Particle Size Distribution of Orange Juice Cloud after Addition of Sensitized Pectin

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The effect of the addition of commercial pectin to orange juice that was de-esterified by alkaline treatment or pectinmethylesterase was evaluated. Pectin at various degrees of esterification (DE) was added to reconstituted frozen orange juice concentrate, and the extent of cloud loss was determined after centrifugation. Of the five pectin treatments, percent transmittance (% T) of juice remained <5%, except for the pectin treatment with a residual methoxyl content (% DE) of <5%. Addition of 100−600 ppm of 5% DE pectin to juice resulted in % T between 36 and 73%. Despite the absence of clarification, increases in cloud particle size were observed and depended on the % DE, the amount of pectin added, and the method used to modify the pectins.

Keywords: *Clarification; de-esterification; laser diffraction; cloud stability; pectinmethylesterase; saponification*

INTRODUCTION

Freshly extracted juice contains a polydisperse distribution of particle sizes, from pulp fragments to particles <2 μm in diameter (*1*). Pulp fragments and large particles tend to settle by gravity, whereas those with a diameter of ∼2 μm produce a stable cloud (*2*, *3*). Cloud particles contain most of the characteristic flavor of the juice, ∼50% protein and ∼5% pectin (*3*−*5*). Considerable effort has been put forth in understanding the mechanisms of stabilization of cloud particles in juice and the means of preventing cloud loss during juice storage. Pectin demethylation by pectinmethylesterase (PME) and the subsequent aggregation of pectin as calcium pectate are considered the main cause of cloud destabilization (*3*). Inactivation of PME or degradation of the endogenous pectin can stabilize citrus cloud. Addition of commercial pectinase to orange juice was as an effective means to stabilize cloud (*6*). Other authors have suggested the addition of PME inhibitors to citrus juice (*7*). In general, during processing, PME is inactivated by thermal treatment of the juice, but this process causes profound changes in several quality parameters (*8*).

Citrus pectins contain polygalacturonic acid chains, and 70−80% of the residues are methyl esterified (*9*). Pectins are polydisperse in molecular weight distribution and the presence of neutral sugar side chains attached to the galacturonic acid backbone. In addition, pectins vary in the total charge and the charge distribution (*10*, *11*). These molecular differences have great impact on the gelling and aggregation behavior of pectins. Whereas pectins containing a high number of methyl esters form gels in the presence of high soluble solids and low pH, pectin gelation with a lower number of methyl esters (low methoxyl pectin) depends on the degree of esterification (% DE), the distribution of charges on the backbone, the average molecular weight of the sample, the ionic strength, the pH, and the presence of cations (*9*, *12*, *13*). Gelation behavior differences are reported for pectins containing similar % DE but prepared chemically versus enzymatically (*12*, *14*). In citrus juice, PME de-esterifies pectins in a blockwise fashion (*15*). Analysis of modified pectins by ion exchange chromatography has demonstrated that fungal PME causes a distribution of charges on the pectin chain different from that of pectin de-esterified by plant PME (*16*).

When a model system containing pectin−hesperidin particles was employed to study juice flocculation by calcium bridging, a critical % DE (<36%) was necessary for aggregation to occur (*17*). Krop and Pilnik (*18*) reported that clarification occurred if ∼20% of the methoxyl groups in pectin were de-esterified by PME. When the clarification potential of commercial pectins with various methoxyl contents was tested in aged, depectinized orange juice, cloud was lost more effectively with pectins that were sensitized by crude extracts of PME than by alkali saponified pectin (*19*). About 40% DE was sufficient to achieve a 60% cloud loss, if commercial pectin was sensitized by PME. When ion exchange chromatography was used to separate pectin and fractions with a well-defined % DE were used to precipitate cloud, no changes occurred with pectin fractions with a DE value as low as 21% and only pectin with <14% DE caused cloud loss (*10*).

Clarification of juice is influenced by the complexity of the PME substrate, pectin, in orange juice. Differences in specific activity, affinity for pectin substrate, and mechanism of de-esterification by PME isozymes are other factors. Furthermore, the polydispersity of distribution of cloud particles in juice confound studies that investigate the mechanism of cloud loss. This work describes a study on the effect of pectins sensitized to different % DE by partially purified PME and alkaline saponification on orange juice clarification by observing the changes in size distribution of orange juice cloud.

