UTILIZING HYDRODYNAMIC CAVITATION AND ULTRAVIOLET IRRADIATION TO IMPROVE THE SAFTEY OF MINIMALLY PROCESSED FLUID FOOD

by

PAUL JESSE MILLY

(Under the Direction of Romeo T. Toledo)

ABSTRACT

A Shockwave Power^{TM} Reactor (SPR) was utilized to induce hydrodynamic cavitation and increase mass transfer of fluid from bulk fluid to an ultraviolet irradiated surface for minimally processing fluid food. Hydrodynamic cavitation is the formation of gas bubbles in a fluid due to pressure fluctuations induced by mechanical means. Details of the novel equipment design are presented and energy consumption was compared to conventional thermal food processing technologies. Energy input was less than 220 kJ/kg and conversion efficiency from electrical to thermal was 55 to 85%. Calcium-fortified apple juice processed at 3000 rpm and 3600 rpm rotor speed on the reactor went through a transient temperature change from 20˚C to 65.6˚C or 76.7˚C and the total process lethality exceeded 5-log reduction of *Lactobacillus plantarum*, *Lactobacillus sakei* and *Zygosaccharomyces bailii* vegetative cells and ascospores. Tomato juice inoculated with *Bacillus coagulans* spores and processed at 3000 rpm and 3600 rpm had transient temperature from 37.8˚C to 93.3˚C or 104.4˚C had viable CFU reduced 0.88 and 3.10 log cycles, respectively. Skim milk inoculated with P.A. 3679 spores and processed at 3000 rpm or 3600 rpm had transient temperature from 48.9˚C to 104.4˚C or 115.6˚C had CFU reduced 0.69 and 2.84 log cycles, respectively. A KI to I_3^- chemical dosimeter for UV was used to quantify photons received by fluid in the annular space of the SPR. UV dose (J/m^2) increased from 97 J/m^2 at 0 rpm to over 700 J/m^2 for SPR speeds above 2400 rpm. Inactivation of *E. coli* 25922 in apple juice and skim milk in the UV-SPR at exit temperatures below 45˚C was greater than 4.5 and 3 logs, respectively. Utilizing hydrodynamic cavitation and ultraviolet radiation to obtain minimally processed pasteurized low acid and commercially sterilized high acid fluid foods is possible with appropriate process considerations for different products.

INDEX WORDS: Hydrodynamic cavitation, ultraviolet, minimally processed foods, actinometry, spoilage bacteria and yeast

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by

PAUL JESSE MILLY

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PAUL JESSE MILLY

Major Professor: Romeo T. Toledo

Committee: Jinru Chen
Mark A. Harrison
William L. Kerr
Ruthann B. Swanson

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

I am dedicating this work to above all GOD. He has given me strength and will power required to pursue my goals in life. Without Him, I would be lost.

I would also like to dedicate all my accomplishments to my mother and father, Patricia Ann Melvin-Milly and Donald Francis Milly, respectively. Without their Love, friendship, advice, tolerance, patience, humor, strength and support, I would have nothing to offer this world. Everything that I am, I owe to them and their Love for each other and our family. I must never forget my two sisters, Janine Ann and Jenna Lynn; they will always be my reminders of how important family is. Dr. Florent Cros, my excellent brother-in-law, remains a constant source of encouragement, inspiration and support. I hope that as I grow, I will be able to exude all the qualities he possesses. I Love you all.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Purpose of Study

The purpose for conducting research included in the subsequent chapters is to prove the suitability of alternative food processing technologies such as hydrodynamic cavitation and ultraviolet light as a pasteurization or commercial sterilization process. The 2000 report published by United States Food and Drug Administration, Center for Food Safety and Applied Nutrition entitled "Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: Executive Summary" states a clear and concise need for additional research pertaining to the exploration and validation of alternative food processing technologies as a means of producing safe food that is sold and consumed in the United States (CFSAN 2000). Innovative and cutting edge technologies will need to be validated through scientific research to provide "proof of principle" to ensure the effectiveness of the new technology.

Consumers are increasingly demanding healthy and more wholesome foods and this demand is driving the market for minimally processed foods. Beyond great taste, today's consumers expect food products to be wholesome, convenient, have extended shelf-life, have acceptable ratio of caloric and essential nutrient content, provide essential dietary nutrients, and be packaged in environmentally sound materials. Minimally processed foods are perceived by consumers to be healthier, more nutritious and more satisfying to the palate than traditionally processed foods. This association is primarily due to the least severe temperature exposure of the product required to render the food free of pathogenic microorganisms. However, novel food processing technologies require a different approach to achieve these benefits to the consumer. The main challenge for food producers or equipment manufacturers that utilize novel

technologies is complying with regulatory constraints on new technology. Regulations for alternative food processing technology implementation do not address specific equipment design or configuration, but rather on sound research on the fundamental scientific principles governing the technologies' efficacy. The basic research studies performed on the innovative technology should cover the scientific basis for the technology, its efficacy in inactivating microorganisms with which it is challenged, and results should yield a convincing foundation from which the technology can be successfully commercialized.

FDA's 1998 final rule (63 FR 37030) for processing fruit and/or vegetable juice requires processors to label products with a warning of potential illness upon consumption of the product unless the product was processed to inactivate the most likely of pathogenic bacteria in the produtct by 5-log. This rule made processors employ conventional heat treatments for pasteurization to ensure that a 5-log reduction has been achieved to avoid the use of a warning label. Processors fear that the warning label may negatively impact consumers' decision to purchase the product. More recently, the food processing industry has turned to non-thermal processing techniques to achieve the 5-log reduction of pathogens while minimizing heat exposure of the product (Morris 2000). In essence, the product can be rendered microbially safe while maintaining the essential nutrients, the phytochemical-health-functional components and organoleptic properties of the original unprocessed juices (Shomer 1994; Rye 2003). In light of these advantages of non-thermal processing technologies on quality, processors must acknowledge that today's consumers are better educated about minimally processed juices and that there is increased consumer demand for health-functional foods (Deliza and others 2005). According to by The Institute of Medicine's Food and Nutrition Board (IOM/FNB), functional

foods are “any food or food ingredient that may provide a health benefit beyond the traditional dietary nutrients it contains” (Senorans and others 2003).

Utilizing non-thermal processing techniques like hydrodynamic cavitation and ultraviolet irradiation would inactivate pathogens and because of the low temperature exposure the nutritional/nutraceutical components in the product are preserved. Certain fruit juices known to be rich in phytochemicals with health-functional properties can be processed with minimal damage to the overall nutritional/functional properties of the product (Konja 1993; McLellan 1993; Shahidi 2004a; Shahidi 2004b). In addition to the preservation of health promoting phytochemicals, research has shown that declaring the use of an alternative food processing technology in conjunction with a brief explanation of the technology elicits a positive response in consumers’ decision to purchase a product (Deliza and others 2005).

Innovative and novel equipment design for food applications must be validated for its efficacy in producing safe and wholesome foods by microbial challenge tests using surrogates for pathogenic microorganisms. The CFSAN’s report on kinetics of microbial inactivation under the conditions applied in the alternative food processing technologies clearly states the rising need for more research in validation of the innovative food processing technology. The following sections address fundamental scientific principles that govern the action of hydrodynamic cavitation by itself and in conjunction with ultraviolet light. Microbial challenge protocols and results are discussed in subsequent chapters. The overall intent of this research is to lay the groundwork for eventual commercialization of this technology for food processing.

1.2 Alternative Food Processing Technologies

Alternative food processing technologies (e.g. high pressure processing, controlled cavitation, high-intensity pulsed electric field, ohmic and inductive heating, high voltage arc

discharge, chemical treatments, oscillating magnetic fields, irradiation, microwave and radio-frequency heating and ultrasound) are defined as food processing tools that do not solely rely upon thermal inactivation to achieve microbial destruction. Instead these alternatives to thermal treatments depend upon other physical processes that affect the biological activity of microorganisms. For example, high pressure processing does not depend upon temperature and time as with heat treatments but rather on time, temperature and pressure to inactivate microorganisms. Alternative food processing technologies are often used as part of a "hurdle" concept in preserving food. The "hurdle" concept utilizes several intrinsic factors (e.g. pH, water activity, oxidation-reduction potential (Eh), nutrient content, antimicrobial components) and extrinsic factors (e.g. temperature of storage, relative humidity of environment, presence and concentration of gases, presence of competitive microorganisms) factors to control the growth of spoilage and/or pathogenic microorganisms. Thus, proliferation of the target species will only occur if microorganisms can "hurdle" several impediments to growth (Jay 1998; Artes and Allende 2005).

In the present study microbial inactivation in an alternative food processing technology (controlled cavitation alone and in conjunction with ultraviolet light) and process efficacy associated with the processing parameters was investigated. Alternative food processing technologies are intended to eliminate and/or inactivate pathogenic and spoilage bacteria at reduced processing temperatures. By lowering processing temperatures product exposure to heat is minimized, development of "cooked" flavors is prevented and the "fresh-picked" flavor of the product is preserved while rendering products microbiologically stable and pathogen free.

1.3 Food and Drug Administration (FDA) Regulations

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) produced a report in 2004 entitled "Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization." The report was written in response to the Farm Security and Rural Investment Act of 2002 (FSRIA) which requested a broadening of the definition of pasteurization. The NACMCF was charged with determining specific parameters for alternative food processing methods intended to pasteurize food products. The NACMCF reached a consensus for releasing an updated definition of pasteurization. The new definition of pasteurization states: "Any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage". The executive summary states further that alternative non-thermal processes and combinations of processes are acknowledged as being just as effective as traditional thermal treatments. In addition, the report states that new pasteurization processes require research on the mechanisms of microbial inactivation and limits of the technology and to validate these processes through the use of biological challenge studies by processing authorities and to develop predictive modeling of the antimicrobial effects of the process (NACMCF 2004).

There are currently no documented regulations addressing the use of hydrodynamic cavitation as a means of pasteurizing and or commercially sterilizing fluid food. The 2004 NACMCF report gave recommendations for evaluating microbial inactivation mechanism resulting from ultrasound and these recommendations could be followed when pursuing studies on hydrodynamic cavitation.

FDA regulations recognize the use of ultraviolet radiation as an alternative food processing technology and approved its' use for "light processing" of fruit juices (Anonymous 2000; Vasavada 2003; Lopez-Malo and Palou 2005). The Code of Federal Regulations (21 CFR 179.39) discusses the specific conditions of UV radiation for disinfecting water and pasteurizing certain juices. The report published by CFSAN (2000) on ultraviolet light as an alternative food processing technology explicitly states that UV radiant exposure must be at least 400 J/m^2 is all parts of the juice to ensure disinfection.

1.4. Thermal Kinetics of Microbial Inactivation

A first order reaction describes the decrease in number of suspended microorganisms when subjected to heating at a constant temperature for a given period of time. The first order reaction rate is governed by Eq. (1):

$$-\frac{dN}{dt} = kN \tag{1}$$

Where N = number of viable organisms, t = time and k = first order rate constant for microbial inactivation.

Using an initial condition of $N = N_0$ at $t = 0$, one may integrate eq. 1 yielding Eq. (2):

$$\ln\frac{N}{N_0} = -kt \tag{2}$$

The rate constant, k, becomes the slope of the natural logarithm of survivors plotted against time. This expression suggests a linear semi-logarithmic plot of N versus t and eq. 2 can be viewed in common logarithms as seen in Eq. (3):

$$2.303 * \log\frac{N}{N_0} = -kt \quad \text{or} \quad \log\frac{N}{N_0} = -\frac{kt}{2.303} \tag{3}$$

This relationship allows for further interpretation of the traditional approach to describing microbial inactivation at a constant temperature as seen in Eq. (4):

$$\log\frac{N}{N_0} = -\frac{t}{D} \tag{4}$$

The relationship between D and the rate constant, k, presents itself in Eq. (5):

$$D = \frac{2.303}{k} \tag{5}$$

The decimal reduction time, D-value, is defined as the time needed to destroy 90% (one log cycle or one order in magnitude) of the microorganisms (reducing their number by a factor of 10) and is determined from a decimal reduction curve. Thermal resistance of cultures is determined using thermal death time (TDT)-capillary tube method procedures described by Stumbo (1973) and kinetics discussed in further detail by Toledo (2007). D-values are determined using Eq. (6):

$$D = \frac{t_2 - t_1}{\log(N_1) - \log(N_2)} \tag{6}$$

Where the difference between t_2 and t_1 denotes the time allocated for thermal treatment at a constant temp and N_1 and N_2 represent the number of survivors at their respective times.

Often in food processing, the temperature of a process is transient in nature. Changes in the D-value are temperature dependent and expressed in terms of the thermal resistance constant known as a z-value. A z-value is used to express the temperature dependence of microbial inactivation or the number of degrees Celsius required to bring about a 10 fold change (one log cycle or one order in magnitude) in the decimal reduction time or D-value. Z-value is determined by constructing a thermal death time (TDT) curve for a culture using mean D-values from varying temperatures. D-value is a function of temperature and is calculated using Eq. (7):

$$D_{NewTemp} = D_0 * 10^{\frac{T_0 - T_{NewTemp}}{z}} \tag{7}$$

Where T_o = reference temp (˚C), D_0 = reference mean D-value at T_0 and z = z-value determined from TDT curve. Eq. 4 can be further useful for determining the inactivation of a microorganism at constant temperatures and/or during transient temperature changes as seen in Eq. (8):

$$Lethality = \int_0^t \frac{1}{D_T} dt + \frac{t}{D_T} \tag{8}$$

The first expression accounts for lethality accumulated during transient temperature change and the second expression reflects lethality accumulated during product residence time at a constant temperature (Toledo 2007).

1.5 Hydrodynamic Cavitation

Cavitation is the formation and collapse of gas or vapor-filled bubbles in a liquid medium. As bubbles rapidly form and collapse, pressurized shock waves, localized heating events and tremendous shearing forces occur. Cavitation is induced acoustically or mechanically. In recent decades, acoustic cavitation gave rise to new disciplines in chemistry referred to as sonochemistry and sonoluminescence. Mechanically induced cavitation is referred to as hydrodynamic cavitation. Hydrodynamic cavitation is induced mechanically when fluids are pressurized and depressurized while flowing around or through an obstacle in the flow field. Innovative hydrodynamic cavitation reactors offer logical applications in the food industry such as degassing of fluids, releasing intracellular enzymes and metabolites, enhanced microbial inactivation, mixing, emulsification and homogenizing of fluid foods (Middelberg 1995; Save and others 1997; Young 1999; Balasundaram and Pandit 2001; Mason and Lorimer 2002).

Hydrodynamic cavitation is characterized for individual fluids by sanctioning a "*cavitation number,*" σ. The cavitation number, σ, is an index of the resistance of the flow to undergo cavitation and is denoted in Eq. (9):

$$\sigma = \frac{P_0 - P_v}{0.5 * \rho * \mu^2} \quad (9)$$

Where P_o = ambient static pressure, P_v = vapor pressure, $(0.5)*\rho*\mu^2$ = dynamic pressure where ρ = density and μ = flow velocity. Cavitation is most likely to occur when the cavitation number is below a critical value characteristic of the fluid. The type of cavitation characterized by the cavitation number transpires in rotating cylinders, propellers, hydrofoils, fluid machinery and rapidly rotating rods (Young 1999).

As fluid is forced through a configuration that induces formation of eddy currents, such as flow around solid surfaces or small and large cross-sectional area in the flow stream, the fluid's kinetic energy is converted into elevated velocities at the expense of a drop in fluid pressure. Localized high and low velocities in a fluid bulk develops momentary gradients of reduced vapor pressures, causing dissolved or trapped gases and fluid vapors to accumulate and expand, forming a bubble. Small particulates, such as dust, debris or biological entities provide a physical surface for bubble nuclei to attach, expand and collapse. Bubbles traveling instantaneously from regions of low to high pressure will implode. The collapse of a vapor-filled cavity is accompanied by intense pressure waves, vigorous shearing forces, localized heating events and in some cases the formation of free radicals (Leighton 1998; Earnshaw 1998; Young 1999; Mason and Lorimer 2002).

Physical stresses resulting from acoustic or hydrodynamic cavitation are understood to be the mechanisms responsible for cellular inactivation. Biological entities in the immediate area of a cavitation event endure stresses that induce severe damage to cell walls and ultimately inactivate the organism (Frizzell 1988; Earnshaw and others 1995; Earnshaw 1998; Young 1999; Geciova and others 2002; Piyasena and others 2003). Earnshaw (1998) cites research suggesting that larger cells such as yeast (5 – 20 μm) are more susceptible to the effects of cavitation due to

their larger surface area. Gram-positive cells were once thought to be more resistant to cavitation than the gram-negative cells due to additional layers of peptidoglycans in the former; however, more recent literature suggests no significant difference between gram-negative and gram-positive microorganisms in their resistance to inactivation by cavitation. Spores of bacteria like *Bacillus* and *Clostridium* possess a higher tolerance to cavitational effects compared to vegetative cells (Earnshaw 1998).

Majority of research investigating cellular disruption via hydrodynamic cavitation employed some variation of venturi configuration or multi-orifice plate, which permitted sample collection after one pass or one cavitation event. These studies examined the efficacy of hydrodynamic cavitation when disinfecting wastewater (Jyoti and Pandit 2001; Gogate 2002; Sivakumar and Pandit 2002; Jyoti and Pandit 2004). In addition to wastewater treatment, increasing focus is being placed on hydrodynamic cavitation as a means of achieving cellular disruption or producing nano sized suspended particles in large scale operations with improved process control and reduced energy costs (Save and others 1994; Save and others 1997).

1.6 Ultraviolet Radiation

The adoption of germicidal UV treatment in water and waste water processing facilities has generated a number of studies validating the efficacy of UV for processing transparent fluids (Liltved and Cripps 1999; Sommer and others 2000; NACMCF 2004). Germicidal ultraviolet light (UVC) at 200 to 280 nm wavelength has proved to be successful for inactivating pathogenic microorganisms like *E. coli* O157:H7 and *Cryptosporidium parvum* in processed fruit juices such as apple juice and cider (Wright and others 2000; Vasavada 2003). Interest in UV treatment of fluid food as an alternative to conventional heat treatment continues to grow. Processors concerned with product flavor and nutrient changes brought on by conventional heat treatment

continue to view UV processing as a favorable alternative. In addition, the equipment required for effective thermal pasteurization may be too high-priced for small operations.

Ultraviolet (UV) radiation is considered a physical treatment for achieving disinfection of certain foods. Primarily used in water disinfection and waste water processing, the intended use is to disinfect, not sterilize. Disinfection refers to the inactivation of vegetative cells and in some cases the inactivation of pathogenic bacterial spores. Radiation is the emission and proliferation of energy through space or a given substance. UV radiation is defined as part of the electromagnetic spectrum displaying wavelengths shorter than the visible region (380 – 800 nm), but longer than X-rays (0.1 – 13 nm). In general, UV radiation wavelengths can range from 1 to 400 nm with subdivisions consisting of long-wave UV (UVA: 315 – 400), medium wave UV (UVB: 280 – 315 nm) and short-wave UV (UVC: 200 – 280 nm) (Lopez-Malo and Palou 2005). Figure 1.1 displays the electromagnetic spectrum in its' entirety. Figure 1.2 allows one to see the subdivisions among the UV radiation range. Bactericidal effects are observed closest to the wavelength of 260 nm (Jay 1998).

The energy provided by UV radiation is non-ionizing and is readily absorbed by proteins and nucleic acids. The photochemical changes at the cellular level are responsible for the bactericidal effect observed in microorganisms exposed to UV radiation. The cell does not die immediately, but rather procreates into mutated and less suited for survival offspring. The exposure to UV radiation results in cross-linking of thymine dimers within the DNA of exposed organism. Figure 1.3 illustrates this photochemical effect.

