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Effect of Acidification on Carrot (*Daucus carota*) Juice Cloud Stability

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ABSTRACT: Effects of acidity on cloud stability in pasteurized carrot juice were examined over the pH range of 3.5−6.2. Cloud sedimentation, particle diameter, and ζ potential were measured at each pH condition to quantify juice cloud stability and clarification during 3 days of storage. Acidification below pH 4.9 resulted in a less negative ζ potential, an increased particle size, and an unstable cloud, leading to juice clarification. As the acidity increased, clarification occurred more rapidly and to a greater extent. Only a weak effect of ionic strength was observed when sodium salts were added to the juice, but the addition of calcium salts significantly reduced the cloud stability.

KEYWORDS: *Daucus carota, carrot juice, acidification, clarification, ζ potential, electrostatic, flocculation, pectin*

INTRODUCTION

The relatively high pH of carrot juice (pH ∼ 6) makes it unique among popular commercial juices, such as orange or apple juices, which have pH values below 4.5. The low-acid nature of carrot juice makes it more susceptible to spoilage and pathogenic organisms, which can be countered by acidification. This issue is of particular interest in fresh carrot juice products that require refrigeration. In addition, carrot juice can be subjected to lower pH conditions when it is mixed with fruit juices in juice blend products. The purpose of this paper is to explore cloud stability in a low-acid juice, such as carrot juice, by examining the effect of pH on colloidal interactions between fine juice particles.

Carrot juice, like all cloudy juices, is a two-phase colloidal system, with a dispersed solid phase called cloud and a continuous liquid phase called serum.[1] The serum contains water-soluble components, and the cloud is composed of insoluble particles of various sizes and shapes from the fruit tissue.[1] Most juice clouds have average particle sizes between 0.2 and 10 μm and are frequently divided into coarse (>10 μm) and fine (0.1−10 μm) cloud fractions.[2,3] The accepted view of cloud particles is that they are a positive protein−carbohydrate complex surrounded by negatively charged pectin, which gives the overall particle a negative charge.[4,5] Pectin is critically important in cloud stability, both because of its charge and its water-binding capabilities. When the pectin coat of particles binds with water, it forms a hydrated pectin layer that has been referred to as a hydration envelope; that is, water molecules are associating with the pectin, effectively enveloping the particle, so that the density of the particle is reduced.[6,7]

Reiter et al.[3] analyzed the effect of acidification at various points during the production of juice from blanched carrots, using citric acid to acidify to pH 4.4. Using turbidity, particle size, and charge measurements, the authors found that acidification at later stages of the production (after fine grinding or in the final juice) increased the particle size and reduced cloud stability. However, stability was enhanced when carrots were acidified earlier in the juice processing, an effect also observed by Sims et al.[8] and Yu and Rupasinghe.[9] The juice components may be affected by a decrease in pH in various ways. Proteins that are acid-sensitive may coagulate and precipitate when the pH is shifted from natural carrot pH (6.2) to 4.4.[3] For pectin, its negative charge is pH-dependent; pectic acid has a pK_a of 3.5−4.0.[6] Near the pK_a, pectin becomes substantially less negative and there is less repulsion between cloud particles.[4,6] More broadly, as juice is acidified and pH is lowered, surface groups become increasingly protonated, which lowers the magnitude of the surface charge of the cloud particles. Reiter et al.[3] concluded that the low pH caused particles to flocculate but that the flocs could be broken up again or removed during processing if acidification occurred at earlier stages of production.

The current study was designed to investigate the underlying interparticle interactions affecting cloud stability in fresh carrot juice, through an analysis of particle size distributions and other cloud characteristics as a function of pH and ionic strength. ζ potential measurements were employed in this study to characterize the electrical charge properties near the surface of the particles in suspension. An inverse relationship between ζ potential magnitude and aggregation has been shown to exist within the natural matrix of orange and apple juices.[4,10−12] There may be a relationship between the degree of methoxylation (DM) and the ζ potential of cloud particles. It was found that apple juice with a DM of 85−88% had a ζ potential from −19 to −20 mV,[4,13] while orange juice with a DM of 71−73% had a more negative ζ potential of −23 mV.[4] For carrots (DM of 55−69%), it is therefore predicted that the ζ potential would be more negative at the same pH than either that of apple or orange juice.[14−17]

Acidification of juices is accomplished in industry by either combining a low-acid juice with a high-acid juice or directly adding acid to the juice. A similar procedure was used to lower the pH of carrot juice in several of our experiments. However, such a procedure also alters the ionic strength and overall concentration of the juice, with the extent of these changes increasing with the degree of acidification. To control for this

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effect, in most experiments, the juice pH was modified using citrate buffer salts at a constant ionic strength. Given the possible role of double-layer interactions between particles on cloud stability, juice ionic strength could well be an important parameter in clarification rates.

