The role of process technology in carrot juice cloud stability

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Abstract

The effect of various processing measures on carrot juice cloud stability was studied on pilot-plant scale using decanter technology. Production steps investigated were mode of acidification, different enzymatic mash treatments and blanching conditions. Acidification showed the strongest effect on cloud stability. Whereas acidifying the coarse mash resulted in cloud stability, acidification following juice extraction even enhanced cloud sedimentation. The production of stable nonacidified juices was also possible. However, sediment formation was less after coarse mash acidification. Further improvement of cloud stability by enzymatic mash maceration or intensified blanching was negligible. Both measures, however, slightly improved the extraction of cloud particles from the mash, thus resulting in higher turbidity. The limited effect of macerating enzymes and pH-dependent sedimentation suggest that proteins rather than pectic substances influence cloud stability of carrot juices.

Keywords: Carrot juices; Cloud stability; Acidification; Blanching; Enzymatic maceration

1. Introduction

Carrot juices are preferably used as a natural source of provitamin A in the production of alpha-tocopherol-beta-carotene drinks (ATBC-drinks) leading to superior physical and chemical stability (Carle, 1999; Marx, Schieber, & Carle, 2000). Investigations on the cloud stability of carrot juices are scarce and difficult to compare due to differing juice extraction and experimental designs. Thermal treatment of carrots prior to juice extraction was found to be an important step in the production of cloud stable juices (Sims, Balaban, & Matthews, 1993). Blanching of carrots, especially in slightly acidified water, improves colour and cloud stability (Bates & Koburger, 1974; Stephens, Saldona, Brown, & Griffiths, 1971). According to Sims et al. (1993), the inactivation of pectinesterase (PE) by blanching or mash heating prior to juice extraction is a necessary step to prevent carrot juices from clarifying. The inactivation of PE is dependent on the carrot cultivar, and is mostly achieved within 10 min at 70°C or 4–6 min at 80°C (Vora, Kyle, & Small, 1999a; Tijskens, Waldron, Ng, Ingham, & van Dijk, 1997). In blanched carrot slices, slow PE inactivation was described even at temperatures as low as 50°C (Tijskens et al., 1997). Heat treatment at 80°C includes inactivation of other deteriorative enzymes such as peroxidase or catechol oxidase, while at 70°C peroxidase shows residual activities after inactivation of PE and catechol oxidase within 10 min (Vora et al., 1999a; Baardseth & Slinde, 1983). Acidification was also reported to be a crucial step during carrot juice production (Sims et al., 1993). When using blanched carrots, clarification can only be prevented by acidifying the mash before juice extraction. Without acidification or when acidifying following juice extraction cloud stability is poor.

Enzymatic mash treatment has been often investigated aiming at improved carrot juice yield (e.g. Anastasakis, Lindamood, Chism, & Hansen, 1987, Vora, Kyle, & Small, 1999b), whereas studies considering its effect on cloud stability are scarce and contradictory. Sims et al. (1993) reported a minor improvement of cloud stability, whereas Vora et al. (1999b) found that intensive mash maceration enhances sediment formation. However, puree-type carrot nectars or juices are stabilized by enzymatic maceration (Borowska, Zadernowski, Zander, Fornal, Markiewicz, Kubiak, & Kowalska, 2000; Pessa & Bailey, 1988). All
studies dealing with cloud stability of carrot juices were based on laboratory-scale pressing techniques. Since today decanter technology is mainly used in industrial practice, those data are of limited use. Therefore, investigations on the effects of blanching, enzymatic mash treatment and different modes of acidification on cloud stability based on semi-industrial scale juice production are required.

2. Materials and methods

2.1. Carrot juices

Carrot juices were produced in autumn/winter 1999/2000 and 2000/2001 using carrots (Daucus carota L. ssp. sativa cv. Karotan) cultivated by the research station of Hohenheim University. To assess the effect of acidification after juice heating, identical raw material of harvest 2000 was provided by an industrial juice manufacturer.

Production of carrot juices. Batches of 27–50 kg of carrots were used. Prior to juice production (Fig. 1), carrot stems and petioles were cut off manually. In preliminary experiments, blanching carrots at 80°C for 10 min was proven to be sufficient for complete PE inactivation. Immediately after removal from the boiler, the core temperature of an “average” carrot (diameter approx. 3 cm) was 60 ± 2°C and increased to 63 ± 2°C after thermal equilibration. The blanched carrots were manually halved prior to grinding.