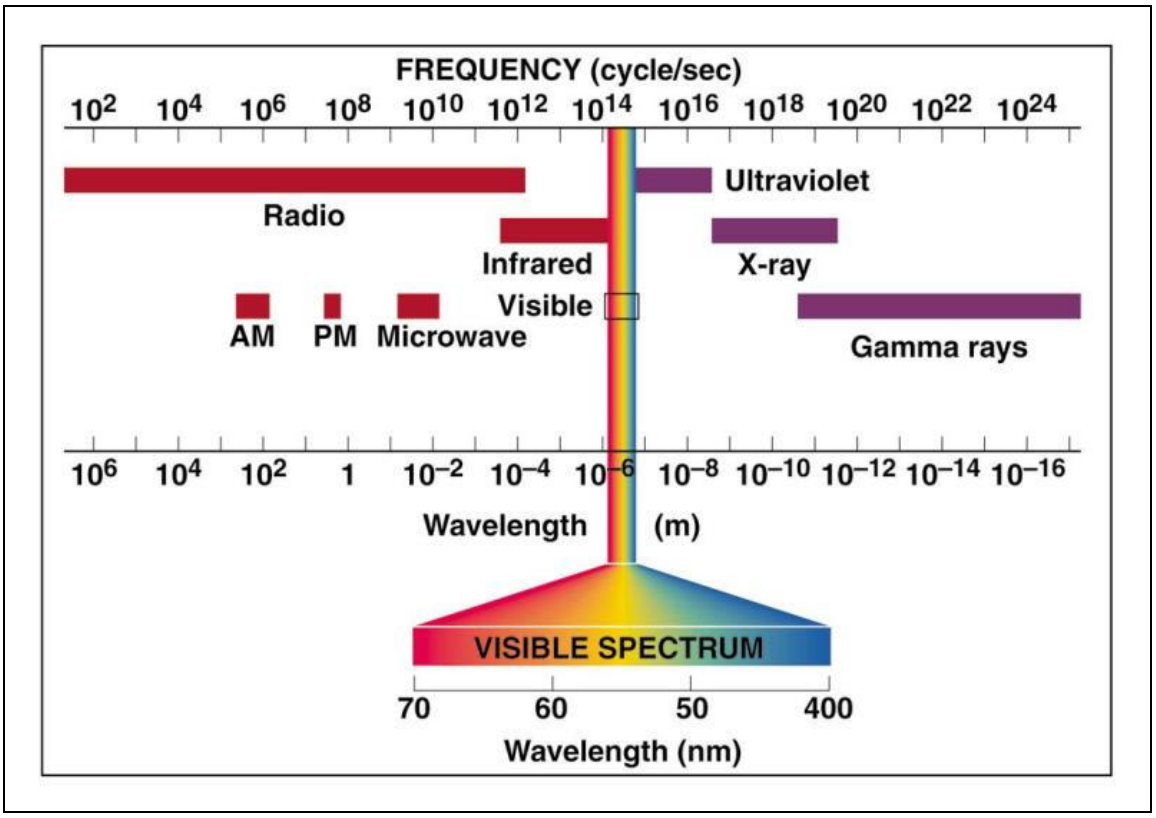

Figure 1.1 – Electromagnetic spectrum (taken from http://rst.gsfc.nasa.gov/Intro/emspec.jpg)

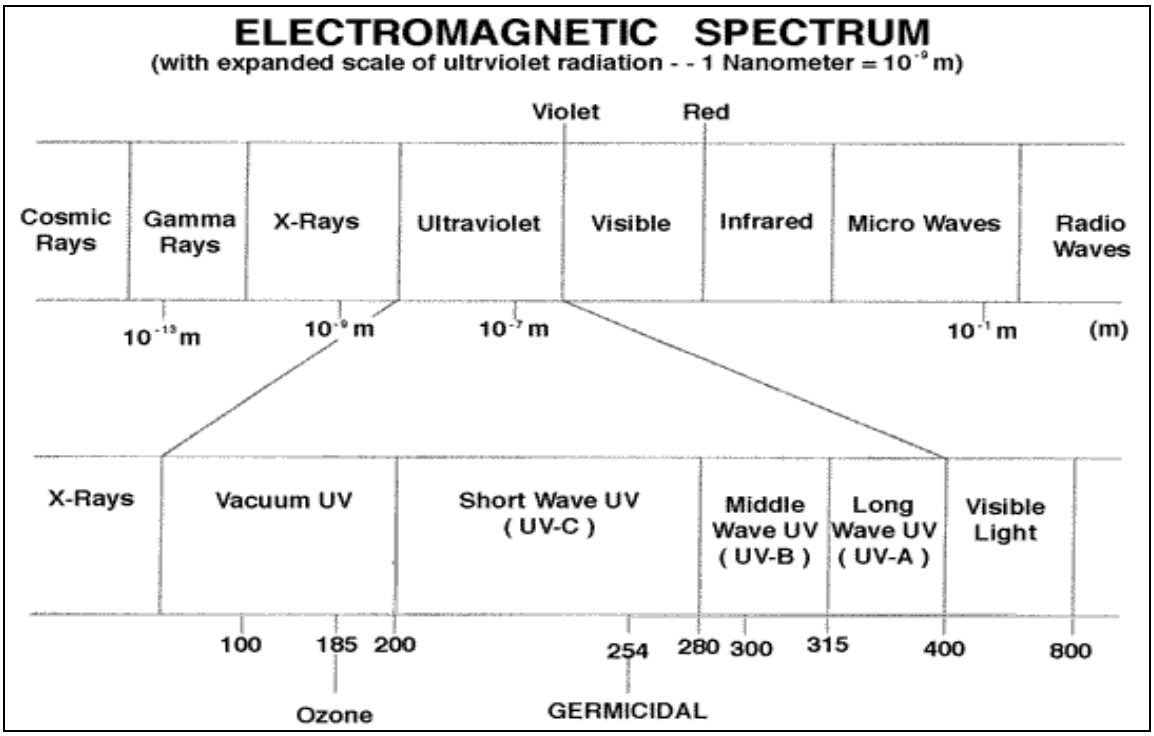

Figure 1.2 – Electromagnetic spectrum subdivisions of ultraviolet region (taken from www.uvlp.ca/images/electromagneticspectrum40x.gif).

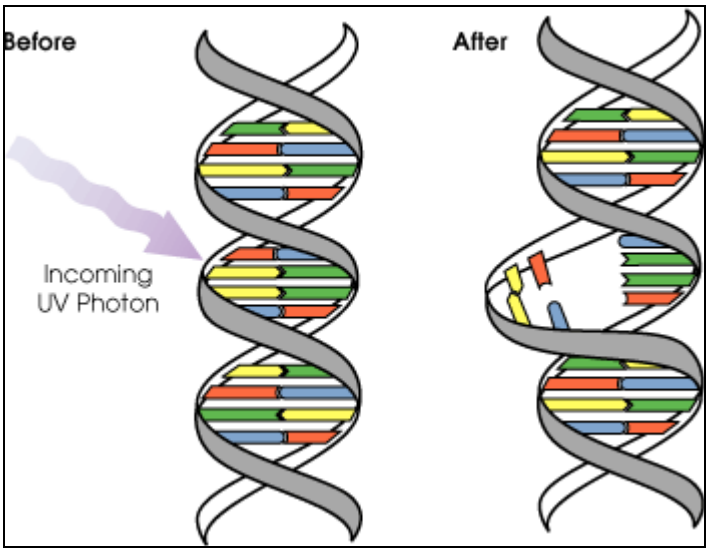

Figure 1.3 – Photochemical mutagenesis in DNA resulting in thymine dimer (taken from www.americanairandwater.com/images/DNA-UV.gif ; accessed March 2007)

This photochemical reaction inhibits the microorganism's capacity to effectively repair injury or successfully reproduce (Vasavada 2003). UV light is documented to produce ozone, a known antimicrobial agent, when processing fruits and vegetables (Jay 1998). However, due to the weak penetration capacity of UV radiation, use of UV technology is limited to surface decontamination and sterilization applications. In addition, opaque fluid foods and/or fluid foods containing particulates, like citrus juices, provide "shading effects" which allow for microbial survival from UV radiation. Most UV applications can be found in waste water treatment plants, where the fluid is "cleaned" of any particulates before exposure to high intensity UV radiation for decontamination and/or sterilization (CFSAN 2000).

1.7 Shockwave Power™ Reactor

A Shockwave Power™ Reactor (SPR) System was constructed by Hydrodynamics Inc., Rome, GA and placed at the University of Georgia, Food Science and Technology pilot plant. The 0.254 m x 0.0508 m unit consisted of concentric cylinders with an annular space (2.91 L volume) separating the stationary outer cylinder from the inner, rotating cylinder. The rotating

cylinder is driven by a 12 HP motor and generates cavitation in the fluid in the annular space when fluid enter and exit cavities on the surface of the spinning cylinder. Fig. 1.4 is a schematic diagram of the innovative SPR equipment design and Fig. 1.5 is a photograph of the unit used in this research. Temperature rise in the SPR is a function of the speed of rotation (mechanical energy input) of the rotating inner cylinder and the rate of fluid flow through the unit. The SPR is equipped with a variable frequency speed controller. Fluid flow rates were regulated in order to achieve the desired end point processing temperatures. A ¼ HP Procon Pump (Series 3 Procon Pump, Procon Productions, Murfreesboro, TN. U.S.A) was used to feed fluid to the SPR and a system of valves recycled the pump output allowing only the desired rate of fluid feed to the SPR. The fluid in the SPR was maintained at 344.738 ± 6.895 kPa (50 ± 1 psi_g) in all trials. The inner, rotating cylinder houses multiple cavities where fluid is pressurized and depressurized many times, causing hydrodynamic cavitation to occur. This type of cavitation is deemed to be "vortex" cavitation and occurs in the cavities of the inner rotor (Fig. 1.6).

This novel design allows for a continuous flow of fluid to replenish multiple cavities as the rotor spins, facilitating cavitation and product flow through the system. Centrifugal forces from the inner, rotating cylinder are countered with centripetal forces to avoid "traveling" cavitation, or cavitation that "spills" out of the cavity and travels co-current through the system. Pressurizing the system during operation ensures continuous product contact with the inner rotor resulting in the fluid undergoing controlled cavitation. As product enters the SPR annular space with the rotor set at a specified operational speed (rpm), the mass rate of flow of product dictates the frequency of cavitational events and ultimately the end exit temperature. As cavitation occurs, the fluid bulk receives energy dissipated by the bubbles in the vapor phase as the bubbles collapse, thus the fluid in immediate contact with the bubbles before collapse will have

instantaneous temperatures higher than the average temperature of fluid leaving the annular space. The exposure to temperatures exceeding that of the system's average allows microbial inactivation to exceed what can be predicted using microbial inactivation kinetics based on the fluid transient time-temperature history in the annular space and the residence time after leaving the SPR prior to sampling.

Fig.1.4 - Schematic of the Shockwave Power™ Reactor (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

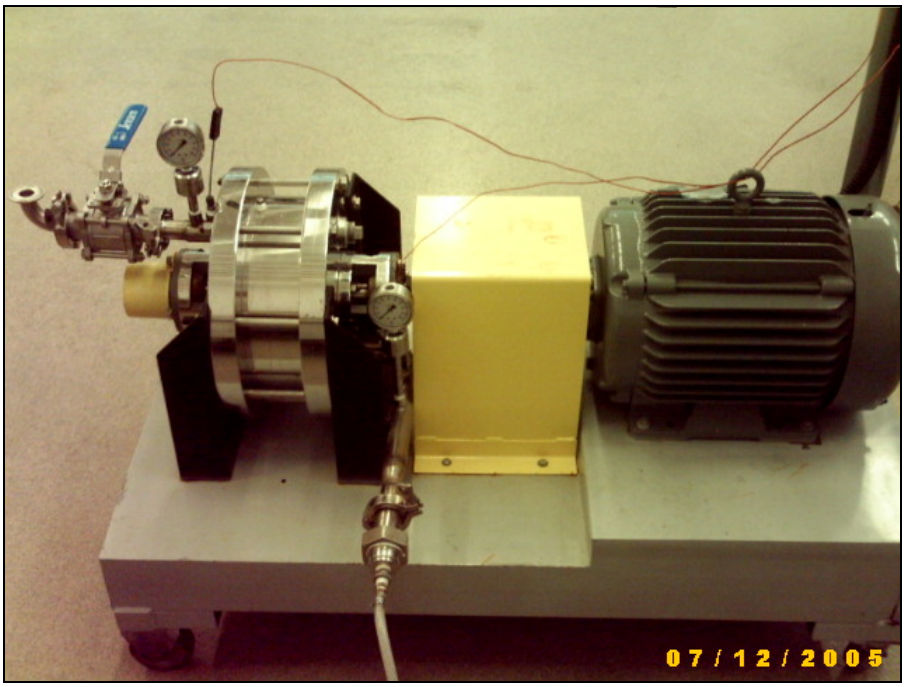

Fig. 1.5 - 12 HP Shockwave Power™ Reactor (10” x 2” pilot scale unit) (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

Fig. 1.6 - Hydrodynamic cavitation within inner rotor cavities (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

1.8 Ultraviolet-Shockwave Power™ Reactor

An Ultraviolet Shockwave™ Power Reactor (UV-SPR™) system constructed by Hydrodynamics Inc., Rome, GA was placed at the University of Georgia, Food Science and Technology pilot plant (Fig. 1.7). The 0.1524 m x 0.19685 m (6 inch diameter x 7.75 inch length) unit consisted of concentric cylinders with an annular space (2.16 L) separating the stationary housing cylinder from the inner, rotating cylinder. The system was powered by a 12 HP motor and generates cavitation in the annular space filled with fluid when the inner rotor which contains multiple cavities on its surface is rotated at high speeds. The unit was surrounded by four 20.32 cm long, high output, low pressure mercury ultraviolet (UV) lamps (Model GPH203T5VH/HO/4P, Lightsources Inc., Orange, CT) each with a rated intensity of 42 $\mu W/cm^2$ at 1 meter plus four 26.3 cm long, high output, low pressure mercury UV lamps (Strahler NNI 60/26 U, Heraeus Noblelight GmbH, Heraeusstraße 12-14 D-63450 Hanau Germany) with a rated intensity of 0.15 mW/cm^2 at 100 cm. Figure 1.8 is the actual pilot scale unit without UV lamps illuminated and Figure 1.9 displays the unit while being irradiated. The stationary outer cylinder was made of 3 mm thick quartz which allowed UV irradiation to penetrate the annular space. The outside surface area of the quartz housing was 0.094 m^2. Nominal power ratings were 18 and 90 W, respectively, for the Lightsource and Heraeus lamps, and the output had a primary wavelength of 254 nm. Lamps were positioned parallel to the outer quartz cylinder axis at 60° intervals around the periphery. Taylor-Couette flow was induced as the inner rotor spins while fluid transferred from the annular space to the cavities on the rotor surface exits the cavity and forced toward the outer cylinder increasing product exposure to UV irradiation. Figure 4.4 illustrates the formation of bubbles as the inner rotor spins. The UV-SPR system pressure was maintained at 75.84 ± 6.895 kPa (11 ± 1 psi_g).

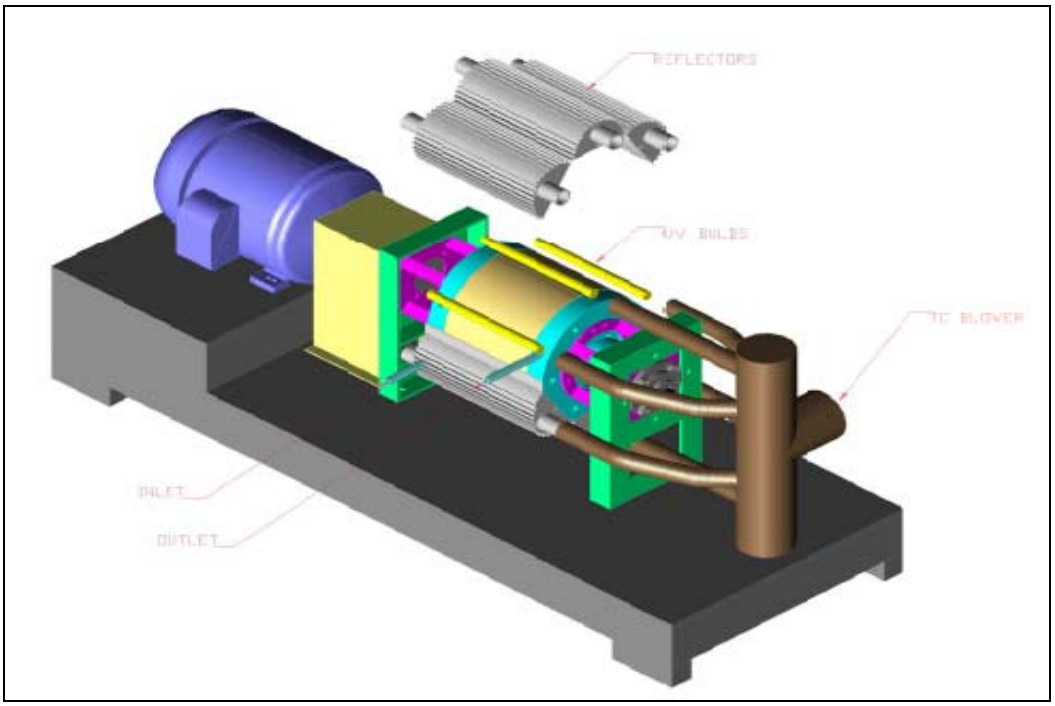

Figure 1.7 - Schematic of the Ultraviolet-Shockwave Power™ Reactor (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

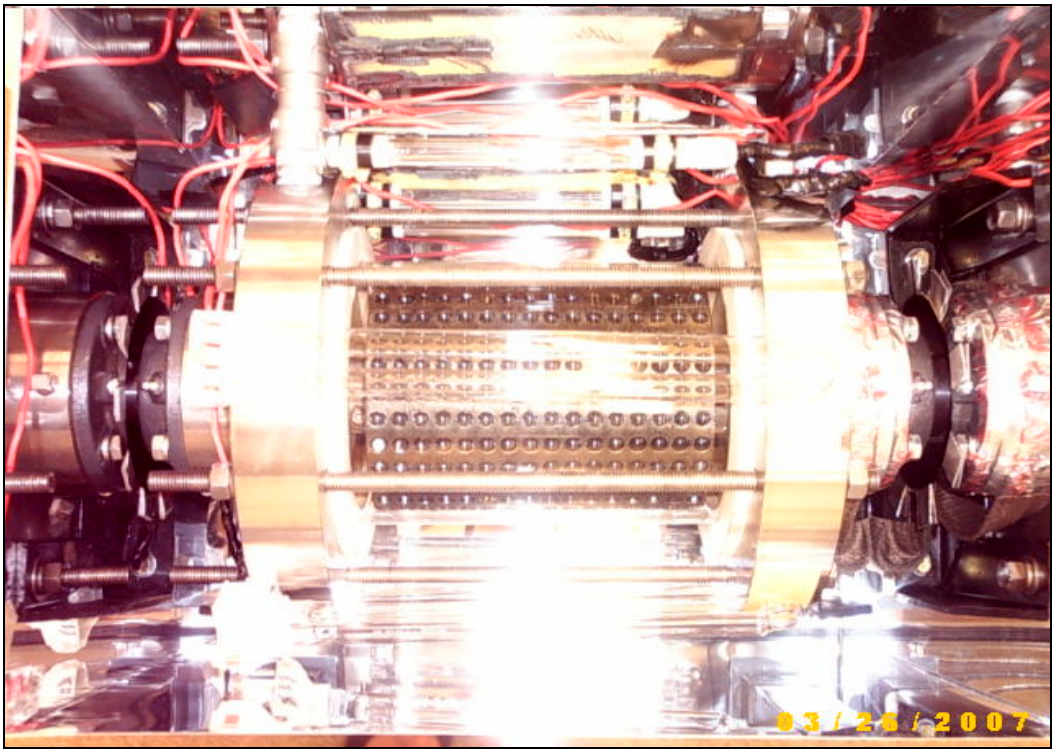

Fig. 1.8 - 12 HP UV-Shockwave Power™ Reactor (6” x 7.75” pilot scale unit) without UV illumination (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

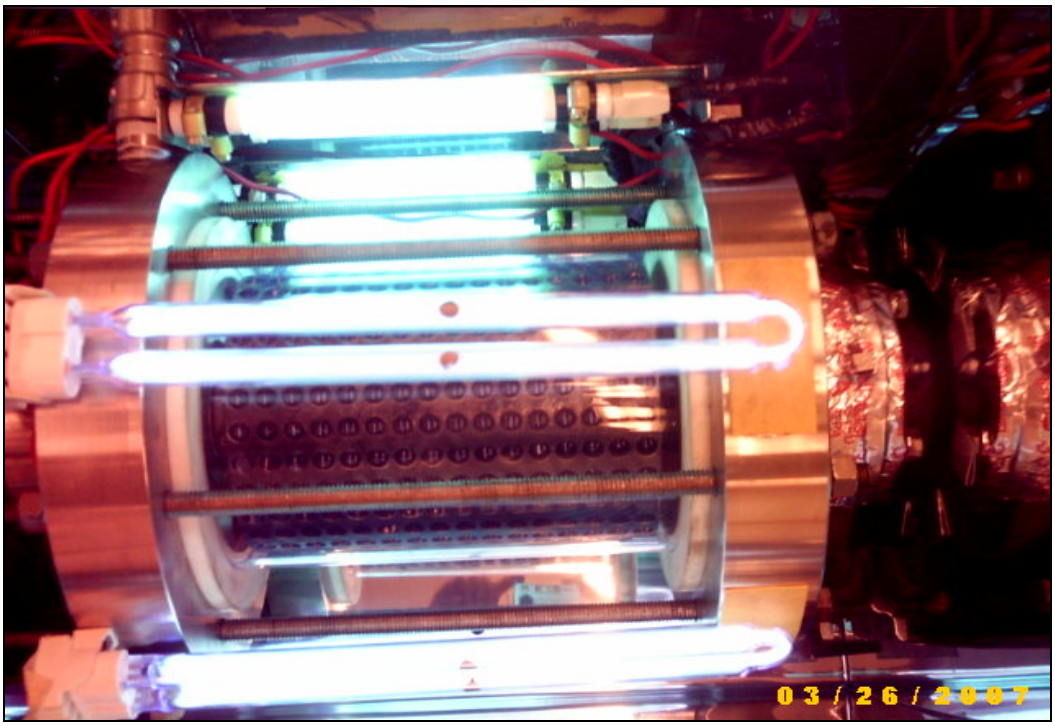

Fig. 1.9 - 12 HP UV-Shockwave Power™ Reactor (6" x 7.75" pilot scale unit) with UV illumination (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

1.9 Chemical Actinometry

The use of a chemical actinometer or dosimeter consisting of a light-induced chemical reaction to manifest a quantum yield, φ, is well known. Quantum yield is defined as the number of events, molecules formed, changed, or destroyed divided by the number of photons absorbed at a particular wavelength in the same period of time (Kuhn and others 2004). The Rahn actinometer (Rahn 1997) permits measurement of UV photons (253.7 nm) that result in the photochemical conversion of iodide (I^-) to triiodide (I_3^-). The actinometer allows determination of UV energy flux or UV dose (J/m^2) absorbed by a solution flowing past an irradiated surface.