Through research presented in this paper, we examine particle interactions as a function of pH, via static light scattering, turbidity, and ζ potential, and discuss the role of interparticle energy barriers in the observed cloud stability. We seek to elucidate cloud stability in low-acid carrot juice, which may have inherent properties different from those of juices previously studied that are naturally more acidic.

■ MATERIALS AND METHODS

Material Sourcing. Refrigerated fresh carrot juice ("ready to drink"), commercially produced in Bakersfield, CA, was purchased from the local grocery store and used for all experiments. Juices prepared by us in the laboratory could not replicate the qualitative features and consistency of the particle size distributions of purchased fresh carrot juices, and thus, a study on commercial juice was preferred. According to the manufacturer, the juice was produced from finely ground fresh carrots that were separated into juice and pomace; the juice was then pasteurized, packed, stored, and distributed under refrigeration. There were no added ingredients, and the juice was not acidified. As indicated by the manufacturer, the average total solids of the commercial juice was 10−20% and the soluble solids was 5−6%. The weight fraction of all solids in carrot juice was estimated in our laboratory to be 0.09 g/g, by drying the juice in a 56 °C oven and weighing the residual. From this concentration, the amount of soluble solids in carrot juice was subtracted, to give a value of ∼0.04 g of insoluble solids/g of juice.[18] Turbidity of the juice was measured in our laboratory using ultraviolet (UV) absorption at 660 nm to be 14 cm^{-1}. The range of pH for carrots used by the manufacturer was reported to be 5.85−6.45, and the pH of the unbuffered juice was measured by us to be 6.2.

Citric acid monohydrate, sodium citrate, sodium chloride, and calcium chloride were obtained from Fisher Scientific (Fair Lawn, NJ).

Acidification. Citric acid and sodium citrate were added directly to commercial carrot juice to obtain seven samples of carrot juice with 0.1 M buffer concentrations and pH values spanning 3.5−6.1. Buffer salts were directly added to the carrot juice to ensure consistent ionic concentrations between the samples. All measurements were taken on the same day after 3 days of storage at 7 °C to ensure that particle flocculation was complete.

Certain juice samples were acidified by adding various small volumes of 2 M citric acid solution to commercial carrot juice, to achieve the desired pH value.

To explore the effect of neutralizing acidified juice back to its natural pH (6.2), 40 mL aliquots of juice were acidified with 2 M citric acid to pH 3.0 and left to sit for 1 day to allow for flocculation to occur. The juice was then neutralized back to pH 6.2 using 2 M NaOH.

Relative Sediment Height. Relative sediment height, also known as the sedimentation test, is a measure of the quantity of visible settling that occurs in the juice.[7,19] It is the height ($H_{sediment}$) of the cloud sediment divided by the total height (H_{total}) of the juice in a vial.

Roughly 5 mL of each juice sample was placed in a 7 mL glass vial with a screw cap and allowed to remain undisturbed, except for gentle transportation of the vials from the refrigerator to the laboratory bench for measurements. The height of the liquid in the vials was between 25 and 30 mm. A caliper was used to make height measurements in triplicate.

Relative Turbidity. Relative turbidity is a spectrophotometric method to determine cloud stability by comparing the turbidity in a juice sample before and after centrifugation.[3,5,7,12,20] For the carrot juices examined here, absorbance at 660 nm was used as the measure of juice turbidity. At this wavelength, absorbance is predominantly determined by the scattering as a result of the particles rather than specific chemical absorption.[21] Relative turbidities were determined by measuring the absorbance values for the homogeneously mixed whole juice and for the supernatants obtained after centrifugation (4200*g* for 15 min at 20 °C). When absorbance values measured in standard 1 cm cuvettes were unacceptably high, shorter path length cuvettes (0.5 mm for whole juice and 5.0 mm for juice supernatants) were used. The absorbance of the whole commercial juice was determined to be 0.68, and the supernatant was 0.81. Turbidity (τ) was calculated as the absorbance divided by the path length used for measurement. Relative turbidities (expressed as a percentage) were calculated as $\tau_{super}/\tau_{total} \times 100$, with τ_{super} obtained from the centrifuged supernatant layer and τ_{total} obtained from the uncentrifuged whole juice. Measurements were performed in triplicate.