MATERIALS AND METHODS

Sample Preparation. The commercial high-methoxyl pectin obtained from Citrus Colloids (Hereford, U.K.) had a uronic acid content of 80%, 72% DE, and a molecular weight average of 212000 Da. The enzyme PME was purified from grapefruit pulp by selective extraction with 1 M NaCl, and purification

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was carried out with ion exchange chromatography as described earlier (*20*). The resulting PME fraction had a measured specific activity of 900 units/mg and was stable after heating at 70 °C for 5 min.

Pectin (2% w/v) was dissolved in 0.1 M NaCl and stored overnight at 4 °C. Aliquots (250 mL) of pectin solution were subjected to alkali saponification at pH 10 (*19*). Such treatment resulted in a statistical distribution of both free carboxyl and ester groups in the pectic chain (*12*). Pectin with a blocklike distribution was obtained by PME demethoxylation at pH 7.5. In all cases the pH was kept constant throughout the reaction with 0.5 M NaOH. The extent of de-esterification was controlled by the amount of NaOH consumed during the reaction. Demethoxylation was terminated by lowering the pH to ~5 with 1 M HCl, precipitating the pectin solution with 95% ethanol, and boiling for 10 min. The pectin was cooled, washed with ethanol, and freeze-dried. A control pectin was precipitated by ethanol and freeze-dried. The amount of galacturonic acid present in the pectin preparation was determined according to the method of Ahmed and Labavitch (*21*). The degree of esterification was calculated by determining the methoxyl percent according to the method of Woods and Siddiqui (*22*) as modified by Hudson and Buescher (*23*).

Cloud Stability Studies. Orange juice (Citrus World, Lake Wales, FL) was reconstituted from PME-negative frozen concentrate at 12 °Brix (pH 4.0) and centrifuged at 3000*g* for 10 min at 4 °C with a Sorvall RC-5B centrifuge (Dupont Instruments, Doraville, GA). The supernatant was separated carefully from the sedimented pulp and stored at 4 °C. Samples of sensitized pectin were prepared by dissolving freeze-dried pectin in deionized water at a concentration of 1% (w/v). Different aliquots of this solution were added to 30 mL of juice, to achieve concentrations from 100 to 600 ppm. To maintain a constant volume, appropriate amounts of deionized water were also added to the samples. Juice samples with added pectins were equilibrated overnight at 4 °C and then centrifuged at 3000*g* for 10 min. The turbidity of the supernatant fractions was determined at 650 nm. Particle size distribution was determined by laser diffraction using a Mastersizer S (Malvern Inst., MA), using 1.73 and 1.33 as the refractive indices of cloud and dispersed phase, respectively, and 0.1 as the absorption index for cloud particles. Samples (10 mL) were introduced into the small volume presentation unit of the instrument, which already contained ~120 mL of deionized water. In this unit, the sample was pumped through the optical cell by a stirrer that rotates at ~2000 rpm. Size distributions (volume fractions against particle size) were calculated, and the weight-average sizes were expressed as $D_{3,2} = \sum_i n_i d_i^3 / \sum_i n_i d_i^2$ and $D_{4,3} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$, where n_i is the number of particles of diameter d_i.

Statistical Analysis. Results from duplicate samples were analyzed by using the general linear model procedure (SAS Institute, version 6.12). The independent variables considered in the statistical analysis of variance were pectin treatment and pectin concentration. Least-squares means were also calculated, and differences were considered to be significant for $p < 0.05$ according to a protected LSD test.

RESULTS AND DISCUSSION

The percent methoxyl content and % DE of the five pectins used in this study are indicated in Table 1. Pectin 1 contained 72% DE and was subjected only to alcohol precipitation and freeze-dried. Samples 2 and 3 had an equivalent number of methoxyl groups, corresponding to a 42% DE, and they were sensitized by PME (*2*) or alkali treatment (*3*). Samples 4 and 5 were pectins treated with NaOH and were characterized by lower DE values (21 and <5%, respectively). After addition of the different sensitized pectins to orange juice, only juices containing pectin 5 showed cloud loss. Table 1 summarizes the results obtained from the clarification study. The addition of pectins 1−4 to juice did not change the apparent stability of the samples, in that the % T was not different from that of samples with no pectin added. At all concentrations of modified pectins 1−4, the % T remained well under 10%. Significant differences were observed in % T of samples containing pectin 5. By analysis of variance of % T, it was also determined that clarification depended on the amount of pectin ($p < 0.05$). As shown in Table 2, clarification did not occur in juices with pectin 2 (PME-sensitized, 42% DE) nor with pectin 4 (alkali-sensitized, 21% DE). In contrast, Baker (*19*) showed that PME-sensitized pectins caused loss of cloud at a DE of ~40%. The difference may be related to the use of a crude or partially purified PME extract, differences in the composition, pH, pectin, or cloud composition of the juice, or differences in the commercial pectin between the two studies.

