Cloud Stabilization of Orange Juice by High Pressure Processing

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ABSTRACT
High pressure processing (HPP) was investigated as a means to preserve cloud in freshly squeezed orange juice. Cloud loss is a major quality defect in orange juice, and methods of preserving cloud without the extreme temperatures used in commercial pasteurization are desirable. Pressures from 500 to 900 MPa were investigated at dwell times of 1 sec, 1 min and 10 min. Higher pressures and longer processing times were more effective at preserving cloud, while all treatments yielded a microbiably stable product. A 90-day shelf life under refrigeration conditions could be achieved using pressures of 700 MPa and higher combined with treatment times of 1 min.

Key Words: orange juice, cloud, high pressure, citrus, pectinesterase.

INTRODUCTION
Juice cloud loss is the result of demethylated pectin interacting with calcium ions, causing a precipitate to form a clear serum layer on top of a viscous layer of settled pectin and insoluble solids. Cloud is retained by protecting natural pectin in the extracted juice from enzymatic deesterification and degradation by pectinesterase (PE) (Rouse and Atkins, 1952). Turbidity is a desired characteristic of citrus juices, and cloud stability is one of the criteria of quality (Rothschild and Karsenty, 1974). Cloud loss is a major quality defect in citrus juices, and its retention is one of the main reasons for the level of heating in commercial pasteurization. High pressure treatment has been shown to inactivate the heat-labile form of pectinesterase in orange- and grapefruit juices (Goodner et al., 1998). Since some pectinesterase activity remains after HPP treatment due to the heat-stable form of this enzyme, it was of interest to determine the cloud stability in juice samples treated by high pressure processing (HPP).

Cloud is considered “definitely” broken or lost in orange juice when light transmittance reaches 36% (Redd et al., 1986). It was the objective of this study to determine if HPP was effective in preventing or lessening cloud loss by inactivation of PE in orange juice, although some enzyme activity remains.

MATERIALS & METHODS
Freshly squeezed Valencia orange juice from mature fruit (Brix/acid = 11) was obtained from an FMC commercial extractor in the Univ. of Florida, CREC pilot plant. The juice was not subjected to a finishing step, and no additional pulp was added. The juice was strained through a #20 U.S. Standard mesh screen and the strained pulp discarded. The juice was homogenized in a blender on low speed for 30 sec to macerate remaining pulp. Straining and blending were employed to simulate a finishing step. Before undergoing HPP, samples were packaged into polyethylene terephthalate bottles (500 mL), capped and chilled in an ice bath to 0°C.

The method for determining cloud loss (Cameron et al., 1997) measured the transmittance of a supernatant at 660 nm after the juice was centrifuged at 10,000 × g for 10 min. Bottles were inverted five times to facilitate mixing before samples (50 mL) were periodically drawn for analysis. Three measurements were taken for each sample and averages reported. There was no variance between readings on the same juice sample. Juice bottles were stored at 4°C between analysis times.

Samples were monitored for microorganism growth or contamination by spread-plating 0.1 mL juice sample on orange serum agar plates (Difco, Detroit, MI). Duplicate plates of samples were plated at days 49 and 89. The plates were incubated at 30°C for 48 h before counting total colony forming units (CFU) using standard counting protocol.

Pressurization
Juice was pressurized using an isostatic high pressure unit (Stansted Fluid Power, Stansted, England), employing pressures from 500 to 900 MPa for 1 sec, 1 min or 10 min dwell time. Dwell time was defined as the time spent at the set point pressure. The packaged samples were kept in an ice bath until they were pressurized. The pressure unit and medium were at 5–10°C before pressurization began. A mixture of ethanol and castor oil (85/15 v/v) (Fisher Scientific, Philadelphia, PA) constituted the pressure medium. Time to reach the desired pressure was 12–15 sec while decompression was 10 sec. The use of a chiller to cool the pressure vessel jacket and the pressure medium ensured that samples remained in the temperature range of 20–50°C (monitored by thermocouple) during HPP. All runs were done in duplicate.