Particle Size Determination. Static light scattering was used to measure the average particle size.[3−5,7,11,22] Measurements were performed using a Microtrac S3500 (Montgomeryville, PA). Results were obtained as the volume fraction of particles contained within a discrete size range (bin). The mean diameter was determined as a volume average:

$$D_{4,3} = \frac{\sum_i n_i d_i^{\,4}}{\sum_i n_i d_i^{\,3}} \qquad (1)$$

where $D_{4,3}$ is the volume mean diameter, n_i is the number of particles in bin *i*, and d_i is their diameter. Carrot juice was inverted gently 10 times to ensure even distribution of particles and then added to the instrument sample port, which was operated at a flow rate of 15% of the maximum. Particles were assumed to be irregular in shape and absorbing, and a refractive index value for water of 1.333 was used. Three replicates were measured for each sample.

ζ Potential. Using a Malvern Zetasizer (Westborough, PA), the ζ potential (ζ) of the cloud particles was determined by measuring the electrophoretic mobility (μ) of the particles in the presence of an electric field. Carrot juice was diluted 1:40 with deionized water to ensure that it was in the appropriate concentration range for measurements, which in our experiments corresponded to a signal intensity of ∼100 kcps. The dielectric constant and viscosity of the continuous phase were set to 78.3 F/m and 0.8903 mPa s, respectively, at 25 °C. A minimum of 10 analysis runs were performed for each sample, the values for which were averaged together and analyzed via Smoluchowski's equation, $\zeta = \varepsilon\mu/\eta$, where ε is the dielectric constant and η is the viscosity of the continuous phase. This model requires the diffuse double layer to be thin compared to the particle radius. Three replicates were taken for each sample.

■ RESULTS AND DISCUSSION

Relative Sediment Height and Relative Turbidity. Cloud stability decreased as juice pH was lowered by the use of citrate buffer. After 3 days of refrigerated storage, there was almost no precipitate in the buffered control juice (pH 6.1) nor in the juices at the two next highest pH values, whereas a separate sediment layer was seen in more acidified juices (Figure 1). The extent of the separation was quantified by

Figure 1. Cloud sedimentation in buffered commercial carrot juice after 3 days in refrigerated storage. pH 6.1 is the control buffered juice.

measuring the height of the sediment layer relative to the total juice height (Figure 2). This relative height increased with

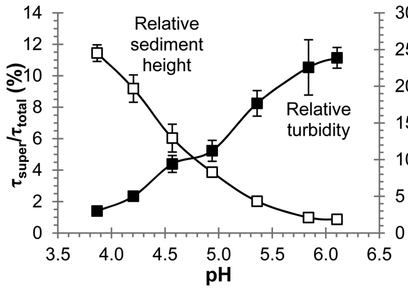

Figure 2. (■) Relative turbidity ($\tau_{super}/\tau_{total}$) and (□) relative sediment height ($H_{sediment}/H_{total}$) as a function of pH of commercial carrot juice with buffer salts added.

decreasing pH. These results were consistent with turbidity measurements of the supernatant before and after centrifugation (Figure 2). With a reduction in juice pH, the relative turbidity decreased monotonically, indicating that, most likely because of particle aggregation, particles sedimented more readily in lower pH samples upon centrifugation. The agreement between the results of relative sediment height and relative turbidity demonstrate that either method would be an appropriate way to measure the cloud instability in carrot juice. Results in Figure 1 also demonstrate that, as previously noted by Reiter et al.,[5] formation of a sediment in this experiment was not accompanied by the loss of visible turbidity in the supernatant, except to a small extent at the two lowest pH values.

Particle Size Distributions. Particle size distributions were measured for the unbuffered juice at pH 6.2 (Figure 3a) as well as the samples with added buffers (panels b−h of Figure 3).