The Rahn actinometer (Rahn 1997) can be used to determine the effect of rotational speed in the UV-SPR™ on the UV energy received by fluid flowing through the annular space. The actinometer is based on the conversion of iodide (I^-) to triiodide (I_3^-), with the latter measured by absorbance at 352 nm. The photochemical reaction is forced out of equilibrium

towards the complete stoichiometric conversion of photons and iodide to triiodide by the presence of KIO_3 and a pH of 9.14 with a 0.01 M $Na_2B_4O_7.10H_2O$ buffer as seen in Eq. (10):

$$8KI + KIO_3 + 3H_2O + hv \rightarrow 3I_3^- + 6OH^- + 9K^+ \quad (10)$$

In order to determine the concentration of triiodide, Beer's law was utilized as seen in Eq. (11):

$$A_{352} = (\varepsilon) * (b) * (c) = -\log \frac{I}{I_0} \quad (11)$$

Where A_{352} = optical density of the solution or the energy of radiation reaching a given area of a detector per second at a wavelength of 352 nm (I_0 = initial radiant intensity and I = remaining radiant energy), ε = molar absorptivity or extinction coefficient ($M^{-1}*cm^{-1}$), b = path length of radiation (cm) and *c* = molar concentration (moles/L). According to Skoog and West (1965), the solubility of Iodine (I_2) in de-ionized water will be slightly higher than 1.0×10^{-3} mol/L at room temperature. Because the molar absorptivity coefficient changes with varying solvent systems, addition of known volumes of saturated Iodine solution to 0.15 *M* KI and Rahn's recommended standard solution allowed for calculation of a molar absorptivity coefficient. Rahn's recommended standard solution consisted of 0.6 *M* KI, 0.1 *M* KIO_3 and 0.01 *M* $Na_2B_4O_7*10H_2O$ yielding a solution with pH of 9.14. With the use of an ultraviolet-visible light spectrophotometer, the wavelength for maximum absorbance and molar absorptivity coefficient (L/mol*cm) can be determined. A wavelength of 352 nm is suggested to provide the maximum absorbance in both solutions. The molar absorptivity coefficient for triiodide in 0.15 *M* KI has been reported to range from 25,800 and 26,400 $M^{-1}*cm^{-1}$, with an average value of 26,100 $M^{-1}*cm^{-1}$ (Rahn and others 2003). Based on the average molar absorptivity value and observed absorbance value for triiodide in 0.15 *M* KI, the molar concentration of the saturated Iodine solution can be determined. A molar absorptivity value of 26,600 $M^{-1}*cm^{-1}$ can be used to

quantify molar concentrations of triiodide in processed samples from obtained absorbance values at 352 nm in a spectrophotometer.

Once the molar concentration of triiodide is determined from processed samples, the energy delivered by the system to the processed fluid can be calculated. The quantum yield of the KI-KIO_3 actinometer is 0.73 moles I_3^-/mole photon (Rahn and others 2003). The UV energy absorbed by the solution is calculated by Eq. (12):

$$E = \frac{c * \alpha * q}{\varphi} \tag{12}$$

where E = energy absorbed by fluid (J/s), *c* = molar concentration of triiodide (moles/L), α = J/mole photon, q = flow rate through UV-SPR (L/s) and φ = quantum yield = 0.73 moles triiodide/mole photon.

In order to use eq. 12, one must determine α, the amount of energy delivered by one mole of photons. This value is calculated using several fundamental equations from introductory physics. The energy of a photon of electromagnetic radiation can be determined by first calculating the frequency of photons delivered by an electromagnetic source. This can be calculated using Eq. (13):

$$\lambda = \frac{c}{f} \tag{13}$$

Where λ = wavelength of electromagnetic energy (254 nm), c = speed of light, 3.00 x 10^8 m/s and f = frequency of photons delivered (s^{-1}). In this case, the frequency of photons delivered is calculated to be 1.18 x 10^{15} s^{-1}. The next step is to determine the amount of energy a photon of specific electromagnetic radiation can provide and is calculated using Eq. (14):

$$E = h * f = \frac{h * c}{\lambda} \tag{14}$$

Where E = energy delivered by electromagnetic radiation source (J), h = Plank's constant (6.63 x 10^{-34} J*s) and the remaining variables have been defined. With a known photon frequency (1.18 x 10^{15} s^{-1}) and Plank's constant, the energy delivered by one photon is calculated to be 7.82 x 10^{-19} J/photon. The amount of energy delivered by one mole of photons or α is calculated by multiplying the energy of one photon by Avogadro's number (6.022 x 10^{23} photons/mole) and is equal to 4.71 x 10^{5} J/mole of photon (Sears and others 1991).

1.10 Microorganisms Used and Characteristics

The present study utilized several microorganisms that are commonly associated with food spoilage and safety concerns in the food industry. Gram-positive and gram-negative bacteria, yeast and mold were exposed to various processing regimens utilizing hydrodynamic cavitation and ultraviolet radiation in order to minimally process fluid foods. The gram-positive organisms were *Bacillus coagulans* ATCC 8038, *Clostridium sporogenes* P.A. 3679 ATCC 7955, *Lactobacillus plantarum* ATCC 21028 and *Lactobacillus sakei* ATCC 15521. The gram-negative organism used in this experiment was *Escherichia coli* ATCC 25922. The yeasts used in this experiment were *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* NRRL Y 7256.

The taxonomy of microorganisms is fickle in the general sense. However, classification for two main types of cells should be known: eukaryotic and prokaryotic. The crucial difference is the presence and absence of a membrane surrounding the cell's genetic information. A prokaryotic cell is defined as a cell that lacks a membrane-encapsulated nucleus and is characterized as usually having a single circular DNA molecule as its chromosomes. Prokaryotic cells lack additional cellular organelles such as: mitochondria, endoplasmic reticulum and golgi apparatus. The size or radius for most prokaryotes ranges from 0.5 to 3 μm with different shapes such as: spherical or coccus, cylindrical or bacillus and spiral or spirillum. Doubling times for

prokaryotic cells range from one-half to several hours and can sustain growth in an array of nutrients supplying a carbon source such as: carbohydrates, hydrocarbons, proteins, and carbon dioxide. A gram-positive cell is defined as a prokaryotic cell whose cell wall is made up of 90 percent peptidoglycan (or murein). Peptidoglycan consist of a thin sheet comprised of two sugar derivatives, N-acetylglucosamine and N-acetylmuramic acid, and amino acids L-alanine, D-alanine, D-glutamic acid and either lysine or diaminopimelic acid (DAP). Teichoic or lipoteichoic acids are associated with gram-positive cell walls and are responsible for gram-positive cells negatively charged surface. In contrast, a gram-negative cell is defined as a prokaryotic cell whose cell wall consists of only 10 percent peptidoglycan, and is surrounded by an outer membrane made up of lipoprotein, lipopolysaccharide, and other intricate macromolecules. All gram-negative have DAP in their peptidoglycan, but some gram-positive cocci have lysine instead. Peptidoglycan is referred to as the glycan tetrapeptide (Madigan and others 1997; Shuler and Kargi 2002).

Gram-positive bacteria are responsible for producing endospores in food. Endospores are cells that have been differentiated resulting in biologically dormant entities capable of withstanding extreme heat, drying, radiation, acids and chemical disinfectants. Several types of endospore morphologies exist and are known as terminal, subterminal and central. The structure of a spore is much more complex compared to vegetative cells due to an increased number of protective layers. The outermost layer of the spore consists of a thin, frail sheath of protein called the exosporium. Below the exosporium there exist several layers of spore coats made up of proteins. The spore coats cover and protect the next internal layer known as the cortex made up of loosely-bound peptidoglycan. The cortex surrounds the core or spore protoplast. The spore protoplast contains the cell wall or core wall, cytoplasmic membrane, cytoplasm and

additional cell constituents. A chemical indicator found in spores and not vegetative cells is known as dipicolinic acid. Calcium ions are found in high amount of spore structures and can be found combined with dipicolinic acid. Madigan and others (1997) report that the calcium-dipicolinic acid complex makes up 10% of endospore dry weight.

Properties of the spore include a partially dehydrated core and high calcium-dipicolinate ratio. Compared to the vegetative cell, the core of a mature spore only contains 10-30% water and is regarded to have a similar consistency to that of a gel. The pH of the core is lower than the vegetative cell and contains high levels of specific small acid-soluble spore proteins (SASPs). One function of SASPs is to bind and protect core DNA from damage induced by ultraviolet radiation, dessication and dry heat. In addition to protecting DNA from severe stresses, SASPs serve as a carbon and energy source for outgrowing vegetative cells from spores. This process is known as germination.

The process of germination involves three primary steps: activation, germination and outgrowth. Activation is achieved by heating a spore suspension at elevated, sublethal temperatures for several minutes and is commonly referred to as "heat shocking." After heat shocking, the spores are placed in a nutrient rich media and allowed to germinate. Germination involves the loss of resistance to heat and chemicals, breakdown of SASPs and loss of calcium-dipicolinate. The spore degrades its' protective barriers and begins the outgrowth step. Outgrowth involves the re-uptake of water and swelling or enlargement of the physical dimensions is visible. In addition to re-hydration, the cell begins to manufacture RNA, necessary proteins and DNA. The cell breaks through the spore coat and reassumes normal growth patterns.

Sporulation occurs when a vegetative cell experiences the lack of a required nutritive constituent. As the environment becomes deprived of available nutrients, the vegetative cell ceases to multiply and initiates sporulation. The sporulation of a vegetative cell involves over 200 genes such as *spo* and *ssp* (genes encoding for SASPs). These genes are triggered by environmental stimuli and result in the formation of spore related proteins needed for survival (Madigan and others 1997).

In contrast to prokaryotic cells, fungi (yeast, mold, and mushrooms) are classified as eukaryotes. Eukaryotic classified cells contain a membrane-enclosed nucleus along with other major organelles like vacuoles and mitochondria. Yeasts are unicellular and are typically classified by the type of reproduction they undergo: budding or fission. The size of radius for most yeast ranges from 5 to 10 μm. The shape of yeasts is usually spherical, cylindrical or oval (Madigan and others 1997; Shuler and Kargi 2002).

1.11 *Bacillus coagulans*

B. coagulans is a gram-positve, nonpathogenic, thermophile aerobe, sporulating microorganism commonly associated with the spoilage of tomato juice and soft drinks. Spores of *B. coagulans* cause flat sour spoilage in low-acid products and is characterized by lowering product pH, sour taste, slightly abnormal aroma and cloudiness in certain products. Spores have been proven difficult to destroy and are good indicators of under processed products. The organism is also associate with spoilage of canned condensed milk (Doyle and others1997; Vasavada 2003)

1.12 *Clostridium sporogenes* P.A. 3679

C. sporogenes is a gram-positive, nonpathogenic, mesophilic anaerobe, sporulating microorganism commonly used as thermal surrogate for *Clostridium botulinum* and is associated

with the spoilage of canned condense milk resulting in cans that swell or possibly explode. The spores of *C. sporogenes* prove difficult to inactivate and prove useful in biological challenges against alternative food processing technologies for minimally processing foods.

1.13 *Escherichia coli*

E. coli ATCC 25922 is a nonpathogenic, UV surrogate form of *E. coli* used as an indicator organism for testing effective means of delivering lethal UV irradiation doses to fluid foods (CFSAN 2000; Worobo 2000). *E. coli spp*. inhabit the intestinal tract of humans and warm blooded animals, and are a part of the standard facultative anaerobic microflora found within the intestines. *Escherichia* species belong to the family *Enterobacteriaceae.* They are characterized as being enteric bacteria, which falls into the phylogenetic phylum of Proteobacteria. This group is classified as having several unique characteristics such as: gram-negative straight rods, motile by body-covering flagella (or nonmotile), nonsporulating, facultative aerobes, acid producing from glucose, catalase-positive, oxidase-negative, optimal growth at 37°C, and sodium is neither required nor stimulatory (Doyle and others 1997). Differences among the surface antigens allow for serological classification between species of *E. coli*. The surface antigens are as such: the O (somatic), H (flagella), and K (capsule) antigens. Certain strains of *E. coli* can cause diarrheal illnesses and is grouped together depending on virulence characteristics, clinical set of symptoms, method of pathogenicity, and specific O:H serogroups. The most notable of the categories is that of the enterohemorrhagic *E. coli* strains (EHEC). *E. coli* O157:H7 is the most predominant cause of EHEC-related disease in the United States. Other categories of *E. coli* strains include: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), and enteroaggregative *E. coli* (EAggEC). A food contaminated with *E. coli* entails possible risk of being contaminated with other enteric

pathogenic bacteria (Doyle and others 1997). Common foods contaminated with *E. coli spp*. are meat and poultry (beef, chicken, and pork), fruits, vegetables, fruit and vegetable beverages, dairy products, water supplies used in plants and farms (Jackson and others 1997; Jay 1998).

1.14 *Lactobacillus plantarum*

L. plantarum is a gram-positive, nonsporulating, non-pathogenic microorganism utilized in this experiment. *L. plantarum* belongs to the family *Lactobacillaceae* and are considered part of the group of lactic acid bacteria. This group is classified as having several characteristics such as: often non-motile, non-sporulating, lactic acid by-product from fermentative metabolism, aerotolerant anaerobes, heterofermentative, typically restricted to environments in which sugars are present, usually catalase negative (some strains positive), and require amino acids, vitamins, purines, and pyrimidines (Madigan and others 1997).

Lactobacilli grow well in acidic habitats and can thrive in a pH range of about 4 to 5. They are often used in the dairy industry to aid in the production of yogurt and acidified milks but can present spoilage problems for certain fruit juices. Other species have been used in the fermentations of sauerkraut, silage, and pickles. They can also be used for fermented meats such as sausage (Jackson and others 1997; Madigan and others 1997; Ricke and Keeton 1997; Jay 1998).

1.15 *Lactobacillus sakei*

L. sakei is a gram-positive, nonsporulating, non-pathogenic microorganism commonly associated with spoilage and contamination of meat products. In addition to meat products, L. sakei is highly associated with plants and plant material. The thermal and pH resistance of the microorganism strengthens its use for biological challenges in high acid products such as fruit and citrus juices (Jackson and others 1997; Madigan and others 1997; Ricke and Keeton 1997).

1.16 *Saccharomyces cerevisiae*

S. cerevisiae is a unicellular fungi belonging to the family *Saccharomycetaceae*. *S. cerevisiae*'s main means of reproducing is through multilateral budding. However, *S. cerevisiae* has been shown to form filamentous growth complexes when subjected to certain growth conditions (Madigan 1997). Other traits of the yeast include an inability to metabolize lactose, optimal growth at 37°C, spherical spore former, and used for baking, wine, and beer fermentations. *S. cerevisiae* is not considered a pathogenic organism, but is associated with the spoilage of fruit juices and fruit concentrates (Deak and Beuchat 1996; Jay 1998).

1.17 *Zygosaccharomyces bailii*

Z. bailii is yeast commonly associated with the spoilage of mayonnaise, pickles, fruit concentrates, soft drinks and wines. The microorganism is non-pathogenic and presents a high tolerance to preservatives, elevated sugar levels, low acid and pasteurization processes. The organism is capable of producing heat resistant ascospores in sacs known as asci. The organism is capable of growing at pH ranges of 2 to 7, sugar contents (>70% v/v), tolerance to benzoic acid (>1000 ppm), sorbic acid (>800 ppm), sulphur dioxide (>3 ppm molecular SO_2) and alcohol tolerance (>20% v/v). The resilience of *Z. bailii* for spoiling a variety of fluid foods makes it an excellent candidate for verifying minimal process regimens (Thomas and Davenport 1985).

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CHAPTER 2

HYDRODYNAMIC CAVITATION: CHARACTERIZATION OF A NOVEL DESIGN WITH ENERGY CONSIDERATIONS FOR THE INACTIVATION OF *SACCHAROMYCES CEREVISIAE* IN APPLE JUICE[1] at 66˚C and 77˚C

[1] Milly, P.J. and R.T. Toledo. To be submitted to *Innovative Food Science and Emerging Technologies*

Abstract

A Shockwave Power™ Reactor consisting of an annulus with a rotating pock-marked inner cylinder was used to induce hydrodynamic cavitation in calcium-fortified apple juice flowing in the annular space. Lethality on *Saccharomyces cerevisiae* was assessed at processing temperatures of 65.6 and 76.7˚C. Details of the novel equipment design are presented and energy consumption was compared to conventional and pulsed electric fields processing technologies. The mean log cycle reduction of *S. cerevisiae* was 6.27 CFU/mL and all treatments resulted in non-recoverable viable cells. Lethality of *S. cerevisiae* exceeded predicted values based on experimentally determined thermal resistance. Energy input was less than 220 kJ/kg. Rotation of 3000 and 3600 rpm at flow rates greater than 1.0 L/min raised product temperature from 20˚C to 65.6 or 76.7˚C. Conversion efficiency from electrical to thermal was 55 to 84%. Hydrodynamic cavitation enhanced lethality of spoilage microorganisms in minimally processed juices and reduced energy usage.

Introduction

Cavitation is the formation and collapse of gas or vapor-filled bubbles in a liquid medium. As bubbles rapidly form and collapse, pressurized shock waves, localized heating events and tremendous shearing forces occur. Cavitation is induced acoustically or mechanically. In recent decades, acoustic cavitation gave rise to new disciplines in chemistry referred to as sonochemistry and sonoluminescence. Mechanically induced cavitation is referred to as hydrodynamic cavitation. Hydrodynamic cavitation is induced mechanically when fluids are pressurized and depressurized while flowing around or through an obstacle in the flow field. Innovative hydrodynamic cavitation reactors offer logical applications in the food industry such as degassing of fluids, releasing intracellular enzymes and metabolites, enhanced microbial inactivation, mixing, emulsification and homogenizing of fluid foods (Middelberg, 1995; Save, Pandit, & Joshi, 1997; Young, 1999; Balasundaram & Pandit, 2001; Mason & Lorimer, 2002).

Hydrodynamic cavitation is characterized for individual fluids by sanctioning a "*cavitation number,*" σ. The cavitation number, σ, is an index of the resistance of the flow to undergo cavitation and is denoted as:

$$\sigma = (P_o - P_v) / (0.5*\rho*\mu^2)$$

Where P_o = ambient static pressure, P_v = vapor pressure, $(0.5)*\rho*\mu^2$ = dynamic pressure where ρ = density and μ = flow velocity. Cavitation is most likely to occur when the cavitation number is below a critical value characteristic of the fluid. The type of cavitation characterized by the cavitation number transpires in rotating cylinders, propellers, hydrofoils, fluid machinery and rapidly rotating rods (Young 1999).

Previous works have been conducted for the modeling of specific hydrodynamic cavitation reactors. The equipment studied is limited to hydrodynamic cavitation induced by

forcing liquid through various flow restrictions and/or plated orifices, allowing a single cavitation event to occur and continue downstream (Moholkar & Pandit, 2001; Kanthale, Gogate, Pandit & Wilhelm, 2005; Gogate & Pandit, 2005). Hydrodynamic cavitation applied on products processed using microwaves eliminates scaling and generates heat instantaneously within the product. We evaluated a pioneering design for a hydrodynamic cavitation reactor which generates "controlled" cavitation with increased cavitational events per unit weight of fluid processed.

