HIGH PRESSURE PASTEURIZATION OF CITRUS JUICES

R. J. Braddock, Professor,
M.E. Parish, Associate Professor,
and J. K. Goodner, Graduate Assistant
University of Florida
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, Fl 33580-2299
Phone: 941\956-1151 Fax: 941\956-4631 Email: RJBK@icon.lal.ufl.edu

ABSTRACT

High hydrostatic pressures affect chemical reactions and phase changes of matter, denaturing proteins, solidifying lipids and disrupting biological membranes. The consequences of this in food systems has importance in killing spoilage microbes without the need for heat. Some applications of high pressure treatment to the processing of citrus juices are included herein. Effective pressures for pasteurization of yeasts and yeast ascospores in citrus juice fall in the range of 43,000 - 72,000 psi. The corresponding D_p (time for 1 log cycle reduction) values for inactivation of ascospores were 10 min at 43,000 psi or 8 sec at 72,000 psi. Pressure treatments of orange and grapefruit juices to by-pass thermal processing for pectinesterase (PE) inactivation were in the range of 72,000 - 130,000 psi. D_p values for orange PE inactivation at 72,000 and 87,000 psi were 83.3 minutes and 2.4 minutes, respectively. Pressures ≥87,000 psi caused instantaneous inactivation of the heat labile form, but did not inactivate the heat stable form of PE. Heat

labile grapefruit PE was also more sensitive than orange to pressure. Orange juice pressurized at 100,000 psi for 1 minute had no cloud loss for >50 days.

INTRODUCTION

Current processing of citrus juice employs a pasteurization step, which has the purpose of reducing microbial load as well as inactivating pectinesterase (PE), the enzyme responsible for cloud loss during storage. Severe commercial pasteurization treatments are necessary to inactivate PE, and these are in excess of what is necessary to make the product microbially safe. As an alternative to heat pasteurization, high pressure has been shown to reduce microbial count (Ogawa et al., 1992), affect properties and functionality of proteins (Messens et al., 1997), and influence enzyme activity (Seyderhelm et al.1996). As such, it is rapidly gaining interest as a tool for food processing. The ability to cause the above mentioned changes in food products without the introduction of extreme heat, which can be deleterious to flavors and nutrients, is the main benefit of high pressure processing. In this paper, both the high pressure technology and its application to citrus juices will be discussed.

DEFINITIONS

The high pressures in the range of those discussed in this paper as well as references to the literature require the reader to be familiar with both British and SI units and some definitions. The following information should allow one to adequately follow the text.

Pressure units.	0.1 MPa = 14.5 psi = 1.0 bar
Temperature.	1.8 °C = °F - 32 0 °C = 273 °K
Volume.	3.785 L = 1 gal
Dwell time.	The time at a set pressure (not considering come-up and come-down).
Pectinesterase.	Activity = PEu x 10^3/min/g juice.
Inactivation.	D_p value = time at a set pressure to decrease the microbial population or enzyme activity by 90% (1 log cycle). z_p value = pressure increase to increase the inactivation rate by 90%.
Isostatic pressure.	Process at constant pressure in a static environment.
Adiabatic.	No loss or gain of heat.

PRESSURE VS THERMAL PROCESSING

here are some practical considerations which may be important if one looks at the differences between pressure and thermal treatment for pasteurization of food products. Pressure increases mainly decrease the volume of the product being pressurized; how-

ever, increasing temperature increases product volume and energy. The latter may have deleterious effects to the chemical constituents of some products. Pressure changes are essentially instantaneous and uniformly distributed throughout a product. Even though Joules-equivalent heating occurs during pressurization, cooling occurs instantly upon depressurization. Also, once the set-point pressure is reached, no additional energy input is required. In thermal processing, heating times to reach setpoints are significant, heat loss may be considerable and some products are difficult, if not impossible to heat uniformly.