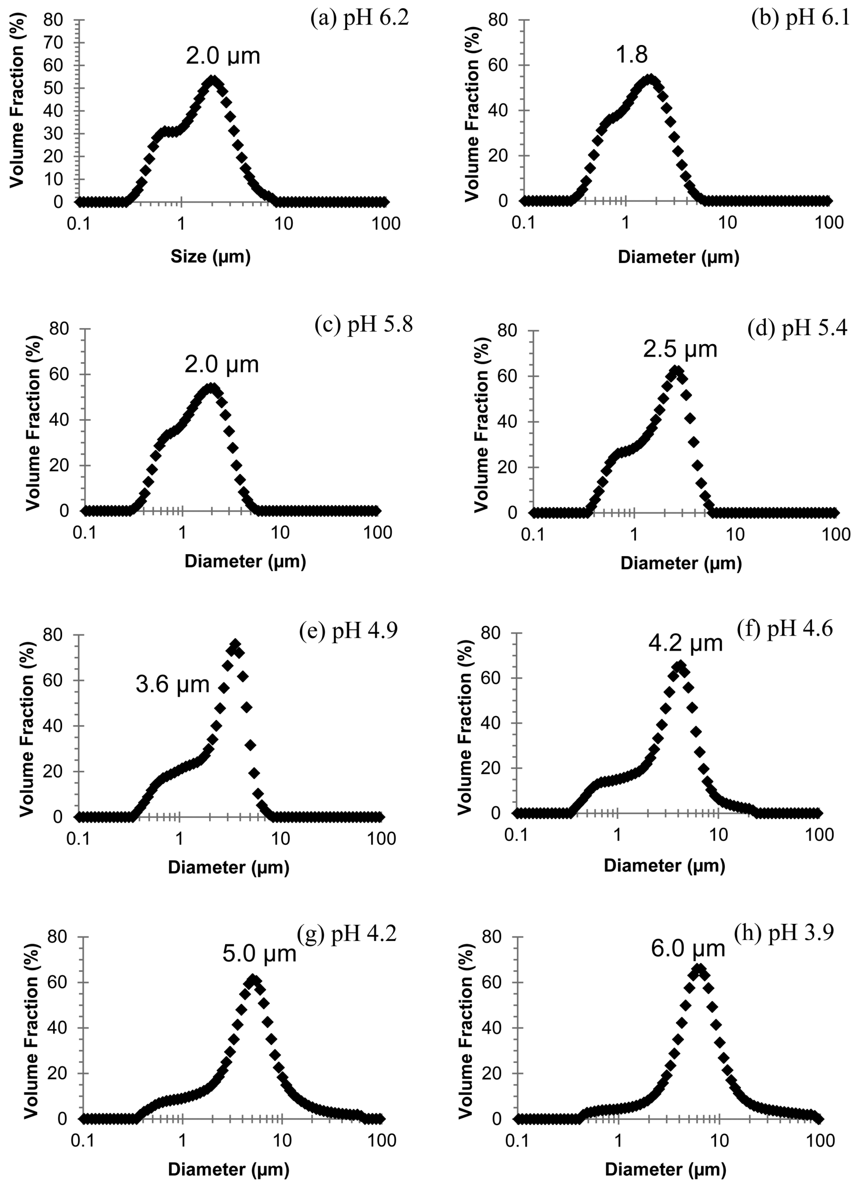

Figure 3. Particle size distribution graphs showing the volume fraction with the associated particle diameter (±0.04 μm) of (a) control unbuffered juice (pH 6.2) and (b−h) juices with added buffer salts at (b) pH 6.1, (c) pH 5.8, (d) pH 5.4, (e) pH 4.9, (f) pH 4.6, (g) pH 4.2, and (h) pH 3.9. The particle diameter at the maximum volume fraction is indicated above the main peak.

The distribution for the control buffered juice (Figure 3b, pH 6.1), shows evidence of two overlapping modes: one with particle sizes of a few micrometers and the other with sub-micrometer sizes. Particles of diameters less than 10 μm only were observed in the control juices, in contrast to typical juices analyzed by Reiter et al.,[5] which had particle sizes ranging from 0.1 to 700 μm. Thus, we did not observe a "coarse particle" fraction (defined as containing particles with a diameter of >10 μm),[5] and effects of acidification on fine particles (<10 μm) could be monitored directly (Figure 3). The average particle size ($D_{4,3}$) of the control juice (pH 6.1) was approximately 2 μm.