Reducing energy consumption, achieving microbial lethality at reduced temperatures and maintaining product integrity during processing drives the need for alternative food processing technologies (CFSAN, 2000). We hypothesized that the unique hydrodynamic cavitation reactor could be used to process apple juice with reduced energy input per unit weight of apple juice to achieve adequate lethality of a common spoilage microorganism (*Saccharomyces cerevisiae*) to prevent spoilage. The cavitation and associated temperature rise is expected to result in improved product quality since the product temperature only reaches 65.6 ˚C to 76.7˚C) compared to conventional pasteurization temperatures of 88°C for 15 s: (Fellows, 1997).

The objectives of this study were to quantify the energy delivered by controlled cavitation per unit weight of apple juice, compare energy requirements between controlled cavitation and conventional pasteurization when processing apple juice and to determine if the level of lethality imposed upon *S. cerevisiae* from hydrodynamic cavitation exceeded that calculated value based on thermal inactivation kinetics of the test organism and fluid temperatures measured.

Materials and Methods

Hydrodynamic cavitation reactor

A Shockwave Power^{TM} Reactor (SPR) System was constructed by Hydrodynamics Inc., Rome, GA and placed at the University of Georgia, Food Science and Technology pilot plant. The 0.254 m x 0.0508 m unit consisted of concentric cylinders with an annular space (2.91 L volume) separating the stationary outer cylinder from the inner, rotating cylinder. The rotating cylinder is driven by a 12 HP motor and generates cavitation in the fluid in the annular space when fluid enter and exit cavities on the surface of the spinning cylinder. Fig. 2.1 is a schematic diagram of the innovative SPR equipment design and Fig. 2.2 is a photograph of the unit used in this research. Temperature rise in the SPR is a function of the speed of rotation (mechanical energy input) of the rotating inner cylinder and the rate of fluid flow through the unit. The SPR is equipped with a variable frequency speed controller. Fluid flow rates were regulated in order to achieve the desired end point processing temperatures. A ¼ HP Procon Pump (Series 3 Procon Pump, Procon Productions, Murfreesboro, TN. U.S.A) was used to feed fluid to the SPR and a system of valves recycled the pump output allowing only the desired rate of fluid feed to the SPR. The fluid in the SPR was maintained at 344.738 ± 6.895 kPa (50 ± 1 psi_g) in all trials.

Culture and sample preparation

Saccharomyces cerevisiae was obtained from Dr. Mark Harrison, University of Georgia, Department of Food Science and Technology, Athens, GA. *S. cerevisiae* was maintained on a Sabouraud agar slant and kept at 4°C until required. To ensure healthy cultures, *S. cerevisiae* was grown in malt extract broth (MEB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) aerobically for 24 h at 37°C and transferred three times before

growing in a large flask to obtain the test inoculum. Following the three transfers, *S. cerevisiae* was grown in MEB broth for 20 h at 37°C. Cells were harvested by centrifugation (Centrific™ Centrifuge Model 225, Fisher Scientific, U.S.A.) at 1,000 x *g* for 10 min, rinsed with 0.1% peptone water (Bacto™ Peptone, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) and centrifuged again as before. The concentrated population of *S. cerevisiae* cells were used immediately while in their late log phase or stored at 4°C for no longer than 1 day before use. Enumeration of viable *S. cerevisiae* cells was carried out on Sabouraud Agar (SA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) and incubated for 48 h at 37°C. Detection limits were set at 1 CFU/mL by directly plating 250 µL of sample onto four Sabouraud agar plates. Samples yielding <1 CFU/mL detection on solid medium were subjected to enrichment and visually checked for turbidity. Aliquots of the original SPR treated juice samples were enriched in MEB incubated aerobically for 48 h at 37°C.

Reconstituted calcium fortified apple juice (Kroger 100% Juice Frozen Concentrated Apple Juice with Calcium and Vitamin C, Kroger Co., Cincinnati, Ohio, U.S.A.) (pH ~ 3.91) was used as the test fluid. Calcium fortified apple juice provides greater heat resistance to microorganisms than unfortified apple juice. Prior to inoculation, warm, heat-sterilized deionized water was added to the frozen apple juice concentrate to obtain a 15 Brix juice at the desired temperature of 20°C used as the fluid temperature entering the SPR. Reconstituted apple juice was inoculated with the *S. cerevisiae* cell suspension to obtain an initial mean $\log_{10}$ CFU/mL ± SD of 6.27 ± 0.04. The inoculated juice was used in the SPR process treatments.

Thermal resistance

Thermal resistance of *S. cerevisiae* in calcium fortified apple juice was determined using thermal death time (TDT)-capillary tube method procedures described by Stumbo (1973) and kinetics discussed in further detail by Toledo (2007). D-value or *decimal reduction time* is the time needed to destroy 90% (one log cycle or one order in magnitude) of the microorganisms (reducing their number by a factor of 10). D-values vary for different organisms and depend upon the medium in which the cells are suspended during the heat treatment. The D-value for *S. cerevisiae* was determined at 55°C using three treatment times (1, 5 and 10 min) in a glass-capillary tube carrying approximately 50 μL of inoculated apple juice. D-values were determined using Eq. (1):

$$D = \frac{t_2 - t_1}{\log(N_1) - \log(N_2)} \quad (1)$$

Where the difference between t_2 and t_1 denotes the time allocated for thermal treatment at a constant temp and N_1 and N_2 represent the number of survivors at their respective times. The calculated mean and standard deviation for the D-value of *S. cerevisiae* in calcium fortified apple juice was determined to be 420 ± 37.2 s (7.00 ± 0.62 min) (raw data not shown). In addition to D-value determinations, a z-value was determined. A z-value is used to express the temperature dependence of microbial inactivation or the number of degrees Celsius required to bring about a 10-fold change (one log cycle or one order in magnitude) in the decimal reduction time or D-value. A z-value was determined by constructing a thermal death time curve for *S. cerevisiae* using mean D-values from three treatment temperatures (55°C, 60°C and 65°C). A z-value for *S. cerevisiae* was determined to be 6°C (raw data not shown).

Process lethality

In order to accurately quantify the lethality delivered by hydrodynamic cavitation, thermal destruction of the microorganism must be integrated during the transient temperature of the product and the residence time at a constant temperature before sample collection. Average change in temperature, D-value and accumulated lethality due to thermal effects must be accounted for before attributing any lethality to hydrodynamic cavitation. The average transient time-temperature change of product was calculated using initial and final product temperature, product flow rate and system volume, using Eq. (2):

$$\Delta T_{avg} = \frac{T_{out} - T_{in}}{\frac{V_{SPR}}{FlowRate}} \tag{2}$$

Where V_{SPR} denotes the volume of the SPR system and flow rate is measured in L/s.

D-value is a function of temperature and is calculated using Eq. (3):

$$D_{NewTemp} = D_{55C} * 10^{\frac{T_o - T_{NewTemp}}{z}} \tag{3}$$

Where T_o = 55°C, $D_{55°C}$ = 420 s and z = 6°C.

The final lethality calculation uses Eq. (4):

$$Lethality\ \ (TRTR\ ) = \int_0^t \frac{1}{D_T} dt + \frac{t}{D_T} \tag{4}$$

The first expression accounts for lethality accumulated in the transient temperature phase and the second expression reflects lethality accumulated as product travels at constant temperature through the piping after the SPR before sample collection. We refer to this value as the *thermal residence time reduction* (TRTR). The difference between the total lethality and the TRTR is the lethality due to hydrodynamic cavitation referred to as Shockwave Power^{TM} Reactor – Lethality

(SPR-L). All equations used for process lethality calculations are reviewed in detail by Toledo (2007).

Energy considerations

The heat content or enthalpy, ΔH, of a food product is expressed in Eq. (5):

$$\Delta H = (Cp)*(T_2 - T_1) \quad (5)$$

Specific heat takes into account the mass of product being evaluated and is expressed using Eq. (6):

$$q = (m')*(Cp)*(T_2 - T_1) \quad (6)$$

Where m' = mass flow rate (L/s). The enthalpy change is largely dependent upon the specific heat capacity, C_p, of apple juice and can be calculated using Siebel's Eq (7):

$$C'_{avg} = 1674.72(Fat) + 837.36(Solids\ Non\text{-}Fat) + 2093.4(Moisture)\ in\ J/(kg*K) \quad (7)$$

The average specific heat of the reconstituted apple juice for all processing temperatures is calculated to be 3.792 (kJ/kg*K) (Toledo, 2007). Setting up an enthalpy balance will only consider the input of mechanical energy and the sensible heat gain received by the mass of apple juice flowing through the system. The enthalpy balance is shown in Eq. (8):

$$q_{in} + Mechanical\ Energy = q_{out} + Energy\ Loss \quad (8)$$

Experimental design

Preliminary tests showed negligible inactivation of vegetative cells when fluid exit temperature was below 54.4˚C. In addition, inner cylinder rotational speeds lower than 3000 rpm would require fluid flow rate < 1.0 L/min for liquid to leave the SPR at 54.4˚C. Inoculated apple juice at 20˚C was processed in the SPR to an exit temperature of 65.6˚C and 76.7˚C at both

3000 and 3600 rpm rotation of the inner cylinder. Each treatment was replicated three times and sampling done in duplicate for each treatment (3 replications x 2 duplications: n = 6). Enumeration of survivors in each sample was performed in duplicate and averaged. Enumeration detection limit for all samples was ≥1 CFU/mL. All appropriate results were analyzed using a 2-way analysis of variance (ANOVA, $\alpha = 0.05$) using the general linear model procedures of SAS (SAS Institute, Inc., Cary, N.C.) with Student-Newman-Kuels (SNK) test, when appropriate.

Results and Discussion

Microbial inactivation

Yeasts such as *S. cerevisiae* are the major spoilage microorganisms in high-acid fluid foods such as beverages, fruit juices and salad dressings (Deak & Beuchat, 1996). Cells of *S. cerevisiae* endured a mean inactivation value of 6.27 logs by the hydrodynamic cavitation treatment where the temperature rise was from 20˚C to 65.6˚C and 76.7˚C with the SPR rotor rotating at 3000 and 3600 rpm. Table 1 displays the combined results for the inactivation of *S. cerevisiae* for all process treatments. Preliminary research suggested that inactivation of *S. cerevisiae* was negligible at end point processing temperatures of 54.4˚C and below (data not shown). Transient temperature changes from 20˚C to 65.6˚C and 76.7˚C when the SPR was operated at 3000 and 3600 rpm yielded mean reductions greater than 6 logs. The relationship between temperature and power setting suggests a significant synergistic effect between temperature and cavitation on the efficacy of microbial inactivation and has been validated in previous research (Save, Pandit & Joshi, 1994; Save, Pandit & Joshi, 1997; Piyasena, Mohareb & McKellar, 2003). Utilizing hydrodynamic cavitation induced by the SPR to process calcium

fortified apple juice inoculated with *S. cerevisiae* at 3000 and 3600 rpm with transient end point temperatures of 65.6˚C and 76.7˚C resulted in a mean reduction $\log_{10}$ CFU ± SD of 6.27 ± 0.04, with populations below the detection limit (≤ 1 CFU/mL). All samples yielded negative for viable cells after enrichment.

Table 1 indicates that a SPR setting of 3000 rpm requires reduced mean flow rates of 1.54 and 1.14 L/min to a achieve transient temperature change to 65.6˚C and 76.7˚C, respectively. At SPR settings of 3600, mean flow rates can be increased to 2.62 and 1.88 L/min to achieve a transient temperature change to 65.6˚C and 76.7˚C, respectively. SPR power settings and mass flow rate through the system dictate the exposure/residence time of product to the vigorous cavitation effects. This relationship is illustrated by the significant differences between TRTR and SPR-L values for SPR settings of 3000 and 3600 rpm at an end point temperature of 65.6˚C. As the SPR rotor speeds increased from 3000 to 3600 rpm, the mass flow rate must be increased in order to obtain the target end point temperature. This increase in mass flow rate reduces the contribution of the TRTR to the total lethality. A SPR rotor speed of 3000 rpm required a mean flow rate of 1.54 L/min to achieve a final processing temperature of 65.6˚C with mean TRTR and SPR-L values of 2.38 and 3.88, respectively. However, a SPR rotor speed of 3600 rpm required a mean flow rate of 2.62 L/min to achieve a final processing temperature of 65.6˚C with mean TRTR and SPR-L values of 1.48 and 4.79, respectively. As the mass flow rate increased through the system, the contribution of TRTR to total lethality diminished. The significant difference between these treatments is attributed to the SPR rotational speed and product residence time of exposure to cavitation inside the SPR. As the SPR rotor speed increased, mass flow rate must be increased to obtain the desired end point temperature, thus TRTR is reduced and SPR-L values predominate on the total cell inactivation. Conversely, as

end point processing temperatures increase (76.7˚C), the residence time needed in the SPR to obtain a target processing temperature increases, off-setting the balance between TRTR and SPR-L in favor of inactivation primarily from TRTR.

Majority of research touching on the subject of microbial inactivation via hydrodynamic cavitation rely upon circulating an inoculated medium continuously through a system with periodic sampling (Save, Pandit & Joshi, 1994; Save, Pandit & Joshi, 1997; Jyoti & Pandit, 2001; Sivakumar & Pandit, 2002; Gogate, 2002; Jyoti & Pandit, 2004). Jyoti and Pandit (2001) reported a 41% and 44% reduction in bacteria cell counts for bore well water treated for 2 and 1 h at 172 kPa and 517 kPa (24.9 and 74.9 psi_g), respectively (in continuous system near ambient temperatures). Initial bacteria cell counts did not exceed 1.0×10^4 CFU/mL and no flow rate values were given. If the system Jyoti and Pandit (2001) discuss was assumed to conservatively operate at 1.0 L/min, the total number of times the fluid would have been exposed to a single hydrodynamic cavitation event would be less than 100 times in one hour. In contrast, with our SPR design, a conservative rotor speed of 3000 rpm, with only one cavity (diameter = 0.0127 m) at one fixed point on the rotor and a product flow rate of 1.0 L/min, the product would be subjected to approximately 5 cavitation events per second. The number of cavitational events is multiplied by the number of cavities in the rotor (i.e. 10 cavities = 50 cavitation events per sec). In addition, the SPR design employs two rows of cavities 2.54 cm apart on the periphery of the rotor.

An adverse effect of hydrodynamic cavitation is severe erosion to surfaces in contact with the fluid undergoing cavitation. This effect has been extensively documented specifically on hydrofoils (Young 1999). A unique design of equipment to induce fluid cavitation would allow harnessing of destructive forces while eliminating equipment erosion and maximizing

hydrodynamic cavitation in the product. The design of our SPR system employs concentric cylinders with fluid in the annular space. The surface of the inner, rotating cylinder contains multiple cavities where fluid is pressurized and depressurized many times, inducing hydrodynamic cavitation in the fluid. This type of cavitation is deemed "vortex" cavitation and occurs in the cavities of the rotor surface (Fig. 2.3). This novel design allows for a continuous flow of fluid to replenish multiple cavities as the rotor spins, facilitating cavitation and product flow through the system. Centrifugal forces from the inner, rotating cylinder are countered with centripetal forces to avoid "traveling" cavitation, or cavitation that "spills" out of the cavity and travels co-current through the system. Pressurizing the system during operation ensures continuous product contact with the inner rotor resulting in the fluid undergoing controlled cavitation. As product enters the SPR annular space with the rotor set at a specified operational speed (rpm), the mass rate of flow of product dictates the frequency of cavitational events and ultimately the end exit temperature. As cavitation occurs, the fluid bulk receives energy dissipated by the bubbles in the vapor phase as the bubbles collapse, thus the fluid in immediate contact with the bubbles before collapse will have instantaneous temperatures higher than the average temperature of fluid leaving the annular space. The exposure to temperatures exceeding that of the system's average allows microbial inactivation to exceed what can be predicted using microbial inactivation kinetics based on the fluid transient time-temperature history in the annular space and the residence time after leaving the SPR prior to sampling. Thermocouples were placed at the entrance and exit of the SPR chamber in order to accurately monitor the change in product temperature entering and leaving the system. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for

3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.40, 0.37, 0.68 and 0.61˚C/s, respectively.

The mechanism responsible for cellular inactivation caused by cavitation induced acoustically is characterized by the thinning cell walls due to immense shear rates, localized heating points, formation of free radicals and compression and decompression cycles (Frizzell, 1988; Earnshaw, 1998; Young, 1999; Piyasena, Mohareb, & McKellar, 2003). Piyasena, Mohareb, and McKellar (2003) report localized temperatures and pressures from acoustic cavitation as intense as 5,500˚C and 50,000 kPa (ca. 7,250 psi_g) (Piyasena, Mohareb, & McKellar, 2003). Although the mechanism in acoustic cavitation is quite similar to that of hydrodynamic cavitation, the main difference falls upon the frequency of cavitational events and scale of operation. Save, Pandit, and Joshi (1994) report that majority of large scale cell disruption processes rely heavily upon mechanical disintegrators to impart cell rupture. Large scale processing techniques employ such equipment such as high-speed agitator bead mills, microfluidizers, high pressure industrial homogenizers or ultrasonic generators (Save, Pandit, & Joshi, 1994; Middelberg, 1995). The drawback for these processing techniques is their decreased efficiency (5-10%) and difficult control over localized heating which causes expensive operations and increased product loss. Hydrodynamic cavitation reactors offer processors improved process efficiency and more control over exposure to cavitational events.

Energy considerations

The food industry is always pursuing novel processing technologies that allow for reduced energy consumption and minimized product exposure to thermal treatments. Excessive thermal treatment of food, although necessary to render the food product safe for consumption

and extend shelf-life, is also a primary reason for reduced product quality and undesirable organoleptic properties. Table 2 depicts the energy consumption for processing calcium-fortified apple juice using a SPR in comparison to conventional heating. Conventional pasteurization of apple juice requires heating the product to 88˚C and holding for 15 s before cooling to eliminate spoilage and pathogenic microorganisms. Table 2 portrays the amount of energy required to achieve a transient temperature change from 20˚C to 65.6˚C, 76.7˚C and 88˚C in order to achieve a 5-log reduction of *S. cerevisiae*. Achieving target lethality at lower temperatures using a SPR results in a reduced energy requirement relative to that for conventional heat treatments to obtain a 5-log reduction of *S. cerevisiae*. The mean value of applied energy per unit weight of product to achieve 6.27 log cycles reduction of *S. cerevisiae* at SPR rotor speeds of 3000 and 3600 rpm at 65.6˚C and 76.7˚C are 173, 215, 173 and 215 kJ/kg, respectively. These values are considerably lower than the conventional heat treatment, which the application of 258 kJ/kg to achieve the equivalent lethality of the SPR. Significant differences among mass flow rates and % energy efficiency at various SPR operating parameters suggests the use of SPR at rotor speeds of 3600 rpm with an end point temperature of 65.6˚C. These parameters require a mass flow rate of 0.044 kg/s and 173 kJ/kg of energy to induce a mean reduction of 6.27 log cycles with the lowest energy loss of 32 kJ/kg and highest energy efficiency of 84%. Operating the SPR at 3600 rpm with an end point temperature of 76.7˚C requires a mass flow rate of 0.031 kg/s and 215 kJ/kg of energy to induce a mean reduction of 6.27 log cycles with an energy loss of 70 kJ/kg and an energy efficiency of 75%. SPR operation at 3000 rpm with end point temperatures of 65.6 and 76.7˚C requires a mass flow rate of 0.026 and 0.019 kg/s and 173 and 215 kJ/kg of energy to induce a mean reduction of 6.27 log cycles with energy losses of 118 and 178 kJ/kg and energy efficiencies of 60 and 55%. These patterns suggest the amount of energy lost to the

system in the form of heat can be reduced by increasing the SPR rotational speed and product mass flow rates through the SPR system. Moreover, these data suggest a more efficient coupling of mechanical energy to the product when the SPR rotor speed was increased along with mass flow rate. As the specific heat of calcium-fortified apple juice is 3.792 (kJ/kg*K), excessive energy generated from hydrodynamic cavitation is lost as heat dissipated from the fluid to the SPR housing. Increasing the mass flow rate of product through the system allows energy to be coupled more efficiently to the product itself. These data support the claim of utilizing a SPR to minimally heat treat apple juice with benefits manifesting in the form of energy savings, superior organoleptic properties and increased retention of heat labile nutrients.

Much research has focused on pulsed electric field (PEF) treatment of fluid foods. Cserhalmi, Vidacs, Beczner and Czukor (2002) report 3.4 log cycles reduction when employing electric field strength of 28 kV/cm (145 kJ/L) at a constant 8.3 pulses for total process time of 100 μs. This process required a flow rate of 84 mL/min. The clear advantage of processing with an SPR unit includes increased product flow rates (≥ 1.0 L/min) with increased log cycle reductions (6.27) when compared to 3.4 and 84 mL/min, respectively. Moreover, Heinz, Toepfl, and Knorr (2003) report 6.2 log cycle reduction when employing electric field strength of 36 kV/cm (70 kJ/kg) at a temperature of 55˚C for a total process time of 7.68 μs. This process required a flow rate of 110 mL/min. Pulsed electric fields processing lacks the evidence for scaling up the equipment needed to make this technology viable for industry needs when compared to hydrodynamic cavitation processing.