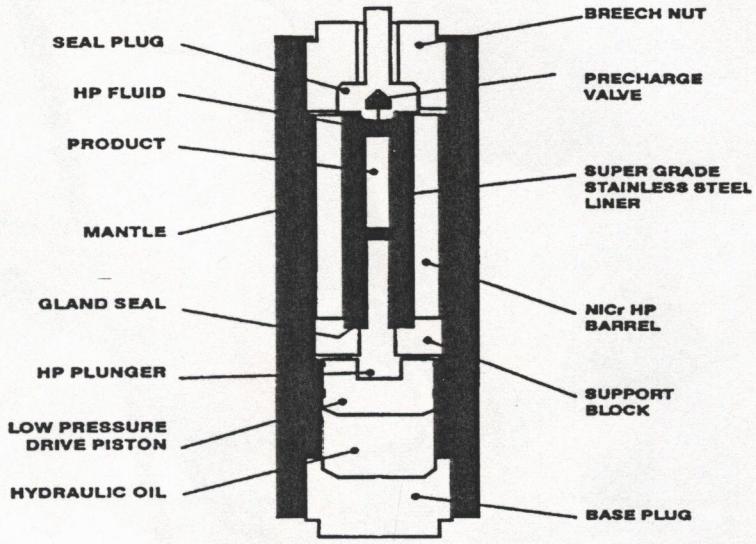
In citrus juice processing, we could compare effects of pressurization of water, a fluid for which there is some pressure data. Such a comparison is valid (although the Department of Citrus might not publicize it), because juice is mostly composed of the nutrient water (90%). At pressures near 100,000 psi, water's volume is reduced by approximately 15%, and if the compression is performed adiabatically, the temperature increase would be in the range 85 - 100 °F. Considering phase changes, water (ice) has the unusual property of decreased melting point with increasing pressure. One potential application of this opens the possibility of defrosting frozen foods very rapidly by a pressure treatment. For chemical reactions, although equilibria may be shifted readily through the application of temperature, only pressures above 40,000 psi may have any significant effect on reaction rates. Volume decreases of acid solutions in water under pressure are accompanied by pH decreases. Since citrus juices are acidic, the pH decrease caused by 100,000 psi pressure would be in the range of 1 pH unit. Keep in mind this is not a permanent effect, but only for the duration of the treatment.

DESIGN

There are now commercial high pressure pasteurization units in applications for fruit juices and a number of products such as jams, preserves and dairy products. Currently batch operation is the mode, although research is well underway for semicontinuous and continuous processes (Moreau, 1995). Operating pressures for foods vary according to the application, but can be in the range of 30,000 -100,000 psi. For metallurgy applications, vessels (3 - 10 L capacity) have been built having working pressures of >200,000 psi . Other important variables to both operation and economics are the cycle time and the dwell time. The cycle time is the time necessary for loading, pressurization, holding, decompression and unloading the vessel. This can be from 1 min for small laboratory units to 30 minutes or more for large commercial systems. The dwell time is the time the product is held at the process pressure, after pressurization. With accumulators to allow for rapid pressurization, the come-up time to the set pressure may be as rapid as 10 - 30 sec., or on the order of minutes.

There are available small-scale, high pressure designs for research purposes. Some characteristics of these and other systems are discussed in the scientific literature (Mertens and Deplace, 1993). The unit at the University of Florida, CREC, Lake Alfred, is a plunger press design having modern features of rapid compression cycles using an accumulator, high pressure capabilities up to 9000 bar (130,000psi), operation at above and below ambient temperatures (-4 to 195 °F) and minimum hold time at set pressures of 0.1 sec. The unit pressure vessel dimensions are 2.75" dia. x 8.00" length, with a usable volume of about 600 mL (20 oz.) juice, which is placed in a flexible container. Figure 1 is a generalized schematic of the plunger press design for the system at Lake Alfred.

FIGURE 1. Stansted "Plunger Press Food Lab 9000" high pressure food processing press schematic. (By permission of P. Freeman, 1997).



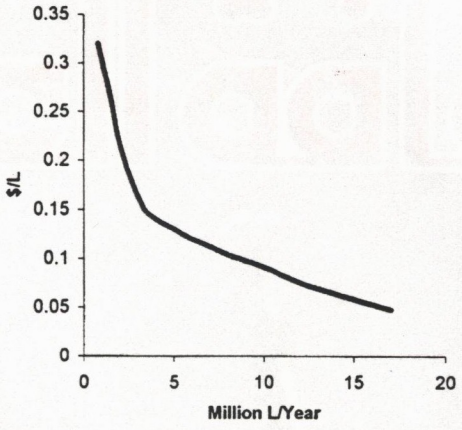
Some other features of this research instrument include hook-up of the pressure medium to a chiller for sub-ambient operation or minimizing product heating during pressurizing. The pressurization may be rapid (< 10 sec.) or direct (< 3 min.), and depressurization may be controlled between 2 sec. – 10 min. The system is designed for PLC, electrical supply 208 v, 60 Hz, 3 phase at 5 hp. The pressure fluid surrounding the product is designed for some lubrication of seals, etc. with an ethanol/castor oil (85/15%) mix in our instrument. Use of this fluid requires that the product be safely isolated from the medium by packaging. For research studies, we have found it expedient to put the sample packages inside a shrink-wrap of flexible plastic material, thus preventing contamination.