Influence of acidification. To assess the effect of acidification, citric acid (500 g/kg) was added at three stages of the process (Fig. 1). Variants 1 and 2 were obtained by spraying 3 g citric acid/kg carrots into the mash. All acidified variants (1–3) were finally adjusted to pH 4.4, having a total acidity (TA, expressed as citric acid, pH 8.1) of 4.40 ± 0.07 g/L. Nonacidified carrot juices (4) with pH 5.6 and TA content of 1.48 ± 0.02 g/L were also produced. Acidified (1–3) and nonacidified (4) variants were produced with and without spraying ascorbic acid (800 mg/kg carrots) into the coarse mash, which resulted in pH reduction of 0.1. To assess the influence of heat on cloud stability, nonacidified juices (4) were pasteurized at 95°C, cooled back to 10°C and acidified (pH 4.4). Additionally, aliquots were taken prior to acidification before and after pasteurization and subsequently sterilized at 121°C (F = 5 min).

Effect of enzymatic mash treatment. Enzymatic maceration of acidified mashes by various technical enzymes was investigated in 1999, based on process 1 (Fig. 1). In order to meet the pH used for enzymatic maceration, the amount of citric acid was reduced to 2.5 g/kg carrots. The pH of the juices was adjusted to 4.4 prior to pasteurization. All enzyme preparations used individually and in combination (Table 1) were recommended for carrot juice production and provided by Röhm (Darmstadt, Germany). The enzymes applied were a pectinmethylotolytic polygalacturonase “Rohament PL” (PG), a pectinendolyase “Rohapect PTE” (PL), and a cellulase “Rohalase 7069” (CL). Enzyme dosages and incubation time were within the recommended range (Table 1). A control was produced without use of enzymes.

Effect of blanching conditions. Apart from the acidified control obtained by carrot blanching at 80°C,
water blanching was carried out at 90°C for 10 min, 20 min, and 30 min with core temperatures reaching 63°C, 81°C and 84°C, respectively. Juices were processed according to variant 1 (Fig. 1).

### 2.2. Characterization of juices

Prior to further investigation, all juices were adjusted to 8°Brix by dilution with demineralized water.

**Separation of coarse particles.** Particles prone to rapid sedimentation were termed coarse particles. Coarse particle content was determined gravimetrically by centrifugation of approx. 38g carrot juice at 4200 g for 15 min (20°C). The supernatant (serum) was removed with a pipette, and the wet sediment was weighed. The average of four determinations was expressed in gram coarse particles per kilogram juice.

**Particle size distribution.** Particle size distributions (PSD) of carrot juices and their respective sera were determined in duplicates by laser light scattering with a Mastersizer E (Malvern Instruments GmbH, Herrenberg, Germany). Data obtained were evaluated using supplier-provided software. Surface mean diameter $D_{3,2}$ and volume mean diameter $D_{4,3}$ were determined for all samples.

**Particle charge measurement.** Particle charge was determined in duplicates using a particle charge detector PCD 02 (Mütek, Herrsching, Germany) combined with an automatic titration system Titrino 702 SM (Mettler, Herisau, Switzerland) and Mütek PCD titration software version 1.2. Coarse particles separated from approx. 37mL carrot juice were resuspended in 25 mL dilution medium and titrated with 0.001N polydimethyl diallyl ammonium chloride. The dilution medium was composed according to the sugar and acid composition of carrot juices (Otteneder, 1982) and adjusted to pH 5.5 with 1N NaOH. Specific charge densities (μeq/g) of coarse particles were calculated as reported earlier (Mensah-Wilson, Reiter, Bail, Neidhart, & Carle, 2000) and transformed to their total charge densities (C/g).

**Particle density.** Buoyant densities of the carrot juice particles were determined in duplicates by isopycnic density gradient centrifugation. Carrot juices (8°Brix) were diluted with water (1:4, v:v), and 5mL of the diluted sample was placed on top of a sucrose gradient consisting of six layers of 5 mL of sucrose solutions of 5°Brix, 15°Brix, 25°Brix, 35°Brix, 45°Brix and 55°Brix. During centrifugation at 60000 x g for 2 h, temperature increased from 0°C to approx. 20°C. Layers containing cloud particles were fractionated. Sugar content was determined refractometrically and the corresponding densities were assigned according to Weast (1978). For relative quantification of the density fractions, weighting factors (1–8) were visually allotted to each layer according to its particle content.

**Turbidity measurement.** The turbidities of juices and sera were measured nephelometrically with an LTP 5 two-beam-photometer (Dr. Lange, Düsseldorf, Germany) using 5cm round cuvettes at colour correction mode. Samples were diluted with dilution medium. Turbidity was expressed in nephelometric turbidity units (NTU). The tendency to clarification was deduced from the turbidity of the supernatant after separation of coarse particles ($T_s$) and from the relative turbidity,

$$T_{rel} = \frac{T_s}{T_0} \times 100$$

where $T_0$ is the turbidity before centrifugation.

