remaining after proteolysis. The results were 64.7 ± 3.5% and 64.8 ± 2.5% (n = 4, P = 0.05), respectively.

The concept of a conformational change in cloud protein upon treatment at pH 7.0 and with the chaotropic agent urea has been proposed earlier (Klavons and Bennett, 1985). We stated that no cloud protein becomes soluble at pH 7.0 but that it all solubilizes in urea–citrate. There are at least three plausible reasons for the solubility of approximately half of the cloud pectin at pH 7.0: (1) Half of the cloud pectin exists as a pectin–protein complex that dissociates at pH 7.0. (2) Half of the cloud pectin is inherently insoluble at pH 2.5 and soluble at pH 7.0 but is not associated with cloud protein (3) Half of the cloud pectin is soluble pectin that has become physically entrapped within the matrix of cloud protein. The greatest solubilization of cloud pectin was accomplished with urea–citrate, that being 77.9 and 92.6% of the original cloud pectin in samples A and B, respectively. This could imply that only one of the three explanations described above is correct and that urea–citrate is just more efficient in solubilizing this type of pectin or that more than one reason exists for the pectin solubility.

The failure of the pH 7.0 solubilized pectin to reprecipitate when dialyzed against 6% citric acid, pH 2.5, indicates the absence of a pectin–protein complex that dissociates at pH 7.0. The dialysis of the urea–citrate-soluble pectin against 6% citric acid, pH 2.5, yielded a small amount of reprecipitated material (16.4% of the original cloud pectin) in sample A but failed to produce any in sample B. Pectin–protein complexes have been demonstrated previously in tomato products (Takada and Nelson, 1983) and in model systems (Imeson et al., 1977). Both papers discuss electrostatic forces between pectin and protein as being responsible for such interactions. In view of the high ionic strength (approximately 6% citric acid) in lemon juice and the data from the reversal of the pH 7.0 and urea–citrate-soluble pectin, it is unlikely that pectin–protein complexes exist in commercial lemon juice cloud. It is also unlikely that pectin is complexed to a low molecular weight organic component, which might become lost upon dialysis, as the two prior organic extractions (with methanol and with Me_2SO/isopropyl alcohol) would have removed them. The washed and extracted cloud showed no solubilized pectin in 6% citric acid, pH 2.5, resulting from such an association.

Ben-Shalom et al. (1985) have demonstrated a complex between pectin and hesperidin in a model system. They state, however, that this complex remains stable, without flocculation, only when the pectin is in the molecular weight range of 76 000–84 000. Our group, using two commercially available citrus pectins covering a broad molecular weight range, was unable to cause a complex to form, but rather we only observed the precipitation of hesperidin, which was free of pectin. Hesperidin is highly insoluble in aqueous systems, and the presence of pectin is not required for its crystallization. Due to the heterogeneous nature of citrus cloud pectin, in molecular weight, composition, etc., it is highly unlikely that such a complex occurs in processed juice to any appreciable extent.

The treatment of the cloud with protease resulted in a 58.4 and 67.0% solubilization of cloud pectin in samples A and B, respectively. This treatment hydrolyzed approximately 95% of the cloud protein. Treatment of control cloud samples with 0.6% citric acid, pH 4.5, showed no solubilization of pectin, and the protease was shown not to contain a pectinase contaminant. This result indicates a clear association of this part of the cloud pectin with the cloud protein.

On the basis of the solubilization studies of the cloud pectin thus described, between half and two-thirds of the cloud pectin may be accounted for as soluble pectin that is physically entrapped within the protein matrix. The

cloud pectin solubilized at pH 7.0 is believed to be due to a conformational change in the cloud protein during its transition from pH 2.5 to pH 7.0, in which the protein remains insoluble (Klavons and Bennett, 1985) and soluble, entrapped pectin is released. This process does not seem to be as efficient as the protease release of soluble pectin, but these two processes appear to be releasing the same type of pectin. When these processes were performed simultaneously (protease treatment at pH 7.0), on the same sample, the soluble pectin released was essentially the same as if the cloud had been treated with pronase alone (Table I).

The pectin that remained insoluble when treated with urea–citrate, 22.1 and 7.4% for samples A and B, respectively, may be classified as "protopectin". Protopectin is regarded as an insoluble precursor to water-soluble pectin and is found in the cell walls (Sinclair, 1984, p 400). Due to the rigorous conditions of juice processing, much of this cell wall material is found in the juice. The exact structure and composition of protopectin are unknown (Sinclair, 1984, p 400), but it has been suggested that protopectin may be composed of two continuous portions: one rich in rhamnose and interposed between blocks of α-1,4-linked D-galacturonic acid and another with a continuous chain of galacturonic acid with various neutral sugars present in side chains (Sinclair, 1984, p 361). The presence of these neutral sugars could account for the water insolubility of protopectin (Sinclair, 1984, p 361). It has been suggested that protopectin plays a role in water retention in fruit tissue and could thus be important in establishing and maintaining fruit consistancy (Sinclair, 1984, p 373).

The remainder of the cloud pectin may then be classified as being inherently insoluble and distinct, that is, not associated with other cloud constituents. Pectin tends to aggregate via polyvalent ions such as calcium, through hydrogen bonding and through other mechanisms (Nelson, 1977; Fishman et al., 1984). Calcium pectate and pectin containing interchain and intrachain hydrogen bonds would be included in this class. The inherently insoluble pectin may be quantitatively represented as that pectin that is solubilized in urea–citrate minus the pectin that is solubilized via protease treatment. Calcium pectate content was determined by its solubilization in sodium oxalate and accounts for this difference.

The degrees of esterification of the total cloud pectin and of that portion of the cloud pectin that may be physically entrapped soluble pectin were not significantly different, and their compositional and/or structural differences remain to be shown.

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Registry No. Pectin, 9000-69-5; citric acid, 77-92-9; urea, 57-13-6; protease, 9001-92-7; sodium oxalate, 62-76-0; protopectin, 9012-27-5; calcium pectate, 12672-40-1.

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Quantitative Analysis of Orange Juice Flavor Volatiles by Direct-Injection Gas Chromatography

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A distillate was prepared from fresh Valencia or Temple orange juice that possessed all of the fresh orange aroma of the juice. This distillate was analyzed by direct injection into a capillary gas chromatographic column. Of 24 volatile constituents identified, 21 of these were quantitatively measured. Temple orange juice contained lesser quantities of most of these components than did Valencia juice. These values were compared to quantitative estimates of volatile constituents reported earlier in fresh orange juice. The technique can be used to study changes in volatile flavor components due to processing and storage of orange juice products.

The popularity of orange flavor has caused processed orange juice to become the major fruit juice consumed in the United States (Gunter, 1985). Over 200 million boxes of oranges are harvested annually in the United States, making it the largest fruit crop in the country. Extensive research studies have been conducted during the past 30 years in an effort to determine the identities and quantities of volatile components that are important contributors to natural orange flavor and aroma. Such knowledge would be useful for determining flavor changes that occur during processing and storage of orange juice products.

Studies of volatile orange flavor and aroma constituents have historically concentrated on separation and isolation of these compounds from cold-pressed and distilled orange peel oils and from aqueous orange essence, which is the

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