ECONOMICS

Manufacturing costs increase with the lifetime for the total number of cycles of the pressure vessel and the steel used. Large forged steel vessels used in the materials industry can be designed for an estimated lifetime of 100,000 cycles and depreciation in greater than 10 - 13 years, based on the assumption that the vessel is always pressurized to its maximum operating pressure (Deplace, 1995). Typical volumes for commercial pressure vessels are in the range from 25 – 150 gal. Process parameters are reliably automated and reproduced and pressure/temperature variables are measured directly in the high pressure media (Olsson, 1995). In some equipment, the steel pressure vessel has a stainless liner depending on the target product.

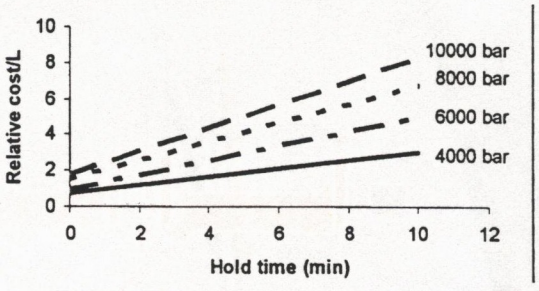
Figure 2 illustrates the cost of pressure processing a food vs the capacity of the equipment (Olsson, 1995). The process cost includes the estimate for capital cost, energy, floor space, labor and maintenance and 5 yr. depreciation. A citrus plant pasteurizing the juice from 4-5 million boxes of fruit would fall in the 10 million L/ year range. Generally, since pasteurization of microbes and enzyme inactivation are more efficient at higher pressures, economics favor use of higher pressures, due to shorter process times. Because of quality considerations, the most efficient and cheapest combination of size, pressure, temperature and time for each product must be established.

FIGURE 2. High pressure food processing cost vs plant capacity (ref. Olsson, 1995).



The cost/volume is also lower in one larger production unit, than installation of smaller parallel units, because of fabrication costs associated with the pressure vessels. The hold time at the process pressure is also very important to the cost, as shown in Figure 3. One can see that a 10 min process time requirement at 4000 bar (58,000 psi) is nearly twice as expensive as that at 8000 bar (116,000 psi) with no hold time. This scenario may be appropriate since there is some indication that certain enzymes and microbes are inactivated instantaneously at high pressures. Realistically, one would expect that this process would be optimized for each specific product to be processed, since the characteristics of microbial contamination or enzyme activity may vary.

FIGURE 3. Relative processing cost vs hold time @ pressures (ref. Olsson, 1995).



APPLICATIONS

Foods. Any food that can conveniently be packaged into a flexible container, plastic bottle, shrink film, or pumped, may be pasteurized by pressure. The first demonstration of this process for preserving peaches was in 1899, and some successful commercial applications include jam and preserves and a semicontinuous process for juices with a capacity of 4 tons/hour (Knorr, 1995).

Citrus Juice. *Inactivation of microorganisms.* Our research at CREC indicates that high pressure treatment can render single strength orange juice as microbially stable as thermal

and Atkins (1955) using 100 mL of a 1% pectin solution in 10% 1M NaCl. Pectin solution was kept at a constant temperature of 82°F. Results were reported in the conventional manner for citrus PE as pectinesterase units x 10^3 min^{-1} g^{-1} juice (PEu x 10^3). All samples were titrated in duplicate.

DATA ANALYSIS

D_p values were calculated from the regression equation of the plot of log PE activity x 10^3 versus time pressurized at each pressure indicated. The negative reciprocal of the slope of this plot is the D_p value. The z_p value is obtained from the negative reciprocal of the slope of log D_p versus pressure and is an indication of the pressure increase necessary to lower the D_p value by one log cycle.