**Serum viscosity.** The dynamic viscosity of sera was termed serum viscosity. Serum viscosity of samples 1999 was determined using a CVO 120 HR rotary rheometer (Bohlin Instruments, Lund, Sweden) with a cone-plate geometry (60 mm, 2°). Viscosities were calculated from the average of four points of the flow curves obtained in the shear rate range between 13 s$^{-1}$ and 60 s$^{-1}$. Since all samples 1999 had shown Newtonian flow behaviour, the samples 2000 were measured using an Ostwald capillary viscosimeter (Schott, Hofheim, Germany). Kinematic viscosities were transformed to dynamic viscosities using the densities determined with a Paar DMA 48 density meter (Paar, Graz, Austria). All determinations were carried out in duplicates at 25°C.

### 3. Results and discussion

#### 3.1. Influence of acidification

Depending on the process stage of acidification, the addition of citric acid strongly influenced sediment formation of the juices as shown by the coarse particle content (Table 2). Irrespective of the addition of ascorbic acid as an antioxidant, acidification of the coarse mash resulted in highest cloud stability. In contrast, acidifying the juice prior to pasteurization always resulted in unstable juices (Table 2) as observed by Sims et al. (1993). Despite the large differences in turbidity of juices and respective sera, no visual difference could be observed due to the high absolute turbidities. Therefore, the amount of coarse particles is most crucial for evaluating cloud stability in carrot juices, thus confirming earlier data (Neidhart, Reiter, & Carle, 2000). Sedimentation behaviour of coarse particles can be attributed to different PSD. As shown in Fig. 2a, acidification of juices resulted in the formation of particles with sphere equivalent diameter of around 10μm and increased average particle sizes $D_{3,2}$ and $D_{4,3}$ (Table 2).

Acidifying the fine mash reduced cloud stability (Table 2). Initial turbidities were lower compared to juices resulting from acidifying coarse mash or juice,
<table>
<thead>
<tr>
<th>Mode of acidification</th>
<th>Coarse particles (g/kg)</th>
<th>Initial turbidity (NTU)</th>
<th>Stable turbidity (NTU)</th>
<th>Relative turbidity (%)</th>
<th>Serum viscosity (mPas)</th>
<th>D [3;2] (μm)</th>
<th>D [4;3] (μm)</th>
<th>Particle charge (C/g)</th>
<th>Density of particles of fraction no.</th>
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<td></td>
<td></td>
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<td>5800</td>
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<td>1.16</td>
<td>0.45</td>
<td>2.82</td>
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<td>8000</td>
<td>4900</td>
<td>61.3</td>
<td>1.14</td>
<td>0.47</td>
<td>6.16</td>
<td>-5.49</td>
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<td>2600</td>
<td>40.0</td>
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<td>1.11</td>
<td>12.93</td>
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<td>8400</td>
<td>1800</td>
<td>21.4</td>
<td>1.10</td>
<td>2.98</td>
<td>13.21</td>
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<td>22.9</td>
<td>7600</td>
<td>5000</td>
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<td>0.53</td>
<td>5.23</td>
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<td>1.23</td>
<td>0.72</td>
<td>6.18</td>
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<td>1700</td>
<td>22.1</td>
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<tr>
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<td>4100</td>
<td>45.1</td>
<td>1.17</td>
<td>0.64</td>
<td>11.04</td>
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<td>5100</td>
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<td>0.54</td>
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<tr>
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<td>1900</td>
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<td>2.35</td>
<td>16.90</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, unacidified</td>
<td>14.4</td>
<td>9300</td>
<td>5800</td>
<td>62.4</td>
<td>1.16</td>
<td>0.45</td>
<td>2.82</td>
<td>-2.56</td>
<td>1.07</td>
</tr>
<tr>
<td>Past., acidified</td>
<td>18.8</td>
<td>7300</td>
<td>2500</td>
<td>34.2</td>
<td>1.12</td>
<td>0.94</td>
<td>15.68</td>
<td>-1.64</td>
<td>—</td>
</tr>
<tr>
<td>2000&lt;sup&gt;a&lt;/sup&gt;, juice preheating</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control, unacidified</td>
<td>21.0</td>
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<td>58.7</td>
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<td>0.58</td>
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<td>1.06</td>
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<tr>
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<td>7400</td>
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<td>40.5</td>
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<td>0.92</td>
<td>6.74</td>
<td>-3.10</td>
<td>n. d.</td>
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<tr>
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<td>6800</td>
<td>475</td>
<td>7.0</td>
<td>1.14</td>
<td>2.38</td>
<td>14.84</td>
<td>-7.04</td>
<td>1.08</td>
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</table>

—, not detected; n. d., not determined; AA, ascorbic acid.