Conclusions

In conclusion, utilizing hydrodynamic cavitation as a processing technology allows processors to minimally heat treat fluid foods while extending shelf-life of perishable products such as apple juice. Enhanced inactivation of *S. cerevisiae* (6.27 log cycles) can be achieved at reduced processing temperatures (65.6˚C and 76.7˚C) when subjected to sufficient hydrodynamic cavitation compared to what can be predicted using heat inactivation parameters (D and z-values). Utilization of hydrodynamic cavitation in cellular disruption industries validates the implementation of hydrodynamic cavitation in food processing applications. Energy consumption can be reduced significantly (173 and 215 kJ/kg versus 258 kJ/kg) and process efficiency can be significantly increased by processing with hydrodynamic cavitation in fruit juice manufacturing. Hydrodynamic cavitation equipment can be easily scaled up for large industrial purposes with enhanced energy saving and efficiency (55 – 84%). Reducing thermal treatments, retaining heat labile nutrients and flavor components by processing with hydrodynamic cavitation creates superior products in today's market where "fresh picked" flavors and healthy/nutritious products drive consumption trends.

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Table 2.1: Enumeration[1] of *S. cerevisiae* processed in calcium fortified apple juice (pH = 3.9) at two SPR[2] settings (3000 and 3600 rpm) at an initial temperature of 20˚C and two exit temperatures of 65.6˚C and 76.7˚C (n = 6)[3].

Setting/Exit Temp (rpm / ˚C)	FR[4] (L/min)	ΔT_{avg} (˚C/s)	LR[5]	TRTR[6]	SPR-L[7]	LT[8]
3000 / 65.6	1.54 ± 0.02^{c}	0.40	6.27 ± 0.04	2.38 ± 0.05^{a}	3.88 ± 0.02^{b}	0/6
3000 / 76.7	1.14 ± 0.05^{d}	0.37	6.27 ± 0.04	*	**	0/6
3600 / 65.6	2.62 ± 0.03^{a}	0.68	6.27 ± 0.04	1.48 ± 0.06^{b}	4.79 ± 0.09^{a}	0/6
3600 / 76.7	1.88 ± 0.02^{b}	0.61	6.27 ± 0.04	*	**	0/6

[1] Average enumeration (means $\log_{10}$ CFU ± S.D.) data calculated from three separate trials with two samples per treatment (detection ≤1 CFU/mL).
[2] SPR = "Shockwave Power™ Reactor" or controlled cavitation system.
[3] n = 6; means ± SD followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis.
[4] FR = Flow Rate: rate of flow through SPR system and hold tube (L/min).
[5] LR = Log Reduction: total mean log reduction for three separate trials (mean $\log_{10}$ CFU ± SD).
[6] TRTR = TRTR = Thermal Residence Time Reduction: mean log reduction due to integrated lethality during transient temperature change inside SPR plus lethality accumulated during hold time in exit piping of SPR (mean $\log_{10}$ CFU ± SD).
[7] SPR-L = Mean log reduction due to hydrodynamic cavitation treatment after TRTR effects have been removed (mean $\log_{10}$ CFU ± SD).
[8] LT = Lethality Test: incidence of growth after enrichment malt extract broth.
* = TRTR allowed for complete theoretical thermal inactivation.
** = SPR-L values do not reflect potential hydrodynamic cavitation inactivation due to TRTR.

Table 2.2: Energy comparisons of a 12 HP SPR unit during treatment of apple juice inoculated with *S. cerevisiae* at an initial temperature of 20˚C using two SPR[1] settings (3000 and 3600 rpm) and two end point temperatures (65.6˚C and 76.7˚C) to a conventional heat treatment (CHT) with an end point temperature of 88˚C (n = 6)[2].

Process SPR v. CHT	Setting (rpm)	$Temp_{out}$ (˚C)	Mass Flow Rate (kg/s)	h_{input} (kJ/kg)	h_{loss} (kJ/kg)	$\%_{efficiency}$ (%)
SPR	3000	65.6	0.026^{c}	173^{c}	118^{b}	60^{c}
	3000	76.7	0.019^{d}	215^{b}	178^{a}	55^{d}
	3600	65.6	0.044^{a}	173^{c}	32^{d}	84^{a}
	3600	76.7	0.031^{b}	215^{b}	70^{c}	75^{b}
CHT	-----	88.0	(0.019 – 0.44)	258^{a}	-----	-----

[1] SPR = "Shockwave Power™ Reactor" or "controlled" cavitation system.
[2] n = 6; Values calculated from three separate trials with two samples per treatment; means followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis.
----- = Data not applicable.

Fig.2.1 - Schematic of the Shockwave Power™ Reactor (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

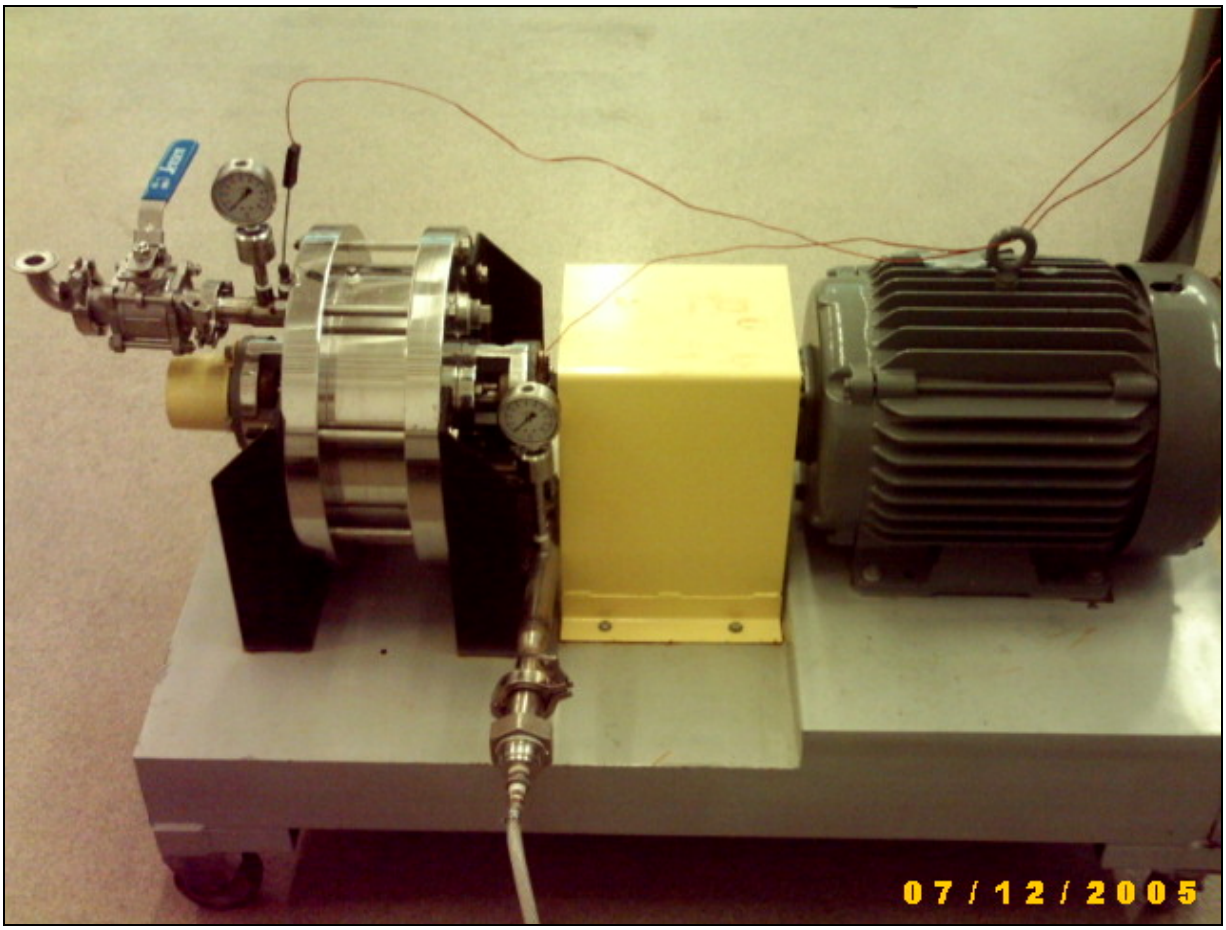

Fig. 2.2 -12 HP Shockwave Power™ Reactor (10” x 2” pilot scale unit) (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

Fig.2.3 - Hydrodynamic cavitation within inner rotor cavities (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

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CHAPTER 3

INACTIVATION OF FOOD SPOILAGE MICROORGANISMS IN FLUID FOODS BY HYDRODYNAMIC CAVITATION AT REDUCED PROCESSING TEMPERATURES[1]

[1] Milly, P.J. and R.T. Toledo. To be submitted to *Journal of Food Science*

Abstract

Various high acid fluid foods were processed in a hydrodynamic cavitation reactor to determine if commercial sterility can be achieved at reduced processing temperatures. Sporicidal properties of the process were also tested on a low acid fluid food. Hydrodynamic cavitation is the formation of gas bubbles in a fluid due to pressure fluctuations induced by mechanical means. Fluid foods were pumped under pressure into a hydrodynamic cavitation reactor, subjected to two rotor speeds and flow rates to achieve two designated exit temperatures. Samples of processed product were enumerated for survivors of inoculated organisms. Thermal inactivation kinetics were used to determine heat-induced lethality. Calcium-fortified apple juice processed at 3000 rpm and 3600 rpm rotor speed on the reactor went through a transient temperature change from 20˚C to 65.6˚C or 76.7˚C and the total process lethality exceeded 5-log reduction of *Lactobacillus plantarum* and *Lactobacillus sakei* vegetative cells and *Zygosaccharomyces bailii* vegetative cells and ascospores. Tomato juice inoculated with *Bacillus coagulans* spores and processed at 3000 rpm and 3600 rpm endured a transient temperature from 37.8˚C to 93.3˚C or 104.4˚C with viable CFU reductions of 0.88 and 3.10 log cycles, respectively. Skim milk inoculated with P.A. 3679 spores and processed at 3000 rpm or 3600 rpm endured a transient temperature from 48.9˚C to 104.4˚C or 115.6˚C with CFU reductions of 0.69 and 2.84 log cycles, respectively. Utilizing hydrodynamic cavitation to obtain minimally processed pasteurized low acid and commercially sterilized high acid fluid foods is possible with appropriate process considerations for different products.

Introduction

The term cavitation is used to describe the formation and rapid collapse of a vapor filled cavity within a fluid due to localized high and low pressure regions within a fluid induced by mechanical means. Cavitation is induced acoustically or mechanically, with mechanical cavitation being referred to as hydrodynamic cavitation. As fluid is forced through a configuration that induces formation of eddy currents, such as flow around solid surfaces or small and large cross-sectional area in the flow stream, the fluid's kinetic energy is converted into elevated velocities at the expense of a drop in fluid pressure. Localized high and low velocities in a fluid bulk develops momentary gradients of reduced vapor pressures, causing dissolved or trapped gases and fluid vapors to accumulate and expand, forming a bubble. Small particulates, such as dust, debris or biological entities provide a physical surface for bubble nuclei to attach, expand and collapse. Bubbles traveling instantaneously from regions of low to high pressure will implode. The collapse of a vapor-filled cavity is accompanied by intense pressure waves, vigorous shearing forces, localized heating events and in some cases the formation of free radicals (Leighton 1998; Earnshaw 1998; Young 1999; Mason and Lorimer 2002).

Physical stresses resulting from acoustic or hydrodynamic cavitation are understood to be the mechanisms responsible for cellular inactivation. Biological entities in the immediate area of a cavitation event endure stresses that induce severe damage to cell walls and ultimately inactivate the organism (Frizzell 1988; Earnshaw and others 1995; Earnshaw 1998; Young 1999; Geciova and others 2002; Piyasena and others 2003). Earnshaw (1998) cites research suggesting that larger cells such as yeast (5 – 20 μm) are more susceptible to the effects of cavitation due to their larger surface area. Gram-positive cells were once thought to be more resistant to

cavitation than the gram-negative cells due to additional layers of peptidoglycans in the former; however, more recent literature suggests no significant difference between gram-negative and gram-positive microorganisms in their resistance to inactivation by cavitation. Spores of bacteria like *Bacillus* and *Clostridium* possess a higher tolerance to cavitational effects when compared to vegetative cells (Earnshaw 1998).

Most of research in which cellular disruption via hydrodynamic cavitation was investigated employed some variation of venturi configuration or multi-orifice plate that permitted sample collection after one pass or one cavitation event. In these studies the efficacy of hydrodynamic cavitation when disinfecting wastewater was examined (Jyoti and Pandit 2001; Gogate 2002; Sivakumar and Pandit 2002; Jyoti and Pandit 2004). In addition to wastewater treatment, increasing focus is being placed on hydrodynamic cavitation as a means of achieving cellular disruption or producing nano sized suspended particles in large scale operations with improved process control and reduced energy costs (Save and others 1994; Save and others 1997).

Reducing energy consumption, achieving microbial lethality at reduced temperatures and maintaining fresh-like product quality during processing drives the need for alternative food processing technologies (CFSAN, 2000). Our hypothesis was that hydrodynamic cavitation can pasteurize and/or sterilize fluid foods at reduced temperatures by the combined thermal and cavitation effects.

The objective of this study was to quantify lethality from hydrodynamic cavitation at temperatures lower than conventional thermal processing on several common spoilage microorganisms and to determine if pasteurization of low acid or commercial sterilization of

high acids fluid foods is possible with the combined hydrodynamic cavitation and thermal effects.

Materials and Methods

Hydrodynamic cavitation reactor

A Shockwave Power™ Reactor (SPR) System constructed by Hydrodynamics Inc., Rome, GA was used for these tests in the University of Georgia, Food Science and Technology pilot plant. The 0.254 m x 0.0508 m (10" x 2") unit consisted of concentric cylinders with a 2.91 L volume annular space separating the stationary outer cylinder from the inner, rotating cylinder. The rotor is powered by a variable frequency drive 12 HP motor to permit controlled and reproducible settings of the rotor speed. Cavitation is generated when the rotor which contains multiple cavities on its surface rotates at high speed in the fluid within the annular space. Fig. 3.1 illustrates the innovative equipment design and Fig. 3.2 is the actual pilot scale unit used in this research. Fluid was fed to the SPR under pressure (345 ± 7 kPa) using a Procon Pump (Series 3 Procon Pump, Procon Productions, Murfreesboro, TN. U.S.A) and rate was varied by a by-pass valve which returned part of the pump output back to the feed reservoir. Rotating the rotor at high speed forces fluid into the cavities on the rotor surface where fluid is depressurized due to expansion because of the sudden increase in the cross-sectional area of the flow stream. . When depressurization occurs, fluid vapor develops and dissolved gas in the fluid combine with the vapors to produce bubbles. Figure 3.3 illustrates the formation of bubbles in the annular space surrounding the spinning rotor.

Mentsrua treatment

Reconstituted calcium fortified apple juice (Kroger 100% Juice Frozen Concentrated Apple Juice with Calcium and Vitamin C, Kroger Co., Cincinnati, Ohio, U.S.A.) (pH = 3.91) was used as the medium for biological challenges on the SPR against vegetative cells of *Lactobacillus plantarum* ATCC 21028, *Lactobacillus sakei* ATCC 15521 and both vegetative cells and ascospores of *Zygosaccharomyces bailii*. Prior to inoculation, deionized water was added to the frozen apple juice concentrate in the manufacturer recommended concentrate to water ratio. Deionized water temperature was adjusted before addition such that the desired temperature of 20°C is obtained in the reconstituted juice. For each SPR speed of 3000 and 3600 rpm, target exit temperatures of processed calcium fortified apple juice were set at 65.6˚C and 76.7˚C by adjusting the feed rate.

Tomato juice (Kroger Tomato Juice From Concentrate, Kroger Co., Cincinnati, OH, 45202) (pH = 4.1) was used as the medium for biological challenges to the SPR against *Bacillus coagulans* spores. Prior to spore inoculation, tomato juice was preheated to the desired initial temperatures of 37.8˚C. For each SPR speeds of 3000 and 3600 rpm, target exit temperatures for tomato juice leaving the SPR were 93.3˚C and 104.4˚C.

Skim milk (Kroger Grade A Vitamin A and D Skim Milk, Kroger Co., Cincinnati, OH, 45202) (pH = 6.0) was used as the medium for biological challenges to the SPR against spores of *Costridium sporogenes* P.A. 3679. Prior to spore inoculation, skim milk was preheated to the desired initial temperature of 49˚C. For both SPR speeds of 3000 and 3600 rpm, target temperatures for processed skim milk leaving the SPR were 104.4˚C and 115.6˚C.

All inocula consisted of greater than 5 log CFU/mL (5.14 – 7.48) of the test organism in the test fluid.

Test microorganisms

Two common food spoilage gram-positive non-spore forming organisms were used as the inocula for calcium fortified apple juice. *Lactobacillus plantarum* ATCC 21028 vegetative cells were grown aerobically in deMan Rogosa Sharpe broth (MRS broth, Difco Laboratories, Div. of Becton, Dickinson and Co., Sparks, Md., U.S.A.) for 24 h at 37˚C and transferred three times before growing in large flasks for 24 h to produce the large numbers of the organism for inoculation. *Lactobacillus sakei* ATCC 15521 vegetative cells were grown in MRS broth for 24 h at 30˚C in anaerobic jars. Five to 10% CO_2 gas packs (BD BBL™ GasPak™ Anaerobic System Envelopes, Difco Laboratories, Div. of Becton, Dickinson and Co., Sparks, Md., U.S.A.) were used to generate anaerobic conditions. At least three transfers were done prior to growing in large flasks under the same environmental conditions to produce the inoculum into the test fluid Enumeration of *L. plantarum* and *L. sakei* was performed on deMan Rogosa Sharpe agar (MRS agar, Difco Laboratories, Div. of Becton, Dickinson and Co., Sparks, Md., U.S.A.) aerobically for 48 h at 37˚C or in anaerobic jars with gas packs 48 h at 30˚C, respectively, SPR processed product where no detectable CFU (detection limit: ≤1 CFU/mL) of *L. plantarum* and *L. sakei* were recovered in colic media were subjected to enrichment in MRS broth at 48 h, 37˚C aerobic and 30˚C anaerobic, respectively. Total inactivation was verified by lack of turbidity in enrichment tubes.

The vegetative cells and ascospores of the yeast *Zygosaccharomyces bailii* in calcium fortified apple juice were used in the SPR challenge because of the high thermal resistance of this organism and its involvement in spoilage of commercially processed high acid fruit juice. *Zygosaccharomyces bailii* NRRL Y 7256 was obtained from Dr. Larry Beuchat from the Center of Food Safety laboratories, Griffin, GA. *Z. bailii* was isolated and checked for purity before

being sent to Athens, GA for use in this research. *Z. bailii* arrived on an agar slant and was kept at 4°C until used. To ensure healthy cultures, *Z. bailii* was grown in malt extract broth (MEB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) aerobically for 24 h at 25°C in three initial transfers. Actively growing *Z. bailii* vegetative cells were grown in MEB broth for 24 h at 25°C before inoculation in the reconstituted calcium-fortified apple juice.

Ascospores of *Z. bailii* were grown by distributing 0.5 mL of actively growing culture (24 h, 25˚C) onto agar plates containing 5 g/L sodium acetate (J.T. Baker, Phillipsburg, NJ 08865) and 10 g/L potassium chloride (J.T. Baker, Phillipsburg, NJ 08865) (Raso and others 1998). Inoculated plates were incubated for 14 days at 25˚C. Ascospores were collected by adding 1 mL of sterile distilled water to plate, scraped with a sterile bent glass rod, collected via pipette and then centrifuged. Ascospores were used immediately or kept at 4˚C for no longer than one day. Enumeration of both vegetative cells and ascospores were carried out on potato dextrose agar (PDA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) for 48 h at 25°C. Samples yielding less than detectable CFU (detection limit: ≤1 CFU/mL) on solid medium were enriched (48 h, 25˚C) in MEB broth and visually checked for turbidity. Non-turbid enrichment cultures indicated complete inactivation of the organism.