Table 1. Effect on Cloud Stability of Reconstituted Orange Juice by Pectinmethylesterase and Alkali-Saponified Pectin[a,b]

	pectin		% transmittance (650 nm) at addition of				
	% CH_3	% DE	0 ppm	100 ppm	200 ppm	400 ppm	600 ppm
1, control	9.1	72	3.6[a]	4.0[a]	4.1[a]	3.5[a]	3.5[a]
2, PME	5.3	42	3.6[a]	4.7[a]	5.0[a]	3.5[a]	3.2[a]
3, NaOH	5.3	42	3.6[a]	3.1[a]	3.5[a]	3.4[a]	3.2[a]
4, NaOH	2.6	21	3.6[a]	5.0[a]	5.1[a]	4.4[a]	4.1[a]
5, NaOH	0	<5	3.6[a]	35.7[b]	62.5[c]	71.5[d]	72.6[d]

[a] Transmittance values of juices after centrifugation at 3000*g* for 10 min are reported as averages of two replicate samples. [b] For the same pectin concentration, treatment means followed by different letters are significantly different ($p < 0.05$).

Table 2. Effect of Different Concentrations of Pectin on the Average Diameter of the Juice Cloud Particles[a]

	particle size, μm, at addition of				
	0 ppm	100 ppm	200 ppm	400 ppm	600 ppm
			$D_{3,2}$		
juice					
control	0.89				
1		1.20[a]	1.30[a]	1.66[a,c]	2.73[a]
2		1.85[b]	2.60[a]	4.69[b]	6.46[b]
3		0.92[a]	0.92[a]	1.13[a]	1.49[a]
4		1.00[a]	1.05[a]	1.55[a]	1.63[a]
5		4.93[c]	12.71[b]	28.83[c]	38.9[c]
			$D_{4,3}$		
juice					
control	1.55				
1		63.26[a]	47.56[a]	71.03[a]	147.20[a]
2		53.37[a]	79.56[b]	124.27[b]	143.86[a]
3		3.36[b]	4.62[c]	20.82[c]	45.18[b]
4		14.17[a,b]	13.7[c]	64.46[a,c]	47.14[b]
5		14.00[a,b]	22.05[d]	41.3[c]	82.77[c]

[a] $D_{3,2}$ and $D_{4,3}$ are averages of two replicate samples; $D_{3,2} = \sum_i n_i d_i^3 / \sum_i n_i d_i^2$, and $D_{4,3} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$ where n_i is the number of particles of diameter d_i. For each concentration level, values with different letters indicate significant differences between treatments ($p < 0.05$).

Despite the apparent stability of the juices containing pectins 1−4, integrated light scattering measurements of juice cloud demonstrated that not only the % DE of pectin but also the pectin concentration affected juice cloud aggregation and particle size distribution. The data in Figure 1 depict the particle size distribution of juice after the addition of 100 ppm of differently sensitized pectins. Juice, with no pectin added, showed a monomodal distribution of particle sizes with an average diameter ($D_{3,2}$) of 0.9 μm. When pectin 5 was added to juice, the average cloud size increased (Figure 1A), showing a monomodal distribution of cloud particles with an average diameter ($D_{3,2}$) of 4.9 μm. Figure 1B shows the particle size distribution of juices containing pectin 1, 2, 3, or 4. Despite their apparent stability,