Juices with buffer at high pH levels, i.e., close to the natural juice pH, had particle distributions that were similar to the control (Figure 3c). As pH decreased, the main peak shifted toward larger particle sizes and increased in height. These particle distributions also exhibited a shoulder to the left of the main peak, and this mode decreased in height as the pH decreased (panels d−h of Figure 3). The rightward shift in the main peak with decreasing pH corresponded to only a moderate increase in the modal diameter (values marked in Figure 3), with this mode remaining below 10 μm for all pH conditions. Thus, it seems likely that, upon acidification, cloud particles of various sizes (sub-micrometer and micrometer) flocculated together to form clusters with just a few members, resulting in fewer sub-micrometer particles in the cloud and an accompanying growth in the size of the larger main mode. In addition, for pH values of ≤4.6, a new mode developed at larger particle sizes above 10 μm (panels f−h of Figure 3). The size of particles in this mode dramatically increased as the juice became more acidic. In contrast to particles in the main peak, it is clear that these largest particles are clusters with a large number of subparticles. These latter findings, in particular, are consistent with the work by Reiter et al.,[3] who observed that acidification of carrot juice to pH 4.4 increased the average diameter of the particles above 10 μm.

Overall, a monotonic increase in the average droplet size was observed with decreasing pH, as shown in Figure 4.

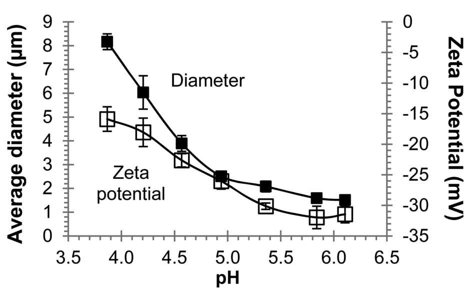

Figure 4. (■) Average particle size $D_{4,3}$ and (□) ζ potential of commercial carrot juice samples with added buffer.

Acidification caused particles to form aggregates that more rapidly sedimented and created an unstable cloud (Figures 1 and 2). The average particle size also increased in juices acidified by a method that was closer to industrial practice: by adding concentrated (2 M) citric acid in increasing amounts to the original juice. Measured results for the average diameter in the entire juice under these conditions are shown in Figure 5a.

Particle sizes in the supernatant and sediment of acidified (unbuffered) juices were also measured, with results in Figure 5a indicating that particles increased in size in both layers as the juices were acidified. During sedimentation of a monodisperse dispersion of particles, the vertical concentration profile exhibits three regions (Figure 5b): a stationary, concentrated region at the bottom of the container (the "sediment"), a region of intermediate concentration, which still falls toward the sediment layer, and above that, a particle-free region. In a polydisperse mixture, such as carrot juice cloud, these three distinct regions will occur simultaneously for different particle size fractions,[23] as shown schematically for a bidisperse suspension in Figure 5c. The "supernatant" region observable above the sediment layer in Figure 1 therefore comprises a mixture of different particle sizes, in sedimenting layers progressing toward the bottom, stationary layer. The influence of pH on the particle size in the supernatant is, thus, complex, because it results from opposing effects to the rate of particle settling and the size distribution remaining within the sedimenting supernatant regions. At high pH (pH ≥ 5.0), little sedimentation occurred over the 3 days of the experiment, and therefore, average sizes in the supernatant and sediment are the same as for the overall juice (Figure 5a). Under more acidic conditions, sedimentation partially separated the large and small particles, yielding smaller averages in the supernatant and larger averages in the sediment. However, because the sedimentation process was incomplete, the supernatant contained the still settling aggregates created at lower pH, and its average particle diameter is therefore larger than that measured at higher pH.

The process of particle separation described above depends upon not only the particle size but also the strength of the forces exerted on the particles. Thus, we expect differences in the extent of particle separation in juices sedimenting as a result of gravity (Figures 1 and 5a) and juices subjected to centrifugation. After centrifugation for 15 min at 4200g, control and acidified carrot juices studied by Reiter et al.[3] formed supernatants that displayed very similar particle size distributions. Large particles with diameters of > ∼5 μm that were present in the intact juices were mainly absent from the supernatant in all cases, leading the authors to conclude that acidification causes only coarse particles to aggregate.[3] However, it is also possible that the centrifugation is effectively removing aggregates and other coarser particle fractions from all of the sample supernatants, regardless of the origins of those aggregates. In our results at lower pH values, we find that supernatants created by sedimentation of commercial carrot juice retained clear evidence of aggregates formed from fine particles.

Aggregate Formation and Reversibility. The effects of neutralizing acidified carrot juice back to its initial pH were also examined. Juice was acidified with 2 M citric acid to pH 3.0, causing the average particle size in the total juice to increase from 1.9 μm at the natural pH of carrot juice (6.2) to 13 μm at pH 3.0 (Figure 5). This aggregate growth led to almost complete clarification upon centrifugation, with a relative turbidity of 5.2% (data not shown). The juice at pH 3.0 was then neutralized back to pH 6.2 with the addition of 2 M NaOH. At 1 day after neutralization, the average particle size had decreased from 13 to 2.6 μm and the relative turbidity increased to 14%, although it did not recover fully to the initial value of 39%.