The spores from *Bacillus coagulans* ATCC 8038 (a.k.a. *Bacillus thermoacidurans* NCA 43P) in tomato juice were used in the SPR challenge test because of their high thermal resistance and association with commercially processed tomato juice spoilage. To produce high viability high heat resistance spores, *B. coagulans* was grown in nutrient broth (NB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) aerobically for 24 h at 37°C and transferred at least 3 times. Spores of *B. coagulans* were prepared by distributing 9 mL of actively growing cells (24 h, 37˚C) into flatbed "Thompson" bottles containing nutrient agar

(NA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) fortified with 500 mg/L of dextrose (Bacto Dextrose, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) and 3 mg/L manganese sulfate (J.T. Baker, Phillipsburg, NJ 08865) (Palop and others 1999). Inoculated plates were incubated for 7 days at 50˚C. Spores were collected by adding 5 mL of sterile distilled water to the flatbed bottle, detached using sterile glass beads, collected via pipette, centrifuged and stored at 4˚C for no longer than one day. Spores were heat shocked in 500 mL of tomato juice at 80˚C for 15 minutes immediately before sample inoculation. Enumeration of spores was carried out on NA for 48 h at 37°C. Processed samples yielding less than detectable CFU (detection limit: ≤1 CFU/mL) were subjected to enrichment (48 h, 37˚C) in NB and visually examined for turbidity.

Spores from *Clostridium sporogenes* P.A. 3679 ATCC 7955 in skim milk were used to determine the lethality induced by the SPR. To ensure healthy cultures, *C. sporogenes* was grown in fortified Clostridium medium (RCM, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) in anaerobic jars with 5-10% CO_2 gas packs for 24 h at 37°C during each of three transfers. After the third transfer, *C. sporogenes* vegetative cells were grown anaerobically in RCM for 24 h at 37°C before sporulation. Spores were produced by distributing actively growing cells into a medium comprised of 3% trypticase soy broth (TSB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.), 0.1% yeast extract (YE, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) and 1% ammonium sulfate (J.T. Baker, Phillipsburg, NJ 08865) (Kalchayanand and others 2004). The medium was incubated for 7 days at 37˚C. Spore suspensions were centrifuged, collected and stored at 4˚C for no longer than one day. Spores were heat shocked in 500 mL of skim milk at 80˚C for 20 minutes immediately before sample inoculation. Enumeration of

spores was carried out anaerobically on brain heart infusion agar (BHIA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) for 48 h at 37°C. Processed samples with less than detectable CFU (detection limit: ≤1 CFU/mL) were enriched (48 h, 37˚C) in RCM and culture tubes were visually examined for turbidity.

Cultures of all vegetative cells were centrifuged (Centrific™ Centrifuge Model 225, Fisher Scientific, U.S.A.) at 1000 x *g* for 10 min, rinsed with 0.1% peptone water (Bacto™ Peptone, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.). Spores were centrifuged as above, washed with sterile distilled water, and centrifuged again as before. Concentrated suspension of cells or spores was used immediately or stored at 4°C for no longer than one day.

Thermal resistance

Thermal resistance of all test microorganisms were determined using thermal death time (TDT)-capillary tube method procedures described by Stumbo (1973) and kinetic data calculated as discussed in detail by Toledo (2007) (Stumbo 1973; Toledo 2007). D-value or *decimal reduction time* is the time needed to destroy 90% (one log cycle or one order in magnitude) of the microorganisms (reducing their number by a factor of 10) and is determined from the decimal reduction curve. D-values vary for different organisms and depend upon the medium in which they are heated. The D-value for test cultures were determined at 55°C or 100˚C using three treatment times in a glass-capillary tube carrying approximately 50 µL of inoculated sample menstrua. D-values were determined using Eq. (1):

$$D = \frac{t_2 - t_1}{\log(N_1) - \log(N_2)} \quad (1)$$

where the difference between t_2 and t_1 denotes the time allocated for thermal treatment at a constant temperature and N_1 and N_2 represent the number of survivors at their respective times. In addition to D-value determinations, z-values were determined. A z-value is used to express the temperature dependence of microbial inactivation or the number of degrees Celsius required to bring about a 10 fold change (one log cycle or one order in magnitude) in the decimal reduction time or D-value. Z-values were determined by constructing a thermal death time (TDT) curve for test cultures using mean D-values from varying temperatures (data not shown).

Process lethality

In order to accurately quantify the lethality delivered by hydrodynamic cavitation, thermal destruction of microorganisms must be integrated during the transient temperature of the product in the SPR and the residence time at a constant temperature in the pipe post-SPR before sample collection. Average liquid temperature in the SPR and post-SPR, and the D-value of the organism are used to determine accumulated lethality due to thermal effects. The average transient time-temperature change of product was calculated using initial and final product temperature, product flow rate and system volume, using Eq. (2):

$$\Delta T_{avg} = \frac{T_{out} - T_{in}}{\frac{V_{SPR}}{FlowRate}} \quad (2)$$

where V_{SPR} denotes the volume of the SPR system and flow rate is measured in L/s.

D-value is a function of temperature and is calculated using Eq. (3):

$$D_{NewTemp} = D_0 * 10^{\frac{T_0 - T_{NewTemp}}{z}} \quad (3)$$

where T_0 = reference temp (˚C), D_0 = reference mean D-value at T_0 and z = z-value determined from TDT curve.

The final lethality calculation uses Eq. (4):

$$Lethality(TRTR) = \int_0^t \frac{1}{D_T} dt + \frac{t}{D_T} \quad (4)$$

The first term is lethality accumulated during the transient product temperature in the SPR and the second term is lethality accumulated during product residence time in the exit piping at constant temperature before collection. The value calculated using eq. 4, is referred to as the *thermal residence time reduction* (TRTR) and when subtracted from the total measured lethality, the difference is the lethality due to cavitation. The lethality attributed to hydrodynamic cavitation is given the term Shockwave Power^{TM} Reactor – Lethality (SPR-L). All equations used for process lethality are reviewed in great detail by Toledo (2007).

Experimental design

Preliminary research suggested inactivation of vegetative cells was negligible at end point temperatures below 54.4˚C (data not shown). In addition, rotor speeds lower than 3000 rpm required very low processing rates (< 1.0 L/min) to reach the target SPR exit temperature (data not shown). In the case of the *Bacillus* and *Clostridium* spores' inoculum, feed temperature of inoculated samples to the SPR was raised so that feed rates > 1 L/min could be used to achieve the target product temperature leaving the SPR. Treatments consisted of two rotor speeds of the SPR, with feed rates adjusted to achieve two exit temperatures at each of the SPR rotor speed. Each treatment was replicated three times and duplicate samples were

collected at each treatment (3 replications x 2 duplications: n = 6). Enumeration of viable CFU in each sample was performed in duplicate and averaged. Enumeration detection limit for all samples was ≤1 CFU/mL. Results were analyzed using a 2-way analysis of variance (ANOVA, $\alpha = 0.05$) using the general linear model procedures of SAS (SAS Institute, Inc., Cary, N.C.) and Student-Newman-Kuels (SNK) test, when appropriate.

Results and Discussion

Thermal resistance

D and z values for all test microorganisms in their heating medium are presented in Table 3.1. In the high acid foods, *Z. bailii* ascospores was the most resistant with a mean D value of 13.08 min at 55 ºC. The mean D values at 55˚C for the remaining microorganisms are: *Z. bailii* vegetative cells (2.38 min), *L. sakei* (1.67 min) and *L. plantarum* (1.45 min). *B. coagulans* and *C. sporogenes* P.A. 3679 spores had mean D values of 2.20 and 22.30 min, respectively, at 100 ºC.

Due to different D and z values, inactivation of individual organisms from each test is not compared against one another. D and z values are specific properties of individual microorganisms therefore inactivation values between organisms could not be statistically analyzed without bias. The relationship between exit temperature and rotor speed suggests significant synergism between temperature and cavitation on the efficacy of microbial inactivation. This confirms findings in prior research by other authors (Save and others 1994; Save and others 1997; Piyasena and others 2003).

Microbial inactivation

Inactivation of all microorganisms inoculated in apple juice and processed with a SPR at 3000 and 3600 rpm to exit temperatures of 65.6˚C and 76.7˚C are illustrated in Table 3.2. Table 3.3 presents the inactivation of *B. coagulans* spores inoculated in tomato juice and processed at 3000 and 3600 rpm with SPR exit temperatures of 93.3˚C and 104.4˚C. Table 3.4 displays results collected from the inactivation of *C. sporogenes* P.A. 3679 spores inoculated in skim milk and processed at 3000 and 3600 rpm to exit temperatures of 104.4˚C and 115.6˚C.

When evaluating lethality obtained with individual organisms, flow rates differed significantly for all combinations of rotor speed (3000 and 3600 rpm) and exit temperatures. This significant difference supports the claim that different SPR settings convert mechanical energy in significantly different degrees to cavitation depending upon the mass flow rate of product flowing through the annular space of the SPR. Product temperature leaving the SPR is a function of mass flow rate (kg/min) and SPR rotor speed

In all vegetative cell inocula, SPR-L values significantly increased as rotor speed increased from 3000 rpm to 3600 rpm at the same exit temperature of 65.6˚C. In all vegetative test organisms except for *L. plantarum*, processing at 3600 rpm to an exit temperature of 76.7˚C had TRTR values equivalent to complete inactivation. SPR treatments to achieve the 20˚C to 76.7˚C temperature rise from feed to exit required a reduction of feed flow rates at both 3000 and 3600 rpm compared to exit temperature of 65.6˚C. Reducing product flow rate increases product residence time in the SPR therefore the thermal lethality (TRTR) completely inactivated the inoculated organism. However, increasing the product flow rate reduced TRTR, for the same exit temperature therefore hydrodynamic cavitation contributed a significant SPR-L value to the total process lethality. At high SPR rotor speeds, product must be fed at a high rate of flow to

achieve the same exit temperature at slower SPR speeds. The increased product feed rate reduces residence time in the SPR, reducing the contribution of thermal effects to the total lethality. Therefore, at higher SPR speeds and increased product flow rates, a majority of the lethality is derived from hydrodynamic cavitation. However, it should be noted that higher lethality from hydrodynamic cavitation requires a certain amount of time in the SPR. Therefore lethality from both thermal and SPR may not be adequate to prevent spoilage. Critical factors for processing depend upon the SPR speed, mass flow rate, initial temperature of product and exit product temperature. The change in enthalpy of fluids in the SPR is due to the conversion of mechanical energy in the rotor to thermal energy in the product. The conversion efficiency is facilitated by multiple cavitation events that take form in high shear rates, localized heating events and pressure waves.

The effect of cavitation on the inactivation of spores differs from that of vegetative cells. Vegetative cells and yeast ascospores display some vulnerability to forces induced by hydrodynamic cavitation, whereas spores from *B. coagulans* and P.A. 3679 proved to resistant to hydrodynamic cavitation. The inherent heat resistance of spores creates challenges for minimally processing sterilized fluid foods. The destructive stresses induced by cavitation however, is synergistic with thermal effects. Destructive forces like high shear, intense pressure fluctuations and localized heating events may cause strains to occur in the spore coat and make the spore more vulnerable to thermal inactivation (Earnshaw 1998; Mason and Lorimer 2002).

Apple juice - *L. plantarum*

Lactic acid producing bacteria are well known spoilage organisms in high acid fruit juices (Tajchakavit and others 1998; Gomez and others 2005). Lactic acid bacteria metabolize

organic acids from fruit juices into diacetyl, an undesirable buttermilk-flavored agent and cause clouding in products (Jay 1998). Table 3.2 displays the combined results for the inactivation of *L. planturum* for all process treatments. Utilizing hydrodynamic cavitation induced by the SPR to process calcium fortified apple juice inoculated with *L. plantarum* at 3000 and 3600 rpm with inlet and exit temperatures of 20˚C and 65.6˚C, or 76.7˚C resulted in a viable cell reduction of 7.48 ± 0.09 logs. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.39, 0.37, 0.67 and 0.61˚C/s, respectively. In addition to the absence of recoverable CFU (detection limit ≤1 CFU/mL), enriched samples showed no recovered cells. Table 3.2 indicates that a SPR setting of 3000 rpm requires mean flow rates of 1.48 and 1.15 L/min to raise feed temperature from 20˚C to 65.6˚C and 76.7˚C, respectively. At SPR speed of 3600, mean flow rates were 2.55 and 1.89 L/min to raise feed temperature from 20˚C to 65.6˚C and 76.7˚C, respectively. As the SPR rpm increases, the mass flow rate must be increased to achieve the same exit temperature since more mechanical energy is being delivered to the product. The mean value of TRTR and SPR-L from 3000 rpm/65.6˚C was 1.62 and 5.87 log cycles, respectively. Processing at 3600 rpm/65.6˚C and 76.7˚C yielded mean TRTR quantities of 0.96 and 5.69 log cycles and SPR-L values of 6.52 and 1.79 log cycles, respectively. All TRTR and SPR-L values differed significantly for each treatment combination. Mass flow rate and SPR speed were responsible for significant differences among TRTR and SPR-L values. Processing with 3600 rpm/65.6˚C required the highest flow rate or reduced residence time in the SPR. As the residence time is reduced, the average thermal lethality is reduced. On the other hand, decreasing the flow rate to 1.15 L/min at 3000 rpm allowed a transient temperature to change from 20˚C to 76.7˚C over an extended time, therefore complete inactivation of *L.*

plantarum could be accounted for by TRTR, and SPR-L was minimal. The higher values of SPR-L compared to TRTR at 3000 rpm and 3600 rpm at an exit temperature of 65.6˚C signify that increased temperatures can also facilitate efficacy of hydrodynamic cavitation to inactivate microorganisms due to increased product residence time in the SPR.

A mean inactivation value of 7.48 log cycles of *L. plantarum* suggests that gram-positive cells are vulnerable to hydrodynamic cavitation when processed in the SPR at 3000 and 3600 rpm and exit temperatures equal to or above 65.6˚C. *L. plantarum* exhibited the highest z value (27.4˚C). Therefore; the thermal resistance of this organism would have prevented complete inactivation from thermal effects alone. Because *L. plantarum* can resist higher processing temperatures, this organism is a likely candidate to test inactivation due to hydrodynamic cavitation when exit temperature is in the 60 to 80˚C range. Additional literature reviewed by Earnshaw (1998) suggest that cocci shaped cells display more resistance to cavitation than rods.

Apple juice – *L. sakei*

L. sakei is a microorganism usually associated with meat products. However, due to its high D-value, a z value of 21.6˚C and rod shape morphology, *L. sakei* was used to evaluate hydrodynamic cavitation in conjunction with moderate heating regimens. Utilizing hydrodynamic cavitation induced by the SPR to process calcium fortified apple juice inoculated with *L. sakei* at 3000 and 3600 rpm with feed to exit temperature range of 20˚C to 65.6˚C and 76.7˚C resulted in a reduction of 7.25 ± 0.11 logs. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.42, 0.36, 0.69 and 0.65˚C/s, respectively. In addition to non-detectable recovered CFU (detection limit ≤1 CFU/mL), enriched samples

also were negative for viable cells. Table 3.2 indicates that a SPR setting of 3000 rpm required mean flow rates of 1.59 and 1.10 L/min to raise feed temperature of 20˚C to exit at 65.6˚C and 76.7˚C, respectively. At SPR speeds of 3600, mean flow rates were 2.65 and 2.00 L/min to raise feed temperature of 20˚C to exit at 65.6˚C and 76.7˚C, respectively. The mean values of TRTR and SPR-L in the 3000 rpm/65.6˚C was 1.79 and 5.46 log cycles, respectively. Processing at 3600 rpm/65.6˚C yielded mean TRTR and SPR-L values of 1.09 and 6.16 log cycles, respectively. Comparing 3000 rpm to 3600 rpm for an end point processing temperature of 65.6˚C reveals a significant difference in TRTR and SPR-L values at each SPR speeds. The TRTR values for 3600 rpm/65.6˚C were significantly lower than TRTR values for 3000 rpm/65.6˚C. This difference is a function of mass flow rate through the system, with a high SPR setting requiring an increased flow rate in order to maintain a target processing temperature. This relationship reinforces the significance of mass flow rate and SPR operation setting. However, this relationship is not observed when examining SPR-L values. A significantly higher SPR-L value is evident with 3600 rpm/65.6˚C when compared to 3000 rpm/65.6˚C. This difference is strongly dependent upon the role of residence time in values of TRTR. The value of SPR-L is determined as the difference between the complete log reduction and the theoretical TRTR. Therefore, as the residence time in the SPR is reduced, the SPR-L value will increase. This relationship was manifested by all organisms inoculated into apple juice. These data are consistent with observations made above with the other inoculated organisms. Elevated temperature is critical to the efficacy of hydrodynamic cavitation as a means of microbial cell inactivation. Processing at 3000 and 3600 rpm at 76.7˚C completely inactivated *L. sakei* due to thermal effects alone.

Apple juice – *Z. bailii* (vegetative cells)

The yeast *Z. bailii* is associated with the spoilage of many acidified fluid foods such as salad dressings, fruit concentrates, soft drinks, wine and high acid fruit juices. Utilizing hydrodynamic cavitation induced by the SPR to process calcium fortified apple juice inoculated with *Z. bailii* at 3000 and 3600 rpm with feed temperature at 20˚C and exit temperature at 65.6˚C and 76.7˚C resulted in a mean reduction CFU ± SD of 6.17 ± 0.11 logs. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.40, 0.37, 0.70 and 0.63˚C/s, respectively. In addition to non-detectable viable CFU in solid medium (detection limit ≤1 CFU/mL), enriched samples exhibited no viable cells. Table 3.2 indicates that a SPR speed of 3000 rpm requires mean flow rates of 1.55 and 1.15 L/min to achieve raise feed temperature of 20˚C to 65.6˚C and 76.7˚C, respectively. At SPR speed of 3600 rpm, mean flow rates were 2.67 and 1.96 L/min to raise feed temperature from 20˚C to 65.6˚C and 76.7˚C, respectively. The mean value of TRTR and SPR-L from 3000 rpm/65.6˚C process was 4.18 and 1.98 log cycles, respectively. Processing at 3600 rpm/65.6˚C yielded mean TRTR and SPR-L values of 2.49 and 3.68 logs, respectively. Values obtained for TRTR and SPR-L for product processed at 3000 and 3600 rpm at 65.6˚C differed significantly. Processing at 3000 and 3600 rpm at 76.7˚C completely inactivated *Z. bailii* due to thermal effects alone. The same relationship between reduced mass flow rates and SPR settings described above for *L. plantarum* and *L. sakei* apply here and is manifested by the data for *Z. bailii* inactivation in the SPR.

Apple juice – *Z. bailii* (ascospores)

Utilizing hydrodynamic cavitation induced by the SPR to process calcium fortified apple juice inoculated with *Z. bailii* ascospores at 3000 and 3600 rpm with feed temperature of 20˚C and exit at 65.6˚C and 76.7˚C resulted in reduction of 5.14 ± 0.09 logs. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.40, 0.37, 0.68 and 0.62˚C/s, respectively. In addition non-detectable CFU in solid media (detection limit ≤1 CFU/mL), all enriched samples were negative for viable organisms. Table 3.2 indicates that a SPR setting of 3000 rpm requires mean flow rates of 1.52 and 1.13 L/min to raise inlet temperature from 20˚C to 65.6˚C and 76.7˚C, respectively. At SPR speed of 3600 rpm, mean flow rates were 2.62 and 1.92 L/min to raise feed temperature from 20˚C to 65.6˚C and 76.7˚C, respectively. The mean value of TRTR and SPR-L from 3000 rpm/65.6˚C was 2.70 and 2.44 log cycles, respectively. Processing at 3600 rpm/65.6˚C yielded mean TRTR and SPR-L values of 1.67 and 3.46 log cycles, respectively. Values obtained for TRTR and SPR-L for product processed at 3000 and 3600 rpm at 65.6˚C differed significantly. Processing at 3000 and 3600 rpm at 76.7˚C completely inactivated of *Z. bailii* ascospores from thermal effects alone. The same relationship between reduced mass flow rates and SPR speeds described for *L. plantarum*, *L. sakei* and *Z. bailii* vegetative cells apply here.

Ascospores of *Z. bailii* proved to be very heat resistant with a mean D-value of 13.08 minutes; however, the ascospores displayed a relatively low tolerance for changes in temperature with a z-value of 5.0˚C. This low tolerance for changes in temperatures and exposure to hydrodynamic cavitation contributed to the inactivation of *Z. bailii*. *Z. bailii* generate several ascospores within a fluid-like sac known as asci. The asci sac houses one to four elliptical

entities. It is postulated that the intact asci sac facilitates the formation of cavitation events in close proximity to ascopores causing their inactivation.