<sup>a</sup> Production year.
thus indicating a lower content of suspended particles. The addition of ascorbic acid did not have measurable effect. Obviously, acidification of carrot juices after extraction causes particle aggregation, while coarse mash acidification does not result in visible cloud instability. Precipitation caused by pH shift is characteristic of proteins. Therefore, cloud stability of acidified carrot juices can be explained as follows: At the genuine pH of carrots (approx. 5.5–5.6), soluble, acid-coagulable proteins are extracted. If juices are subsequently acidified, protein insolubility leading to cloud particle coagulation occurs. If acidification is carried out during coarse mash preparation, coagulates possibly formed are subsequently crushed during fine grinding. Acidification of the finely ground mash results in lower turbidity and intermediate cloud stability. Since protein coagulation is initiated in the fine mash, resulting coagulates are partly retained in the pomace. A smaller quantity of proteins, already released from the fine mash, undergoes coagulation, thus contributing to an increase in particle size. Hitherto, the relevance of proteins for carrot juice cloud stability was only realized with respect to thermal preservation, where heat-coagulable proteins were supposed to cause cloud particle precipitation following heat treatment of juices extracted from unblanched carrots. Juices from blanched carrots, in contrast, did not show sedimentation, since coagulation already took place during blanching (Stephens et al., 1971).

Carrot juices are often acidified after redilution of nonacidified carrot juice concentrates or when blended with fruit juices. Heating of nonacidified juices to 95°C prior to acidification could not prevent cloud particles from acid-induced coagulation (Table 2). However, seasonal differences were observed. While in 1999, coarse particle formation following acidification was partly prevented by heating, clarification occurred in 2000 (Table 2). Compared to nonacidified juices, stable turbidity of preheated juices of both seasons decreased, indicating coagulation to a different extent. The seasonal differences must possibly be ascribed to raw material characteristics.

In contrast to the heterogeneous PSD of differently produced juices (Fig. 2a), PSD of supernatants (Fig. 2c) always showed similar sizes of cloud stable particles. Obviously, particle aggregation following acidification only applies to the coarse particle fraction. Also particle density, greatly influencing cloud stability, showed similarities in all samples (Table 2). One particle density class, which was common to all samples except the juices heated prior to acidification, had a density of about 1.06 g/mL coming close to the serum density (1.03 g/mL). Such low-density particles, shown to be extremely resistant to centrifugal forces, were most frequently found in juices produced by coarse mash acidification (Table 2). In accordance with Maltschev, Petkantschin, and Mollov (1991), particle charge was negative (Table 2). However, cloud particles of samples produced by coarse mash acidification were higher charged compared to those of differently acidified and nonacidified juices (Table 2). Nonacidified juices showed high relative turbidities comparable to juices produced by coarse mash acidification (Table 2). In contrast to earlier reports (Sims et al., 1993), carrot juices produced without acidification
did not clarify. However, compared to juices produced by coarse mash acidification, nonacidified juices showed an increased amount of coarse particles (Table 2). Serum viscosity of all samples was close to an 8°Brix sucrose solution (1.08 mPa s). Therefore, the influence of the continuous phase on cloud stability appears to be negligible.

3.2. Influence of enzymatic treatment of acidified mash

All juices produced by enzymatic mash treatment were characterized by extreme cloud stability (Table 3). Their initial turbidity was always exceeding that of the control (Table 3). Since yield of cloud particles was reduced by stirring and heating of mash (Fig. 1), this control was also inferior to samples produced by coarse mash acidification omitting mash heating (Table 2). However, this thermo-mechanical effect on initial turbidity was compensated by enzymatic mash maceration. Due to high absolute turbidity values, differences could not be recognized visually. Compared to acidified juices without mash treatment (Table 2), enzymatic maceration did not significantly affect cloud stability.

PG produced maximum initial turbidity (Table 3), reflecting the macerating properties of this enzyme preparation. Protopectinolytic properties of PG resulted in significantly higher serum viscosities (Table 3), which, however, did not enhance cloud stability. As expected, PL and CL did not substantially increase serum viscosity. A combination of PG, PL, and CL, recommended for improved mash maceration, was not advantageous to cloud properties. Resulting physical juice properties did not differ from those obtained by using only PG, except for a minor decrease in coarse particle content (Table 3). PG-treated samples tended to higher cloud particle charge, which might be ascribed to a PE-side-activity.