Statistics
Statistical data analyses and graphs were performed using Microsoft Excel.

RESULTS & DISCUSSION
Different pressure levels were applied (Fig. 1–3) and cloud loss over time was compared to that in an untreated control. Treatments of <1 min at 500 MPa did not yield any increased stability over the untreated control, although a 10 min hold time increased cloud...
stability of HPP samples by at least 2 wk. Treatment at 600 MPa (Fig. 1) yielded better results, with a 10 min treatment yielding a cloud-stable product for the duration of the 90 day study. As expected, shorter treatment times were less effective, but 1 min processing afforded much greater cloud stability than the control and extended cloud stability to 49 days. At 700 MPa (Fig. 2), treatment was much more effective, as a 1 min treatment stabilized cloud for 90 days. Samples treated for 10 min at 700 MPa were stable until the last week, when they completely lost all cloud. These samples remained microbiologically stable. Only molds can clarify juice through the production of extracellular enzymes such as pectinesterase (Nussinovitch and Rosen, 1989). Thus, absence of contamination by a yeast or bacteria would not be unusual, as was the case in these samples. It is possible that mold growth was present but not detected by plating. Visible mycelia in the samples (which were absent), would not be necessary to cause cloud loss or clarification of a sample. Cloud was monitored for 90 days because after that time flavor deterioration would be a factor in commercial, packaged juice quality.

Pressures of 800–900 MPa were much more effective in preserving cloud at shorter processing times (800 and 900 MPa data were similar, so only Fig. 3 is shown). Treatment (1 sec) at these higher pressures was effective at preserving cloud for up to 80 days. Longer processing times of 1 or 10 min maintained a juice cloud level that was unchanged from the initial cloud content. Samples at 800 or 900 MPa at processing times of 1 min or above were considered to have no appreciable cloud loss at refrigerated storage (4°C) over a period of 90 days. Shorter processing times would result in higher product through-put.

The question of whether the heat generated by pressurization would be sufficient to inactivate PE (and thus aid in stabilizing cloud) was considered. Samples were placed in the unit at 5–10°C and reached temperatures between 20°C and 50°C (by thermocouple) depending on set-point pressure. Immediate cooling occurred upon decompression. The temperature change due to pressure changes (in water) has been calculated as 1.86 × 10⁻³ K bar⁻¹ (Morild, 1992). After adjusting the equation to the heat capacity of our pressure medium and converting to MPa, the conversion factor becomes 4.8 × 10⁻³ K MPa⁻¹. At the highest pressure we used, 900 MPa, the maximum theoretical temperature increase would be 43.2°C. The maximum temperature reached by any sample was 50°C, suggesting that temperatures generated by pressures were not sufficient to thermally inactivate PE and stabilize cloud. Although temperatures <65°C may partially inactivate the heat-labile form of PE, long hold-times would be necessary, and at least 70°C is necessary to completely inactivate this form of the enzyme (Rouse and Atkins, 1952).

Microbiological results
Plate counts taken during the cloud loss study were compared (Table 1). A dashed line indicates that the sample was not cloud-stable at the time of microbiological sampling, so that sample was eliminated from microbial analysis. The two samples listed as “TNTC” were contaminated post-processing by Rhodotorula yeast, which has not been shown to affect cloud in citrus juice. The other samples were at levels of microorganisms considered to be microbiologically stable. It has been shown (Parish, 1998) that ascospores of Saccharomyces cerevisiae were more resistant to high pressure treatment than were the vegetative cells.

CONCLUSIONS
HIGH PRESSURE TREATMENT WAS EFFECTIVE FOR PRESERVING cloud in orange juice, while maintaining acceptably low levels of microbes. In order to achieve a 90-day shelf-life of packaged juice at refrigerated temperatures, pressures of 700 MPa or greater and processing times of 1 min are recommended.

REFERENCES

Ms received 11/18/98; revised 3/12/99; accepted 3/19/99.

Florida Agricultural Experiment Station Journal Series No.R-06644.