This partial recovery of cloud properties upon neutralization likely indicates that the aggregation of the particles at lower pH is reversible. These results are qualitatively similar to that seen in work performed on the more acidic apple and orange juices. In these systems, adjusting the juices to pH 7 retarded

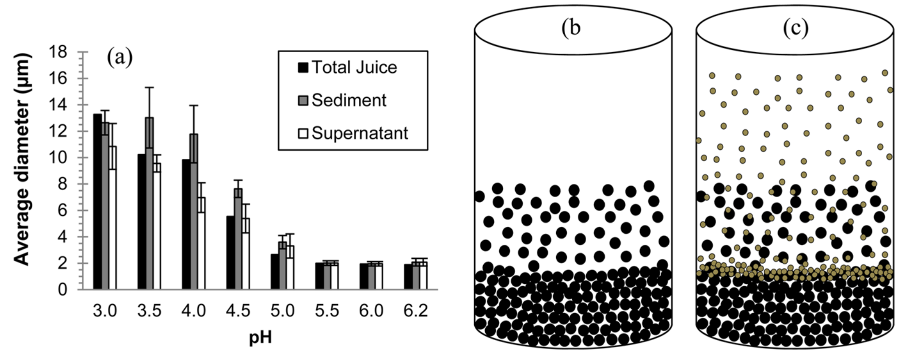

Figure 5. Effect of gravitational separation and pH on particle size: (a) $D_{4,3}$ measured in the entire juice (black bar), in the sediment (gray bar), and in the supernatant (white bar) in commercial juice acidified with 2 M citric acid and allowed to separate for 3 days, (b) particle layers in a monodisperse suspension during settling, and (c) particle layers in a bidisperse suspension during settling.

clarification and bringing the pH back down to the natural pH accelerated clarification.[24,25] Ellerbee and Wicker[11] also observed particle association and dissociation in orange juice aggregates at a fixed pH, which suggests formation of weak flocs. Likewise, carrot particles may flocculate at low pH, because of protonation and neutralization of the negatively charged pectin groups at the surface of the particles, which reduces repulsion sufficiently to allow for particles to be trapped together because of an attractive potential well, as discussed previously for orange juice.[24] When base is added and those groups are again deprotonated, the negative charges of the pectin become exposed again and the particles repel one another. This apparently reverses flocculation, decreasing the particle size. In this study, full recovery of the initial particle size may not have occurred because the attractive energy minimum between particles remained sufficiently large compared to thermal energy to allow for complete cluster separation. The protonation/deprotonation equilibrium of acid or base groups may also be altered within the cluster.

The growth in particle size with acidification can be seen more directly by viewing the particles under a light microscope at 20× magnification (Figure 6). A substantial increase in

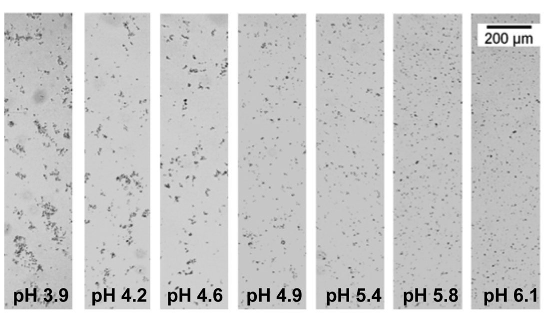

Figure 6. Optical micrographs with 20× magnification of carrot juice cloud particles at different pH values created by acidification of the juice.

particle size between pH 4.9 and 4.6 was particularly visible. At pH values above 4.6, the aggregates appeared to consist of small rather symmetric clusters, whereas at the lower pH values, the clusters appeared to become more irregular and elongated. In addition, the dispersions at the lowest pH displayed some very large aggregates, approaching 100 μm in size, consistent with results in panels f−h of Figure 3.

ζ Potential. The ζ potential decreased in magnitude, from −31 to −15 mV, as pH decreased from 6.1 to 3.9. The average particle size increased over this same range (Figure 4). Below pH 4.6, the particle size increased and the magnitude of the potential decreased more steeply; this change corresponded to conditions where the large particle mode (diameter > 10 μm) appeared and grew on the size distribution graphs (Figure 3) and in the micrographs (Figure 6). From these results, a clear correlation between ζ potential and particle size increase is apparent.