Characteristic PSD of enzymatically treated juices and corresponding sera are shown in Fig. 2b and c, respectively. Except for the CL-treated sample, all juices displayed particle size reduction (>10 μm). This is also reflected by the volume mean diameter \( D_{[4,3]} \) (Table 3), which is strongly influenced by larger particles. As observed for different modes of acidification, the sizes of cloudy stable particles were similar (Fig. 2c). Concerning particle density, stable fraction #1 particles were predominating in all enzymatically treated juices (Table 3), similar to coarse mash acidification (Table 2). Due to their minor frequency, a high-density particle fraction (1.23–1.25 g/mL) exclusively found in some enzymatically treated samples did not affect cloud stability.

Improvement of carrot juice yield by enzymatic maceration is well documented (Munsch, Simard, & Girard, 1986; Anastasakis et al., 1987; Vora et al., 1999b). Confirming earlier investigations (Sims et al., 1993; Vora et al., 1999b), relevance of maceration enzymes was insignificant with respect to cloud stability of carrot juices. However, PG slightly improved liquid–solid separation resulting in higher initial turbidities (Table 3). Whereas enzymatic mash treatment markedly enhanced cloud stability of puree-type carrot nectars and juices (Pessa & Bailey, 1988; Borowska et al., 2000), the role of pectic substances seems to be less important in carrot juices produced by pressing or decanter technology. Since the focus of this study was cloud stabilization, the influence of enzymatic mash maceration on juice yield was not considered.

3.3. Influence of blanching conditions

Differences in cloud stability of the juices obtained after different blanching times at 90°C were less pronounced than expected from deviating core temperatures after blanching (63–84°C). Coarse particles content slightly increased with blanching time (Table 3), not substantially differing from blanching at 80°C. Serum viscosity also increased, indicating thermally induced pectin release from the middle lamellae in the course of carrot tissue breakdown, while particle charges increased. The PSD of the juices (Fig. 3a) and sera (Fig. 3b) did not vary significantly, except for a minor decrease of the relative amount of smaller particles (around 0.5 μm) with harsher blanching conditions. The surface mean diameter \( D_{[3,2]} \) remained constant, whereas the volume mean diameter \( D_{[4,3]} \) changed due to minor differences in large particle content (Table 3). All samples produced from carrots blanched at 90°C displayed identical particle densities, with the major fraction at 1.06 g/mL (Table 3). The quantity of the intermediate density fraction (≥2) decreased with increasing blanching temperature. All samples blanched at 90°C showed higher initial and relative turbidity than the control.

Our analysis of PE-activity of freshly extracted juices from blanched carrots confirmed earlier findings (Tijskens et al., 1997; Vora et al., 1999a), revealing that blanching at 80°C for 10 min enabled complete PE-inactivation and production of cloud stable juices. Provided that inactivation is performed at 80°C, PE was considered the most heat-stable enzyme in carrots, whereas other deteriorative enzymes were supposed to be of less relevance (Vora et al., 1999a). At lower temperatures, e.g. in the core of blanched carrots, peroxidase activity still has to be considered (Baardseth & Slinde, 1983; Vora et al., 1999a). Blanching of carrots for inactivation of PE was reported to be a prerequisite for the production of cloud-stable juices (Sims et al., 1993). Beyond PE-inactivation, more rigorous blanching conditions were insignificant with respect to cloud stability of carrot juices.
4. Conclusion

Acidification of carrot juices resulted in cloud particle coagulation. This effect was avoided by acidification of the coarse mash prior to juice extraction. It is assumed that cloud coagulation was caused by acid-coagulable proteins, which were precipitated and tended to cloud particle aggregation after pH-shifting of juices from 5.5 to 4.4. Acidification of the coarse mash while grinding resulted in superior cloud stability, provided that carrot PE was inactivated by blanching. Enzymatic mash maceration and high-temperature blanching slightly contributed to improved cloud formation, which, however, is of less sensory significance. In contrast to earlier findings based on laboratory juice pressing technique, stable, nonacidified carrot juices could be produced by applying decanter technology. Frequently used in multi-component blended juices, heat-preserved, nonacidified carrot juices are often subjected to acidification prior to bottling. Pasteurization of juices prior to acidification could not prevent cloud coagulation. However, coagulation of cloud particles following juice acidification may be prevented by heating of carrots or mash at elevated temperatures. Consequently, acid-induced cloud precipitation deserves further elucidation.
Therefore, investigations on the chemical composition of cloud and on the supposed mechanism of precipitation are currently under way.

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