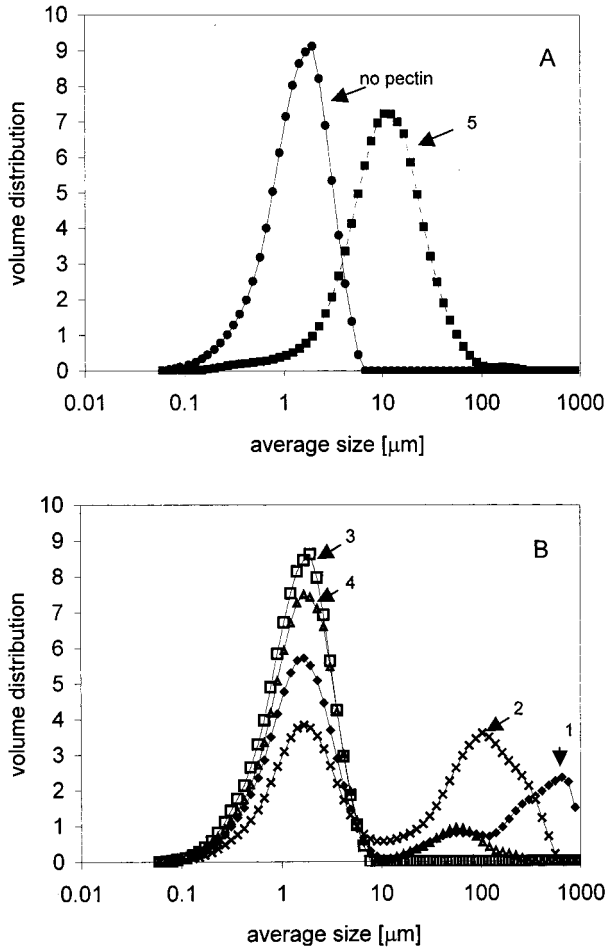

Figure 1. Particle size distribution of orange juice containing 100 ppm of sensitized pectin: (A) juices with no pectin added (no pectin) and juice with pectin 5 (5), <5% DE; (B) juices with pectin 1 (control), 2 (42% DE by PME), 3 (42% DE by alkali), or 4 (21% DE by alkali). Numbers on the curve denote pectin number.

based on % T measurements, all samples showed a certain amount of aggregation, with a small number of particles with diameter >10 μm. Only juice containing pectin 3 was characterized by a monomodal distribution comparable to that of untreated juice. It can be hypothesized that to maintain % T values as low as those indicated in Table 2, a large number of particles with diameters comparable to that of stable juice cloud must be present. In samples containing pectin 5, the % T was >30% and no particles <2 μm were measured. The shift in the cloud particle size of juice containing pectin 5 may have been caused by strong interactions between the highly charged pectin molecules and the cloud constituents. When these juice samples clarified, large visible aggregates formed. However, these aggregates were partly shear sensitive, because the flocculated material was reduced in size during mixing and dilution of the juice in the presentation unit of the instrument, and a monomodal distribution with an average size of 5 μm was measured. Samples with pectin 1 (nonsensitized pectin) added also contained large particles (>80 μm). This distribution showed a population of very large particles, different from that of the other juice treatments, no pectin added (Figure 1A) and pectin added juices (Figure 1A,B). When pectin 2 was added, a distinct bimodal distribution of particle sizes was observed, with a population of particles with a diameter equivalent to that of control cloud and a second population with sizes between 50 and 150 μm. The added pectin seemed to interact with the juice components, forming a second population of aggregated cloud. In contrast to the results reported for pectin 2 (42% DE by PME), when pectin 3 (42% DE by alkali) was added to juice, the particle size distribution was comparable to the monomodal distribution with no pectin added.

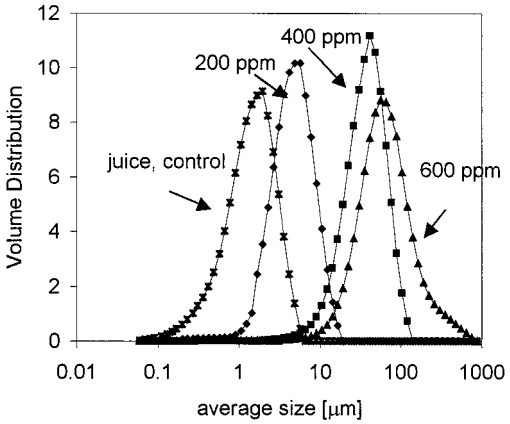

Figure 2. Particle size distribution of juices, as determined by integrated light scattering, as a function of different concentrations of pectin 5 (<5% DE by alkali).