KINETICS

Figure 4 shows remaining PE activity versus the dwell time at four different pressures. Inactivation of PE with higher isostatic pressure was bi-phasic, suggesting more than one form of the enzyme, which was reported for thermal inactivation (Versteeg et al., 1980). It was suspected the initial drop in activity was due to an inactivation of the heat labile form of PE, while the remaining activity illustrated the effect of pressure on the heat stable form. The heat labile form of PE comprises from 86 - 94.4% of the total enzyme in Valencia juice (Snir et al., 1996), and at the higher pressures the rapid inactivation is very close to this percentage.

FIGURE 4. Inactivation of orange juice PE at 600, 700, 800, and 900 MPa pressure for three dwell times. Values are the average of two experiments

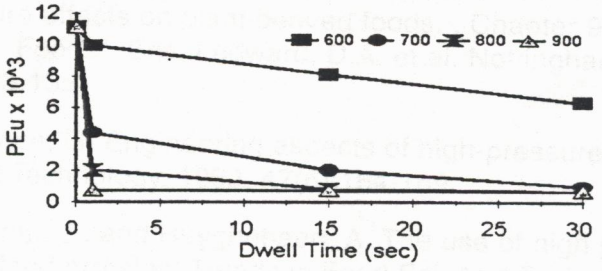


Table 2 is a summary of the inactivation percentages for orange and grapefruit juice at varying pressures. Subsequent heating of a pressurized (1 minute at 700 MPa) sample for 2 minutes at 160°F only reduced PE activity from 0.21 to 0.18 PEu; while heating for 2 minutes at 195°F resulted in a 0.21 to 0.08 PEu decline in activity, suggesting that only the heat stable form remained after pressurization.

Table 2. Inactivation of PE by pressure and heat treatments in orange and grapefruit juice.

	% Inactivation	
Treatment	Orange	Grapefruit
600 MPa (1 sec)	10%	50%
700 MPa (1 sec)	61%	82%
800 Mpa (1 sec)	82%	87%
900 Mpa (1 sec)	93%	85%

pasteurization. There is data to indicate that yeast ascospores are more thermally and pressure tolerant than vegetative cells. Ascospores are reproductive bodies of yeasts, similar to bacterial spores. D- and z-values are measures for thermal tolerance of microorganisms and enzyme inactivation, which we have applied to pressure studies. D_p-value is the time at a specific pressure to reduce the population of organisms (or enzyme activity) by 1 log cycle, or 90%. The z_p-value is the pressure increase required to increase the inactivation rate 10-fold. Table 1 contains results of experiments which suggest that ascospores of the strain of *S. cerevisiae* isolated from orange juice could be reduced from 10,000 CFU/mL to 0.01 CFU/mL (CFU = Colony Forming Units) by the 500 MPa treatment for 1.08 min (6 D_p). The z_p-value indicates that a 117 MPa pressure increase would effectively increase the inactivation rate by a factor of 10.

Citrus Juice. *Inactivation of enzymes.* Samples of orange and grapefruit juice were extracted in the pilot plant of the Citrus Research and Education Center in Lake Alfred, Florida. The pulpy juice was not subjected to finishing in order to have additional PE activity. These samples were homogenized with a blender for two minutes to insure small, relatively uniform particle size distribution and maximum enzyme activity. The resulting pulpy juice was stirred before packaging samples (1 oz.) into sterile polyethylene bags and heat sealed, retaining as little headspace as possible.

TABLE 1. D_p and z_p-values for high pressure inactivation of *S. cerevisiae* ascospores[a]

Pressure (MPa)	Pressure (psi)	D_p-values (minutes)	
		Orange Juice	Apple Juice
500	72,500	0.18	0.15
450	65,250	0.50	0.48
400	58,000	0.97	0.88
350	50,750	2.80	2.51
300	43,500	10.81	9.97
z_p (MPa)		117	115

[a] D_p-value is the time needed at a specific pressure to reduce the ascospore population 90%. The z_p-value is the pressure increase needed to increase the inactivation rate 90%. Results are averages for a minimum of 3 experiments. D_p-values obtained from the linear portion of inactivation curves.

PRESSURIZATION OF JUICE

Juice (1oz.) was pressurized using an isostatic high pressure unit (Stansted Fluid Power, Stansted, England) at 600, 700, 800, or 900 Mpa (0.1Mpa = 14.5 psi = 1.0 bar) for 1, 15 or 30 second dwell (time spent at the set point pressure). The packaged samples were kept at 40°F until they were pressurized. The pressure unit was at 40-50°F before pressurization began. Time to reach the desired pressure was 12-15 seconds while decompression was approximately 10 seconds. The use of a chiller to cool the pressure vessel jacket and the pressure medium ensured that samples remained in the temperature range of 68-120°F during processing. PE was assayed using the titration method of Rouse

Grapefruit PE was initially more sensitive to pressure treatment than orange. Table 2 shows that grapefruit PE inactivation is greater at the lower pressures than for orange, but was not greater than ~ 85% even at the highest pressures used.