Similar trends have been seen previously in apple and orange juices, which are naturally more acidic than carrot juice. Croak and Corredig[4] found that, when they adjusted the pH of orange juice over the range of pH 2.5−6.0, the most negative ζ potential existed when the pH was 6.0 and its magnitude decreased steeply until it nearly reached 0 mV at pH 2.5. Ellerbee and Wicker[11] also report a more negative ζ potential for pH 5.5 orange juice compared to that at the natural pH of 4.0. Larger average particle sizes and increased clarification were also observed at the lower pH condition. In apple juice, Benítez et al.[26] measured ζ potential and turbidity in diafiltered apple juice after allowing 1 h for sedimentation, comparing the control juice (pH 4.5) to those acidified with HCl to lower pH. Turbidity was the greatest, and thus, the cloud was most stable at the highest pH of 4.5, where the ζ potential was also most negative. The ζ potential for carrot juice at pH 4 was found to be only slightly more negative (−17 mV) than the value (−19 mV) published for the fruit juices; this difference may reflect the lower DM for carrot pectin.

For all three types of juice, decreasing the pH decreased the magnitude of the negative charge on the particle, and this change likely reduced the energy barrier between the particles. Thus, particle flocculation and cloud instability were enhanced at lower pH in these three types of juice. Interestingly, Mensah-Wilson et al.[7] demonstrated in a very different way the connection between the particle charge and cloud stability, by measuring the effects of adding pectin to pineapple juice and passion fruit nectar. The fruit particles were initially very weakly (positively) charged and would flocculate and separate but became negatively charged and stabilized upon the addition of pectin.

Acidification and Ionic Strength Effects. The practice of acidification by the addition of different amounts of strong acid to the juice modifies both the concentration and ionic strength of the juice, in addition to its pH. Modification of pH by the addition of 2 M citric acid required adding no more than 3% by volume of the concentrated acid, and thus, the dilution factor was insignificant. However, concentrations of the citric acid in

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the final juice ranged up to 0.062 M at the lowest pH, and this would increase the juice ionic strength by up to ~0.04 M at pH values below 5.5.

These acidified juices can then be compared to those in which buffer salts were added directly to keep the ionic strength uniform between samples. The relative turbidity results from Figure 2, in which citric acid/sodium citrate buffers were used to alter pH, are replotted and compared in Figure 7 to carrot juice to which 2 M citric acid was added to decrease pH. Juices acidified using citrate buffer salts (prepared at a constant ionic strength of 0.1 M) showed a smoother increase in turbidity over the entire pH range, while the turbidity data of the juice acidified with 2 M citric acid displayed a more sigmoidal shape. The latter juices were slightly less stable at high and low pH than the juices containing buffer salts. This result is somewhat surprising, because the higher ionic strength in the buffer salt-containing mixtures might be expected to promote flocculation by decreasing the Debye length and lowering the energy barrier between particles.[10,13]

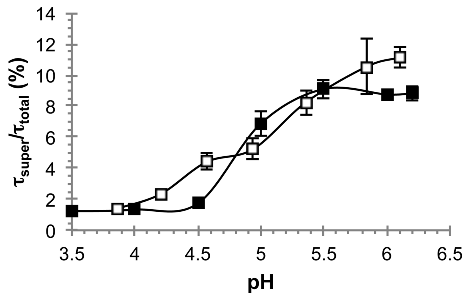

Figure 7. Relative turbidity ($\tau_{super}/\tau_{total}$) as a function of pH of commercial carrot juice with (■) 2 M citric acid solution or (□) buffer salts added. Buffer salts contributed 0.1 M to the ionic strength at all pH values.

To probe this effect further, the extent of clarification of juices was compared when sodium chloride versus calcium chloride was added in specific amounts to unbuffered commercial carrot juice at pH 6.2 (Figure 8). The juices were stored at 4 °C for 6 days, undisturbed, before relative sediment height was measured. The results in Figure 8 indicate that $CaCl_2$ significantly induced clarification, in contrast to NaCl, which had little effect on the cloud stability. Results with the sodium salt are consistent with those in Figure 7 and indicate that any effect of Na^+ on the particle diffuse double layer thicknesses does not seem to impact the particle stability.