Clarification of juice occurred when stable cloud showed aggregation by shifting the particle size distribution to larger diameters. Figure 2 illustrates the particle size distribution of juice with increasing concentrations of pectin 5. The average diameter increased significantly with increasing amount of pectin added. This confirmed the increase in the loss of cloud as measured by % T (Table 1) increased with the concentration of pectin added. Table 2 summarizes the average $D_{3,2}$ and $D_{4,3}$ of particles in juice containing sensitized pectins. At all concentrations up to 600 ppm, pectin 5 increased the average particle size of the juice cloud. As shown in Figure 1, cloud treated by PME-pectin (pectin 2) also showed significant differences in average sizes from cloud treated with NaOH pectin with a similar % DE. For all pectins added to juice, analysis of variance within one pectin showed that the average particle size was dependent on pectin concentration ($p < 0.05$). The differences in aggregation behavior of cloud treated with pectins 2 and 3 (PME-treated and alkali-treated pectin) were in agreement with previous reports (*19*). Results depicted in Figure 3 demonstrate the differences in cloud aggregation for juice samples after addition of pectins 2 and 3 as a function of pectin concentration. The cloud size distribution was bimodal in juice containing pectin 2 (PME-sensitized, Figure 3A). In contrast, less aggregation seemed to occur when pectin 3 (NaOH-treated) was added to juice (Figure 3B); in this case the size distribution of cloud particles was similar to that of control samples. Pectins sensitized with PME have a greater propensity to clarify juice when compared to alkali-sensitized pectin (*19*). These results show that PME-sensitized pectins result in larger particle size when added to juice than NaOH-sensitized pectins.

CONCLUSIONS

The determination of particle size by integrated light scattering allowed a detailed study of the aggregation occurring in orange juice after the addition of de-esterified pectin. The presence of calcium reactive pectin is fundamental for the clarification of orange juice (*3*).

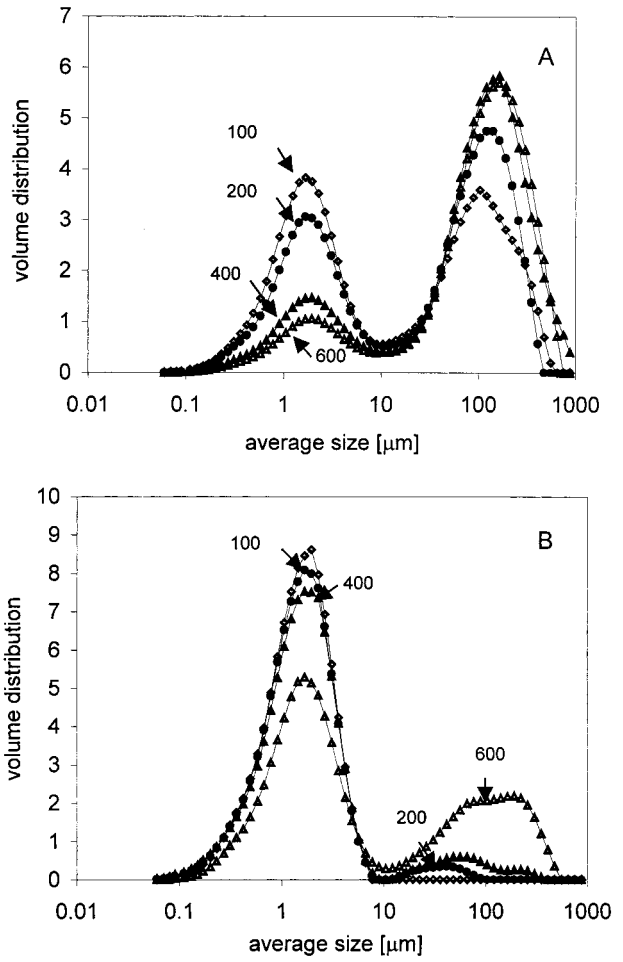

Figure 3. Particle size distribution of juices containing increasing amounts of (A) pectin 2 (42% DE by PME) and (B) pectin 3 (42% DE by alkali).

Integrated light scattering analysis of changes in cloud size demonstrated that strong interactions exist between charged pectin and cloud particles. For cloud loss to occur, most of the cloud particles must be aggregated as shown by their shift in particle size distribution to larger diameters. The aggregation is likely caused by bridging of charged pectins to cloud particles. Pectin fractions increase in heterogeneity after enzymatic treatment and retain the same level of homogeneity after chemical de-esterification (*12*, *14*, *16*). PME sequentially demethylates the methoxyl groups of pectin within a molecule (*15*), and some molecules may be demethylated more than others. In addition, the distribution of DE may play a role, creating more reactive sites and bridging at higher DE values. The enhanced bimodal distribution of particle size of PME-sensitized pectins in juice compared to alkali-sensitized pectins in juice is further evidence that PME-sensitized pectins are more reactive than alkali-modified pectin. Results presented herein demonstrate that a small population of sensitized pectins may play a more critical role in cloud loss than previously appreciated.

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