Tomato juice – *B. coagulans* (spores)

Utilizing hydrodynamic cavitation induced by the SPR to process tomato juice inoculated with *B. coagulans* spores at 3000 and 3600 rpm with feed temperature at 37.7˚C and exit temperature at 93.3˚C and 104.4˚C resulted in various levels of reduction of *B. coagulans* spores and is presented in Table 3.3. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.38, 0.27, 0.74 and 0.60˚C/s, respectively. In addition to viable CFU enumerated on solid medium (detection limit ≤1 CFU/mL), all enriched samples were positive for viable organisms. Table 3.3 indicates that a SPR speed of 3000 rpm requires mean flow rates of 0.66 and 0.40 L/min to raise feed temperature from 37.7˚C to 93.3˚C and 104.4˚C, respectively at the exit. At SPR speed of 3600 rpm, mean flow rates 1.30 and 0.88 L/min to raise feed temperature from 37.7˚C to 93.3˚C and 104.4˚C at the exit, respectively. Processing at 3000 rpm and 104.4˚C provided for the greatest mean log reduction of 3.10 log cycles. The second highest log reduction came from processing at 3600 rpm and 104.4˚C with a mean log reduction of 2.29 log cycles. This difference can be accounted for by the product flow rate and product residence time in the SPR. Processing at 3000 rpm at 104.4˚C required almost double the residence time when compared to processing with 3600 rpm at 104.4˚C. This increase in residence time is not reflected in the SPR-L values, but rather in increased TRTR. This relationship illustrates how critical operational setting and product flow rate are on total microorganism inactivation. The mean quantity value of TRTR and SPR-L from 3000

rpm/93.3˚C was 0.05 and 0.84 log cycles, respectively. These values significantly differed from processing at 3000 rpm/104.4˚C and yielded mean TRTR and SPR-L values of 1.74 and 1.35 log cycles, respectively. The increase in TRTR and SPR-L values for 3000 rpm/104.4˚C when compared to 3000 rpm/93.3˚C is due to increased cavitation exposure time coupled with elevated processing temperatures. Reducing product flow rates allowed additional TRTR values to accumulate and enhanced exposure times to hydrodynamic cavitation increase SPR-L values. The same pattern can be seen when evaluating TRTR and SPR-L relationships between the SPR speeds of 3600 rpm and exit processing temperatures of 93.3˚C and 104.4˚C.

The survival of *B. coagulans* spores indicates that more developments are needed in hydrodynamic cavitation processing for commercial tomato juice processing. Achieving log reductions between 0.5 and 3.1 logs presents promising potential for commercial sterilization of tomato juice at reduced processing temperatures. Increasing the diameter and length of the inner rotor may allow increased product flow rates at reduced exit temperature by increasing residence time to ensuring adequate lethal values for *B. coagulans* spores. In addition, an appropriately designed SPR could have applications in aseptic processing.

Skim milk – *C. sporogenes* P.A. 3679 (spores)

Utilizing hydrodynamic cavitation induced by the SPR to process skim milk inoculated with P.A. 3679 spores at 3000 and 3600 rpm with feed temperatures of 37.7˚C to exit temperatures of 104.4˚C and 115.6˚C, resulted in various levels of reduction of P.A. 3679 spores. Values are reported in Table 3.4. Average transient time-temperature values were calculated using eq. 2. Average transient time-temperature values for 3000 and 3600 rpm with end exit temperatures of 65.6 and 76.7˚C were 0.44, 0.36, 0.81 and 0.70˚C/s, respectively. In addition to

viable CFU recovered in solid medium (detection limit ≤1 CFU/mL), all enriched samples were positive for viable organisms. Results in Table 3.4 indicate that a SPR at 3000 rpm required mean flow rates of 0.0.76 and 0.52 L/min to change a feed temperature of 37.7˚C to exit temperatures of 104.4˚C and 115.6˚C, respectively. At SPR speeds of 3600, mean flow rates were 1.42 and 1.02 L/min to change feed temperature change from 37.7˚C to exit temperatures of 104.4˚C and 115.6˚C, respectively. Processing at 3000 rpm and 115.6˚C resulted in the greatest mean log reduction of 2.84 logs. The second highest log reduction came from processing at 3600 rpm and 115.6˚C with a mean log reduction of 2.16. This difference can be accounted by the product flow rate and the consequent product residence time in the SPR. Processing at 3000 rpm at 115.6˚C required almost double the residence time in the SPR when compared to processing at 3600 rpm at 115.6˚C. This increase in residence time is not reflected in the SPR-L values, but in an increase in TRTR. The data for P.A. 3679 spore inactivation can be explained as above for *B. coagulans* spores.

The survival of P.A. 3679 spores in the current SPR design reinforces the need for further development in the design and operation of a hydrodynamic cavitation processing. Although log reductions between 0.7 and 2.84 is low for commercial sterilization of low acid foods, the data on vegetative cell inactivation in the SPR at relatively low temperatures indicate potential milk pasteurization and with proper system modification, for commercial sterilization of skim milk at reduced processing temperatures.

Conclusions

Hydrodynamic cavitation provides processors an effective method for pasteurizing low acid and commercial sterilization of high acid foods. Hydrodynamic cavitation induced adequate

destructive forces to inactivate vegetative cells of bacteria, yeast, yeast ascospores and heat resistant bacteria spores. Common spoilage microorganisms such as lactic acid bacteria and yeast can be eliminated at reduced temperatures due to the synergistic effect of temperature and hydrodynamic cavitation. Adequate lethality was obtained for commercial sterility of high acid foods using the current SPR design. However, system modification and operating procedures need to be developed to achieve commercial sterility in high acid foods. Differences in resistance to hydrodynamic cavitation were not observed between gram-positive bacteria and yeast because thermally induced lethality predominated under the conditions of the test. Bacteria spores proved to be the most resistant to both thermal and hydrodynamic cavitation effects. With hydrodynamic cavitation spore inactivation was achievable at reduced processing temperatures; however the total lethality was not adequate for commercial sterility. Alteration of SPR design and consideration for in-process residence time must be investigated to achieve commercial sterility in low acid foods. Increased fluid feed temperature is an option for processors to improve total lethality at relatively low speeds and low exit temperature.

Hydrodynamic cavitation induced microbial lethality greater than that accounted for by thermal effects. Lethality from hydrodynamic cavitation is strongly dependent upon moderate processing temperatures (65.6˚C and above), elevated product exit temperature with maximal SPR residence time, and rotational speed of the SPR rotor. Foods such as acidic fruit juices, salad dressings and milk can be safely processed at reduced processing temperatures. Reduction of processing temperature translates into superior products.

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Table 3.1: D and z values for spoilage microorganisms using capillary tube determination method (n = 3; means ± SD).

Spoilage Microorganism	Food Medium	$D_{55^{\circ}C}$ (mins)	$D_{100^{\circ}C}$ (mins)	z vlaue[a] (˚C / ˚F)
B. coagulans (spores)	Tomato Juice	-------	2.20 ± 0.53	8.0 / 14.4
C. sporogenes (spores)	Skim Milk	-------	22.30 ± 1.18	9.8 / 17.6
L. plantarum	Apple Juice[b]	1.45 ± 0.51	-------	15.2 / 27.4
L. sakei	Apple Juice[b]	1.67 ± 0.33	-------	12.0 / 21.6
Z. bailii (veg cells)	Apple Juice[b]	2.38 ± 0.19	-------	7.0 / 12.6
Z. bailii (ascospores)	Apple Juice[b]	13.08 ± 0.47	-------	5.0 / 9.0

[a] = z values determined from thermal death curves constructed from surviving mean $\log_{10}$ CFU values.
[b] = Calcium fortified.

Table 3.2: Enumeration[a] of high acid fruit juice spoilage microorganisms processed in calcium fortified apple juice (pH = 3.9) at two SPR[b] settings (3000 and 3600 rpm) at an initial temperature of 20˚C and two exit temperatures of 65.6˚C and 76.7˚C, respectively (n = 6)[c].

Spoilage Organism	Setting/Exit Temp (rpm / ˚C)	FR[d] (L/min)	ΔT_{avg} (˚C/s)	LR[e]	TRTR[f]	SPR-L[g]	LT[h]
L. plantarum (veg cells)	3000 / 65.6	1.48 ± 0.07^{o}	0.39	7.48 ± 0.09	1.62 ± 0.08^{n}	5.87 ± 0.12^{n}	0/6
	3000 / 76.7	1.15 ± 0.05^{p}	0.37	7.48 ± 0.09	*	**	0/6
	3600 / 65.6	2.55 ± 0.13^{m}	0.67	7.48 ± 0.09	0.96 ± 0.05^{o}	6.52 ± 0.12^{m}	0/6
	3600 / 76.7	1.89 ± 0.09^{n}	0.61	7.48 ± 0.09	5.69 ± 0.71^{m}	1.79 ± 0.76^{o}	0/6
L. sakei (veg cells)	3000 / 65.6	1.59 ± 0.02^{o}	0.42	7.25 ± 0.11	1.79 ± 0.01^{m}	5.46 ± 0.11^{n}	0/6
	3000 / 76.7	1.10 ± 0.02^{p}	0.36	7.25 ± 0.11	*	**	0/6
	3600 / 65.6	2.65 ± 0.05^{m}	0.69	7.25 ± 0.11	1.09 ± 0.04^{n}	6.16 ± 0.13^{m}	0/6
	3600 / 76.7	2.00 ± 0.00^{n}	0.65	7.25 ± 0.11	*	**	0/6
Z. bailii (veg cells)	3000 / 65.6	1.55 ± 0.04^{o}	0.40	6.17 ± 0.09	4.18 ± 0.02^{m}	1.98 ± 0.09^{n}	0/6
	3000 / 76.7	1.15 ± 0.04^{p}	0.37	6.17 ± 0.09	*	**	0/6
	3600 / 65.6	2.67 ± 0.05^{m}	0.70	6.17 ± 0.09	2.49 ± 0.12^{n}	3.68 ± 0.14^{m}	0/6
	3600 / 76.6	1.96 ± 0.04^{n}	0.63	6.17 ± 0.09	*	**	0/6
Z. bailii (ascospores)	3000 / 65.6	1.52 ± 0.04^{o}	0.40	5.14 ± 0.09	2.70 ± 0.06^{m}	2.44 ± 0.14^{n}	0/6
	3000 / 76.7	1.13 ± 0.04^{p}	0.37	5.14 ± 0.09	*	**	0/6
	3600 / 65.6	2.62 ± 0.05^{m}	0.68	5.14 ± 0.09	1.67 ± 0.04^{n}	3.46 ± 0.09^{m}	0/6
	3600 / 76.7	1.92 ± 0.05^{n}	0.62	5.14 ± 0.09	*	**	0/6

[a] Average enumeration (means $\log_{10}$ CFU ± S.D.) data calculated from three separate trials with two samples per treatment (detection ≤1 CFU/mL).

[b] SPR = "Shockwave Power^{TM} Reactor" or controlled cavitation system.

[c] n = 6; means ± SD followed by same letter in column for each organism do not differ significantly (α = 0.05) according to ANOVA and SNK analysis.

[d] FR = Flow Rate: rate of flow through SPR system and hold tube (L/min).

[e] LR = Log Reduction: total mean log reduction for three separate trials (mean $\log_{10}$ CFU ± SD).

[f] TRTR = TRTR = Thermal Residence Time Reduction: mean log reduction due to integrated lethality during transient temperature change inside SPR plus lethality accumulated during hold time in exit piping of SPR (mean $\log_{10}$ CFU ± SD).

[g] SPR-L = Mean log reduction due to hydrodynamic cavitation treatment after TRTR effects have been removed (mean $\log_{10}$ CFU ± SD).

[h] LT = Lethality Test: incidence of growth after enrichment malt extract broth.

* = TRTR allowed for complete theoretical thermal inactivation.

** = SPR-L values do not reflect potential hydrodynamic cavitation inactivation due to TRTR.

Table 3.3: Enumeration[a] of *Bacillus coagulans* spores in tomato juice (pH = 4.1) processed at two SPR[b] settings (3000 and 3600 rpm) at an initial temperature 38˚C and two exit temperatures of 93˚C and 104 ˚C, respectively (n = 6)[c].

Spoilage Organism	Setting/Exit Temp (rpm/˚C)	FR[d] (L/min)	ΔT_{avg} (˚C/s)	LR[e]	TRTR[f]	SPR-L[g]	LT[h]
B. coagulans (spores)	3000/93	0.66 ± 0.01^{o}	0.38	0.88 ± 0.10^{p}	0.05 ± 0.00^{o}	0.84 ± 0.10^{p}	6/6
	3000/104	0.40 ± 0.02^{p}	0.27	3.10 ± 0.21^{m}	1.74 ± 0.08^{m}	1.35 ± 0.19^{n}	6/6
	3600/93	1.30 ± 0.02^{m}	0.74	0.92 ± 0.26^{o}	0.02 ± 0.00^{p}	0.90 ± 0.26^{o}	6/6
	3600/104	0.88 ± 0.04^{n}	0.60	2.29 ± 0.20^{n}	0.80 ± 0.03^{n}	1.49 ± 0.17^{m}	6/6

[a] Average enumeration (means $\log_{10}$ CFU ± S.D.) data calculated from three separate trials with two samples per treatment (detection ≤1 CFU/mL).
[b] SPR = "Shockwave Power™ Reactor" or controlled cavitation system.
[c] n = 6; means ± SD followed by same letter in column do not differ significantly ($\alpha = 0.05$) according to ANOVA and SNK analysis.
[d] FR = Flow Rate: rate of flow through SPR system and hold tube (L/min).
[e] LR = Log Reduction: total mean log reduction for three separate trials (mean $\log_{10}$ CFU ± SD).
[f] TRTR = TRTR = Thermal Residence Time Reduction: mean log reduction due to integrated lethality during transient temperature change inside SPR plus lethality accumulated during hold time in exit piping of SPR (mean $\log_{10}$ CFU ± SD).
[g] SPR-L = Mean log reduction due to hydrodynamic cavitation treatment after TRTR effects have been removed (mean $\log_{10}$ CFU ± SD).
[h] LT = Lethality Test: incidence of growth after enrichment in nutrient broth.

Table 3.4: Enumeration[a] of *Clostridium sporogenes* P.A. 3679 spores in skim milk (pH = 6.0) processed at two SPR[b] settings (3000 and 3600 rpm) at an initial temperature of 49˚C and two exit temperatures of 104˚C and 116˚C, respectively (n = 6)[c].

Spoilage Organism	Setting/Exit Temp (rpm/˚C)	FR[d] (L/min)	ΔT_{avg} (˚C/s)	LR[e]	TRTR[f]	SPR-L[g]	LT[h]
P.A. 3679 (spores)	3000/104	0.76 ± 0.04^{o}	0.44	0.70 ± 0.18^{o}	0.04 ± 0.00^{o}	0.64 ± 0.18^{n}	6/6
	3000/116	0.52 ± 0.02^{p}	0.36	2.84 ± 0.11^{m}	1.57 ± 0.06^{m}	1.28 ± 0.17^{m}	6/6
	3600/104	1.42 ± 0.04^{m}	0.81	0.69 ± 0.07^{o}	0.05 ± 0.00^{o}	0.64 ± 0.07^{n}	6/6
	3600/116	1.02 ± 0.06^{n}	0.70	2.16 ± 0.18^{n}	0.80 ± 0.05^{n}	1.36 ± 0.20^{m}	6/6

[a] Average enumeration (means $\log_{10}$ CFU ± S.D.) data calculated from three separate trials with two samples per treatment (detection ≤1 CFU/mL).
[b] SPR = "Shockwave Power™ Reactor" or controlled cavitation system.
[c] n = 6; means ± SD followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis.
[d] FR = Flow Rate: rate of flow through SPR system and hold tube (L/min).
[e] LR = Log Reduction: total mean log reduction for three separate trials (mean $\log_{10}$ CFU ± SD).
[f] TRTR = TRTR = Thermal Residence Time Reduction: mean log reduction due to integrated lethality during transient temperature change inside SPR plus lethality accumulated during hold time in exit piping of SPR (mean $\log_{10}$ CFU ± SD).
[g] SPR-L = Mean log reduction due to hydrodynamic cavitation treatment after TRTR effects have been removed (mean $\log_{10}$ CFU ± SD).
[h] LT = Lethality Test: incidence of growth after enrichment in reinforced clostridium medium.

Fig.3.1 - Schematic of the Shockwave Power™ Reactor (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

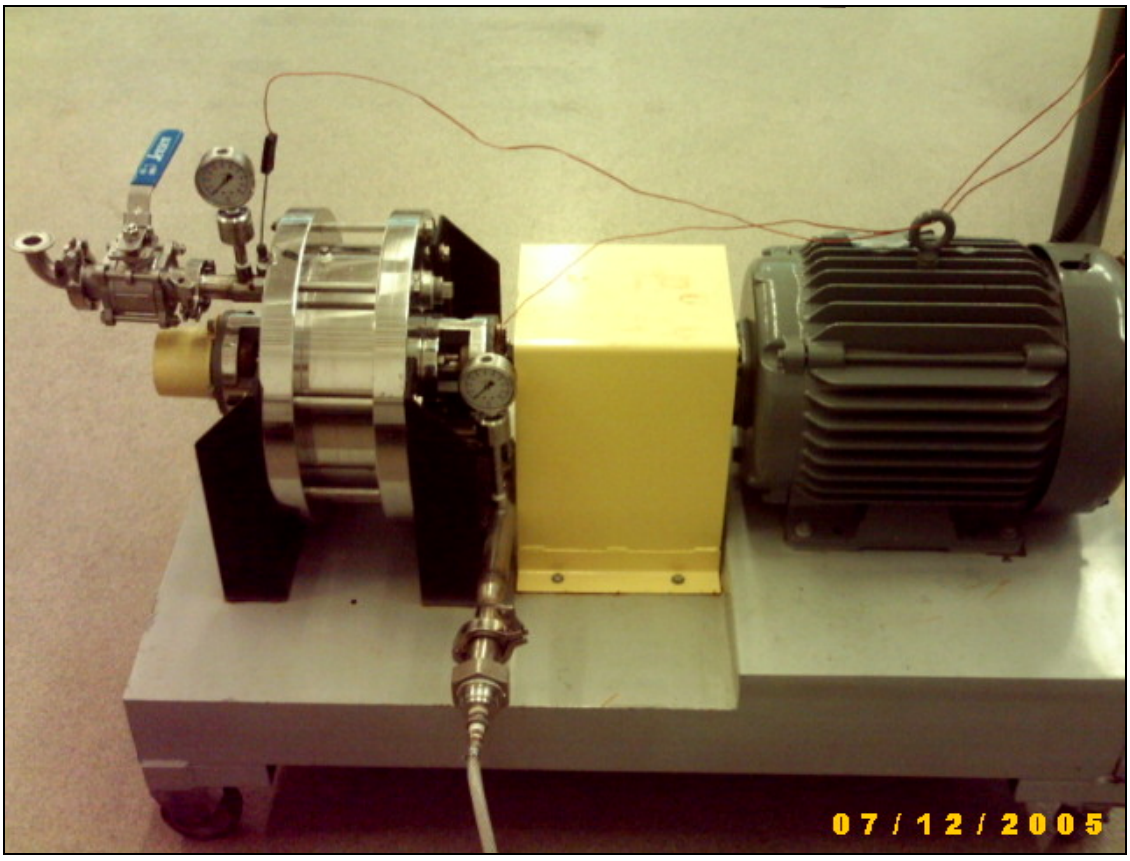

Fig. 3.2 - 12 HP Shockwave Power™ Reactor (10” x 2” pilot scale unit) (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

Fig.3.3 - Hydrodynamic cavitation within inner rotor cavities (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

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CHAPTER 4

HYDRODYNAMIC CAVITATION TO IMPROVE BULK FLUID TO SURFACE MASS TRANSFER IN A NON-IMMERSED ULTRAVIOLET SYSTEM FOR MINIMAL PROCESSING OF OPAQUE AND TRANSPARENT FLUID FOODS[1]

[1] Milly, P.J. and R.T. Toledo. To be submitted to *Journal of Food Science*

Abstract

Ultraviolet (UV) induced chemical reactions and inactivation of microorganisms in transparent and opaque fluids is strongly dependent upon on the homogenous exposure of the target species to the UV irradiation. Current UV technologies used in water disinfection and food preservation applications have limited efficacy due to suspended particles shading target species. An Ultraviolet-Shockwave Power^{TM} Reactor (UV-SPR) consisting of an inner rotating rotor and a stationary quartz housing and two end plates was used to induce "controlled cavitation." Eight UV low pressure mercury lamps spaced uniformly were installed lengthwise around the quartz housing periphery. A KI to I_3^- chemical dosimeter for UV was used to quantify photons received by fluid in the annular space of the SPR. UV dose (J/m^2) increased from 97 J/m^2 at 0 rpm to over 700 J/m^2 for SPR speeds above 2400 rpm. Inactivation of *E. coli* 25922 in apple juice and skim milk in the UV-SPR at exit temperatures below 45˚C was greater than 4.5 and 3 logs, respectively. The UV-SPR system proved successful in increasing the mass transfer of transparent and opaque fluid to the UV irradiated surface.