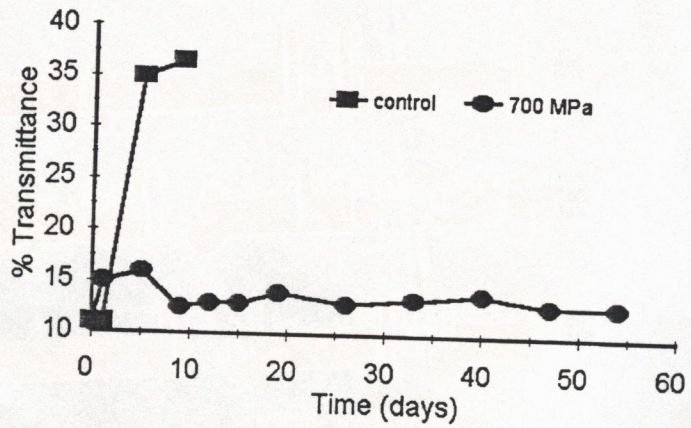
The question of whether or not the heat generated by pressurization was sufficient to inactivate PE was considered. Samples placed in the unit at 40°- 50°F reached temperatures between 68° - 120°F (measured by a thermocouple) depending on set point pressure. Immediate cooling occurred upon decompression. Morild (1992) described the temperature change due to pressure changes as 1.86×10^{-3} °K bar^{-1}. After adjusting the equation to the heat capacity of our pressure medium and converting to MPa, the conversion factor becomes 4.8×10^{-2} °K MPa^{-1}. At the highest pressure used, 900 MPa (130,000 psi), the maximum theoretical temperature increase is 43.2°C, suggesting that temperatures generated by pressures used in these studies were not sufficient to thermally inactivate PE.

The time necessary to reduce activity one log cycle, or 90% at a given pressure, is defined as the D_p value. The D_p value of PE in orange juice for 600 MPa was 143 seconds (2.4 min), while the D_p value for 500 MPa was 5000 seconds (83.3 minutes). This represents inactivation of the heat (and pressure) labile forms of PE. The corresponding zp value was 65 MPa. Basak and Ramaswamy (1996) report a D_p value of 260 minutes at pH 3.7 and 14 minutes at pH 3.2 at 400 MPA. Our juice was in the middle of this pH range at 3.45. For comparison, the temperature necessary to accomplish 90% PE inactivation in less than a minute was reported as 185°F by Rouse and Atkins (1952).

CLOUD LOSS

Since some PE activity remained in juice, it was of interest to determine the stability of the cloud after pressure treatment. Cloud is considered "definitely" broken, or lost, when percent transmittance reaches 36% (Redd et al., 1986). Figure 5 summarizes the cloud loss over time in pressurized as well as untreated orange juice. PE activity was 1.3 PEu x 10^3 (g^{-1} min^{-1}) before pressure treatment and 0.24 PEu x 10^3 (g^{-1} min^{-1}) after. High pressure is very effective in preventing cloud loss for >50 days and is associated with inactivation of PE in orange juice, even though 18% of the initial activity remains.

FIGURE 5. Cloud loss in pressurized (700 MPa for 1 minute) and untreated orange juice stored at 40°F



CONCLUSIONS

High pressure has been shown to be useful for the inactivation of microorganisms and PE in orange and grapefruit juice, and is a potentially useful tool for extending the shelf life of juice. Since very little heat is generated, the process should result in preserving fresh taste and color of juice products, a definite advantage over thermal processing. Experimental results indicate pressure inactivation of a high percentage of PE and stabilization of orange juice cloud for an extended period. For most efficient inactivation of PE in these juices, pressures greater than 600 MPa (87,000 psi) should be used; however, lower pressures may inactivate certain yeasts and ascospores. Future engineering research related to commercial implementation of this process should deal with the possibilities of making this a continuous process. Quality studies might deal with the fact that not all food enzymes or microbes are inactivated by pressure and the consequences of having remaining active enzymes in a packaged product.

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