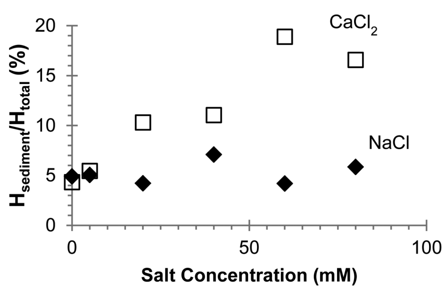

Figure 8. Relative sediment height ($H_{sediment}/H_{total}$) in commercial carrot juice (no acidification) after 3 days of storage at 4 °C, with added (□) $CaCl_2$ or (◆) NaCl.

Benítez et al.[10] explored the effect of the ionic strength on apple juice cloud stability, by first separating apple juice cloud particles from the juice serum and reconstituting them in aqueous solutions containing potassium chloride concentrations between 10^{-4} and 10^{-1} M. Turbidity and, hence, cloud stability were greatest at the lowest ionic strength, as expected.

A key difference between the results in Figure 8 and those by Benítez et al.[10] is that, in the latter study, the contributions of the native juice serum have been removed. To account for the surprisingly weak effect of ionic strength on our carrot juice cloud results, it is important to recognize that the native carrot juice serum will itself contain ions that contribute to the overall ionic strength. Data from the United States Department of Agriculture (USDA) National Nutrient Database[27] as well as measurements of electrolyte concentrations in other types of juices[28−32] suggest that native ionic strength values of the order of 20−100 mM[10] are likely. At such concentrations, the diffuse double layer is already less than 1−2 nm and further thinning upon addition of further ions may not have a large effect on modifying interparticle repulsion. In addition, with such small Debye lengths, electrostatic repulsion may occur over length scales comparable to short-range forces, such as steric interactions, giving the latter an important contributing role.[33] Benítez et al.[26] found that, even at very low pH values, where the ζ potential of isolated cloudy apple juice particles was close to zero, particles remained significantly stable because of a short-range repulsion. Ackerley and Wicker[24] also concluded that, at high pH values (pH 7), steric stabilization as a result of a hydrated pectin layer accompanies repulsive forces in preventing aggregation and clarification of orange juice. Thus, despite the high pH and high negative charge in the native carrot juice, such short-range interactions may be playing a significant role in carrot juice cloud stability, relative to the longer range double-layer interactions.

Results in Figure 8 also indicate an important contribution because of specific ion effects. Previous research has demonstrated that the presence of divalent cations increases fruit juice cloud instability, consistent with our results for carrot juice.[11,34−36] Calcium bridging of pectin is an important mechanism in the clarification of orange juice.[37] Such particle destabilization has been attributed to the formation of junction zones, in which divalent cations, such as calcium, induce pectin chain association.[34] Consequently, one explanation for the greater stability of the juices modified with buffer salts (Figure 7), despite their higher ionic strength, is that the higher concentration of sodium ions in the system competes with native calcium for binding to pectin. This would make calcium-induced flocculation less likely in these samples compared to juices acidified with citric acid.

pH Threshold for Carrot Cloud Instability. In summary, results from this study demonstrated that acidification of carrot juice caused clarification of the cloud because of particle flocculation. The growth in particle size was attributed to the particle ζ potential becoming less negative, which reduced the repulsion between particles and allowed them to approach one another and aggregate. Effects on ζ potential and particle clustering were observed over the entire pH of 3−6; however, the impact on cloud stability became more marked for pH values below 5. At these lower pH values, particle clusters between 10 and 100 μm developed as a significant portion of the size distribution. As the ζ potential increases more strongly below pH 5, these larger clusters may be promoted by a reduction in the energy barrier below a threshold value. However, the attractive interactions between particles in the cluster also appear quite weak, because the aggregation process was at least partially reversible upon returning the juice to neutral pH.

However, salts had a complex effect on cloud stability. Small differences were only seen between juices acidified with buffer salts at a constant ionic strength versus those acidified by the addition of various amounts of concentrated citric acid; surprisingly, the juices at higher ionic strength were slightly more stable. The addition of monovalent sodium chloride to the juice had no clear influence on the stability, whereas the addition of divalent calcium chloride promoted clarification, likely because of its ability to bind to pectin and form bridges between different pectin molecules. Further work is needed to elucidate the types and concentrations of ions present in carrot juice, to understand how these ions influence interparticle interactions. Factors (such as chelators) that reduce the contribution of these ions in the juice as well as factors enhancing the negative charge of the particles could push the threshold pH for cloud stability lower and promote carrot juice stability in the presence of acidification.

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■ ABBREVIATIONS USED

DM, degree of methoxylation

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