Introduction

The adoption of germicidal UV treatment in water and waste water processing facilities has generated a number of studies validating the efficacy of UV for processing transparent fluids (Liltved and Cripps 1999; Sommer and others 2000; NACMCF 2004). Germicidal ultraviolet light (UVC) at 200 to 280 nm wavelength has been shown to be successful for inactivating pathogenic microorganisms like *E. coli* O157:H7 and *Cryptosporidium parvum* in processed fruit juices such as apple juice and cider (Wright and others 2000; Vasavada 2003). Interest in UV treatment of fluid food as an alternative to conventional heat treatment continues to grow. Processors concerned with product flavor and nutrient changes brought on by conventional heat treatment continue to view UV processing as a favorable alternative (Vasavada 2003). In addition, the equipment required for effective thermal pasteurization may be too high-priced for small operations.

The use of a chemical actinometer or dosimeter consisting of a light-induced chemical reaction to manifest a quantum yield, φ, is well known. Quantum yield is defined as the number of events, molecules formed, changed, or destroyed divided by the number of photons absorbed at a particular wavelength in the same period of time (Kuhn and others 2004). The Rahn actinometer (Rahn 1997) permits measurement of UV photons (253.7 nm) that result in the photochemical conversion of iodide (I^-) to triiodide (I_3^-). The actinometer allows determination of UV energy flux or UV dose (J/m^2) absorbed by a solution flowing past an irradiated surface.

Currently available UV systems can effectively process only transparent fluids. Opaque fluids such as milk prove difficult to process with UVC because depth of UVC penetration is small and most microorganisms do not receive direct UVC exposure. In a well-mixed system,

shadowing effects can be minimized and more of the target species would be exposed to UVC rays.

An Ultraviolet-Shockwave Power™ Reactor (UV-SPR) consisting of an inner rotating cylinder with surface cavities surrounded by a stationary quartz housing and two quartz end plates was used to induce "controlled cavitation." The UV source for the UV-SPR consisted of eight pressure mercury lamps installed lengthwise and spaced uniformly around the periphery of the quartz cylinder. The hypothesis of this research is that turbulence induced by cavitation in a fluid flowing through a UV-SPR unit at varying speeds will increase mass transfer of fluid from the fluid bulk towards the irradiated surface resulting in higher UV absorption by the fluid compared to flow-induced turbulence alone. Increased UV absorption would be manifested by increased formation of triiodide in a chemical actinometer and increased microbial inactivation of a target microbial species. The objective of this study was to quantify the UV dose (J/m^2) delivered to the actinometer solution at varying UV-SPR speeds (rpm) and to quantify inactivation of an E. coli O157:H7 surrogate, *E. coli* ATCC 25922, in apple juice and skim milk at varying operational speeds and exit temperatures below 45˚C.

Materials and Methods

Ultraviolet hydrodynamic cavitation reactor

An Ultraviolet Shockwave™ Power Reactor (UV-SPR™) system constructed by Hydrodynamics Inc., Rome, GA was placed at the University of Georgia, Food Science and Technology pilot plant (Fig. 4.1). The 0.1524 m x 0.19685 m (6 inch diameter x 7.75 inch length) unit consisted of concentric cylinders with an annular space (2.16 L) separating the stationary housing cylinder from the inner, rotating cylinder. The system was powered by a 12

HP motor and generates cavitation in the annular space filled with fluid when the inner rotor which contains multiple cavities on its surface is rotated at high speeds. The unit was surrounded by four 20.32 cm long, high output, low pressure mercury ultraviolet (UV) lamps (Model GPH203T5VH/HO/4P, Lightsources Inc., Orange, CT) each with a rated intensity of 42 $\mu W/cm^2$ at 1 meter plus four 26.3 cm long, high output, low pressure mercury UV lamps (Strahler NNI 60/26 U, Heraeus Noblelight GmbH, Heraeusstraße 12-14 D-63450 Hanau Germany) with a rated intensity of 0.15 mW/cm^2 at 100 cm. Figure 4.2 is the actual pilot scale unit without UV lamps illuminated and Figure 4.3 displays the unit while being irradiated. The stationary outer cylinder was made of 3 mm thick quartz which allowed UV irradiation to penetrate the annular space. The outside surface area of the quartz housing was 0.094 m^2. Nominal power ratings were 18 and 90 W, respectively, for the Lightsource and Heraeus lamps, and the output had a primary wavelength of 254 nm. Lamps were positioned parallel to the outer quartz cylinder axis at 60° intervals around the periphery. Taylor-Couette flow was induced as the inner rotor spins while fluid transferred from the annular space to the cavities on the rotor surface exits the cavity and forced toward the outer cylinder increasing product exposure to UV irradiation. Figure 4.4 illustrates the formation of bubbles as the inner rotor spins. The UV-SPR system pressure was maintained at 75.84 ± 6.895 kPa (11 ± 1 psi_g). Fluid feed rates for the actinometry solution and the inoculated test food fluids were maintained at 1.5 L/min in all trials.

Ultraviolet actinometry

The Rahn actinometer (Rahn, 1997) was used to determine the effect of rotational speed in the UV-SPR on the UV energy received by fluid flowing through the annular space. The actinometer is based on the conversion of iodide (I^-) to triiodide (I_3^-), with the latter measured by

absorbance at 352 nm. The photochemical reaction is forced out of equilibrium towards the complete stoichiometric conversion of photons and iodide to triiodide by the presence of potassium iodate (KIO_3) and a pH of 9.14 with a 0.01 M sodium borate ($Na_2B_4O_7.10H_2O$) buffer as seen in Eq. (1):

$$8KI + KIO_3 + 3H_2O + hv \rightarrow 3I_3^- + 6OH^- + 9K^+ \quad (1)$$

In order to determine the concentration of triiodide formed, Beer's law was utilized as seen in Eq. (2):

$$A_{352} = (\varepsilon) * (b) * (c) = -\log \frac{I}{I_0} \quad (2)$$

where A_{352} = optical density of the solution or the energy of radiation reaching a given area of a detector per second at a wavelength of 352 nm (I_0 = initial radiant intensity and I = remaining radiant energy), ε = molar absorptivity or extinction coefficient ($M^{-1}*cm^{-1}$), b = path length of radiation (cm) and *c* = molar concentration (moles/L). According to Skoog and West (1965), the solubility of Iodine (I_2^-) in de-ionized water will be slightly higher than 1.0×10^{-3} mol/L at room temperature. Therefore, a saturated solution of Iodine (Iodine, EM Science, Gibbstown, NJ) in de-ionized water was prepared and allowed to dissolve overnight. Because the molar absorptivity coefficient changes with varying solvent systems, addition of known volumes of saturated Iodine solution to 0.15 *M* KI (Potassium Iodide, J.T. Baker, Phillipsburg, NJ) and Rahn's recommended standard solution allowed for calculation of a molar absorptivity coefficient for triiodide in the actinometer solution. Rahn's recommended standard solution consisted of 0.6 *M* KI, 0.1 *M* KIO_3 (Sodium Iodate, J.T. Baker, Phillipsburg, NJ) and 0.01 *M* $Na_2B_4O_7*10H_2O$ (Sodium Borate, 10-Hydrate, Crystal, J.T. Baker, Phillipsburg, NJ) yielding a solution with pH of 9.14. Using an ultraviolet-visible light spectrophotometer (8453 UV-Visible

Spectrophotometer, Agilent Technologies, Inc., Santa Clara, CA), the wavelength for maximum absorbance and molar absorptivity coefficient (L/mol*cm) were determined. A wavelength of 352 nm was determined to provide the maximum absorbance in both solutions. The molar absorptivity coefficient for triiodide in 0.15 *M* KI has been reported to range from 25,800 and 26,400 M^{-1}*cm^{-1}, with an average value of 26,100 M^{-1}*cm^{-1} (Rahn and others 2003). Based on the average molar absorptivity value and observed absorbance value for triiodide in 0.15 *M* KI, the molar concentration of the saturated Iodine solution was determined to be 1.157 x 10^{-3} *M*. The absorbance of triiodide in Rahn's standard solution was 1.018 times that of triiodide in 0.15 *M* KI. Therefore, the molar absorption coefficient in Rahn's standard solution was calculated: 26,100 x 1.018 = 26,600 M^{-1}*cm^{-1}. The observed molar absorptivity coefficient for triiodide in Rahn's standard solution was 26,570 M^{-1}*cm^{-1}, in close agreement with previous calculations. A molar absorptivity value of 26,600 M^{-1}*cm^{-1} was used to quantify molar concentrations of triiodide in processed samples from obtained absorbance values at 352 nm in a spectrophotometer (Visible Spectrophotometer, Bausch and Lomb (Milton Roy) Spectronic 20, U.S.A.).

Once the molar concentration of triiodide was determined from processed samples, the energy delivered by the system to the processed fluid could be calculated. The quantum yield of the KI-KIO_3 actinometer is 0.73 moles I_3^-/mole photon (Rahn and others 2003). The UV energy absorbed by the solution is calculated by Eq. (3):

$$E = \frac{c * \alpha * q}{\varphi} \tag{3}$$

where E = energy absorbed by fluid (J/s), *c* = molar concentration of triiodide (moles/L), α =

4.71 x10^5 J/mole photon, q = flow rate through UV-SPR (L/s) and φ = quantum yield = 0.73 moles triiodide/mole photon.

Before processing, the KI/KIO_3 with buffer solution was pre mixed until all solutes were dissolved in reverse osmosis treated water and allowed to recycle through UV-SPR system without ultraviolet light until the temperature in the feed reservoir reached 37.8˚C.

Preparation of microbial inoculum

E. coli ATCC 25922 has been deemed an acceptable surrogate for *E. coli* O157:H7 due to their similarity in ultraviolet irradiation sensitivity. The strain is resistant to nalidixic acid in quantities less than or equal to 50 μg/mL, making it ideal for biological challenge tests in milk products. To ensure healthy *E. coli* ATCC 25922 inocula, *E. coli* was grown in tryptic soy broth (TSB, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) aerobically for 24 h at 37°C and transferred to new broth cultures three times. Actively growing *E. coli* was inoculated in TSB supplemented with increasing concentrations of nalidixic acid (NA, Sigma-Aldrich, Inc., U.S.A.) until sustainable growth was achieved in the presence of 50 μg/mL nalidixic acid. The *E. coli* cells used for inoculation were grown in TSB supplemented with 50 μg/mL nalidixic acid for 20 h at 37˚C. Enumeration of *E. coli* CFU was performed on tryptic soy agar (TSA, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.) supplemented with 50 μg/mL nalidixic acid.

The actively growing culture of *E. coli* was centrifuged (Centrific™ Centrifuge Model 225, Fisher Scientific, U.S.A.) at 1000 x g for 10 min, rinsed with 0.1% peptone water (Bacto™ Peptone, Difco Laboratories, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.), then

centrifuged as before. Concentrated populations of microorganisms were used immediately or stored at 4°C for no longer than one day.

Test food fluids

Reconstituted calcium fortified apple juice (Kroger 100% Juice Frozen Concentrated Apple Juice with Calcium and Vitamin C, Kroger Co., Cincinnati, Ohio, U.S.A.) (pH = 3.91) and skim milk (Kroger Grade A Vitamin A and D Skim Milk, Kroger Co., Cincinnati, OH, 45202) (pH = 6.0) were used as test food fluids for biological challenge tests of the UV-SPR against the vegetative cells of *E. coli* ATCC 25922. Inlet temperatures for apple juice and skim milk were 18˚C ± 1˚C and 15˚C ± 1˚C, respectively.

The test fluids were inoculated separately with *E. coli* ATCC 25922 to obtain an initial mean inoculum level greater than 5 logs. The inoculated test fluids were used immediately in the UV-SPR process treatments.

Experimental design

Actinometry experiments were conducted three times with each treatment having two samples collected and analyzed accordingly (n =6). Preliminary research suggested inactivation of vegetative cells due to hydrodynamic cavitation alone was negligible at end point processing temperatures below 54.4˚C (data not shown). *E. coli* inoculated test fluids were processed in the UV-SPR at varying speeds and exit temperatures below 54.4˚C. Samples from each treatment were collected and enumerated for surviving microorganisms. Tests were repeated twice with two samples collected per test and surviving organisms were enumerated in each sample (n = 4). Number of CFU's in each sample was determined in duplicate and averaged. Enumeration

detection limit for all samples was ≥10 CFU/mL. Results were analyzed using a 2-way analysis of variance (ANOVA, $\alpha = 0.05$) using the general linear model procedures of SAS (SAS Institute, Inc., Cary, N.C.) and Student-Newman-Kuels (SNK) test, when appropriate.

Results and discussion

UV-SPR: UV actinometry

Preliminary studies (data not shown) suggested that power absorption by the fluid (in Watts) is dependent on the fluid flow rate through the UV-SPR unit and the rotational speed, but the specific energy absorption in J/kg fluid is independent of the flow rate or the concentration of reagents in the actinometer.

Fluid traveling through an annular space between concentric cylinders, one of which is rotating, experiences unique flow patterns known as Taylor-Couette flow. The flow pattern generated between a rotating, inner cylinder and a stationary, outer cylinder has been characterized as generating Taylor vortices. Taylor vortices increase surface refreshing of fluid from the fluid bulk toward the outer housing. However, inside of a Taylor vortex, liquid just continuously re-circulates within the vortex never reaching the surface of the outer cylinder unless disrupted (Chossat and Iooss 1994). Coupling Taylor-Couette flow with hydrodynamic cavitation generated by embedding cavities into the inner, rotating cylinder allows disruption of such Taylor vortices, increasing extent of surface refreshing of fluid at the outer cylinder surface. Table 4.1 displays the UV dose (J/m^2) or UV radiant exposure, H, delivered by the UV-SPR to a solution in the annular space, as determined by the chemical actinometer. The UV-SPR unit delivered a UV dose of 97 J/m^2 when the inner cylinder was stationary. This value is significantly increased to 407 J/m^2 when the UV-SPR speed was 600 rpm. In addition,

increasing the rotor speed to 1200, 1800 and 2400 rpm yields significantly different UV doses with values of 491, 584 and 742 J/m^2, respectively. At low speeds, (600 to 2400 rpm), Taylor vortices are created in Taylor-Couette flow and did not seem to be completely disrupted by hydrodynamic cavitation. However, increasing the inner rotor speed from 600 to 2400 rpm increasingly refreshed fluid from the bulk to the inner surface of the outer cylinder, significantly enhancing amount of fluid directly exposed to UV light. UV-SPR speeds of 3000 and 3600 rpm yielded UV dose values of 779 and 705 J/m^2, respectively. However, UV doses at UV-SPR speeds of 2400, 3000 and 3600 rpm were not significantly different. This suggests that at UV-SPR speeds of 2400 rpm or higher, the influence of hydrodynamic cavitation on refreshing of fluid at the UV-irradiated surface, plateaus. At high rates of hydrodynamic cavitation (2400 – 3600 rpm), the expulsion of fluid out of the cavity into the annular space results in unstable Taylor-Couette flow. Extent of Taylor vortex formation was maximized at the constant mass rate of flow of fluid through the annular section, therefore the role of hydrodynamic cavitation in breaking the Taylor vortices was no longer relevant in refreshing of fluid at the irradiated surface. This relationship is evident in the non-significant difference in UV dose received by the actinometer solution at UV-SPR speeds of 2400 to 3600 rpm.

This phenomenon of diminishing returns on UV dose levels with increasing UV-SPR speeds offers advantages such as: reduced energy consumption to achieve a target UV dose. Furthermore, enhanced mixing due to hydrodynamic cavitation for certain fluids, such as emulsions, might be possible while maintaining UV doses greater than 400 J/m^2. For some products quality degradation reactions may increase with increased UV dose and elevated temperatures from the mechanical energy input at high speeds, suggesting that there may be an optimum UV dose and mechanical energy input to maximize product quality.

Apple juice

Radiant exposure, H, is defined by FDA's Center for Food Safety and Applied Nutrition as "the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere divided by the sphere cross-sectional area dA. H is expressed as the average fluence rate multiplied by the exposure time in s. The term UV dose is often used in UV disinfection literature. It represents UV exposure in J/m^2 of a given organism in the germicidal range (CFSAN 2000b). The latter report states that in order to effectively pasteurize fluid foods, the UV radiant exposure must be at least 400 J/m^2 in all parts of the processed fluid. Several parameters must be considered when utilizing UV light as a processing tool, such as: immersed versus non-immersed UV light sources, product transmissivity and flow profile through reactor, geometric configuration of the equipment, the power, wavelength and spatial arrangement of UV light sources, and radiation path length to product. Disinfection of water dominates the application of UV light as a means of processing fluids. However, elevated interest in alternative processing technologies such as UV light processing from apple cider and juice processors continues to emerge (Vasavada 2003; Koutchma and others 2004).

Inactivation of *E. coli* ATCC 25922 in apple juice processed in an UV-SPR is displayed in Table 4.2. Operating the UV-SPR at 600 rpm in conjunction with UV light resulted in a 3.45 log reduction of viable organisms. This value was significantly higher than 0.09 log inactivation when the rotor was stationary. This result validates the argument that the UV-SPR increased mass transfer to replenish fluid at the irradiated surface from the fluid bulk in the annular space. Increasing the inner rotor speed to 1200 rpm significantly increased fluid refreshment at the irradiated surface and increased inactivation of *E. coli* to 4.20 log. UV-SPR speed of 1800 rpm resulted in 4.34 log reduction but was not significantly different from inactivation at 1200 rpm.

Increasing the UV-SPR speed to 2400, 3000 and 3600 rpm did not result in significantly different mean inactivation values of 4.71, 4.96 and 4.72 log, respectively, but were significantly higher than at 1200 and 1800 rpm. These results are consistent with the actinometer results which showed no increase in UV dose received by the fluid in the annular space when UV-SPR speeds were 2400 to 3600 rpm. Although not significantly different, the mean inactivation value for *E. coli* when processed at 3600 rpm is lower than when processed at 3000 rpm. This mean reduction suggests that at high operating speeds, the mixing capacity of the UV-SPR supercedes the ability to refresh the surface for UV exposure. Inactivation of *E. coli* by UV irradiation is validated by a 0.14 log inactivation when the UV-SPR is operated at 3600 rpm with the UV source turned off.

Skim milk

Transmissivity of fluid food often dictates the efficacy of UV radiant exposure in inactivating microorganisms. Opaque fluids such as milk limit the penetration depth of UV light which reduces the microbial exposure to the energy source. In addition, particulates in milk such as protein and fat globules offer microorganisms shading and light scattering protection from UV light. Fluids with high light absorptivity remain difficult to process using UV irradiation (Lopez-Malo and Palou 2005).

Table 4.3 displays results for the inactivation of *E. coli* ATCC 25922 in skim milk due to processing in an UV-SPR unit. The UV-SPR unit proves successful in surface refreshing for enhanced exposure to UV light exposure for opaque fluids such as skim milk. Operating the UV-SPR unit with zero rotation of the inner rotor while UV irradiated produced a minimal mean inactivation value of 0.08 log for *E. coli* 25922. Increasing the inner rotor speed to 600 rpm

significantly increased the mean inactivation value of *E. coli* to 0.33 log. The mean inactivation value for 600 rpm while UV irradiated is lower but not significantly different from the mean inactivation value of 0.54 log for operation at 1200 rpm. The ability to increase refreshing capacity of opaque fluids at the irradiated surface becomes evident when considering the mean inactivation values at 600 and 1200 rpm. This trend is significantly enhanced for operation at 1800 rpm, yielding a mean inactivation value of 1.33 log. The trend for increased surface refreshing for enhanced absorption of UV irradiation is further validated as the UV-SPR operation speed is increased to 2400, 3000 and 3600 rpm. The mean inactivation values for operating speeds of 2400, 3000 and 3600 rpm are 3.25, 3.24 and 3.24, respectively and did not significantly differ. The level of UV irradiation absorbed by skim milk was constant during operation speeds of 2400 and above. This tendency for UV irradiation absorption to level off at operating speeds at 2400 rpm or higher is observed in experiments with the actinometer and apple juice biological challenge tests.

Lower mean inactivation values for *E. coli* in milk suggest that medium transmissivity plays a significant role in the efficacy of delivering a lethal UV dose to the processed medium. The opaque nature of skim milk limits the amount of UV dose able to be delivered to the product during processing. This limitation may be overcome by optimizing the product flow rate and making modifications on the geometry of the UV-SPR.

Conclusions

The UV-SPR unit increases the UV energy absorbed by fluids traveling in the annular space under an irradiated surface. The amount of UV energy absorbed is dependent on rotor speed and may also be dependent on the flow rate. Fluid is in turbulent flow in the UV-SPR

causing fluid transfer from the fluid bulk to the irradiated surface. The UV dose received by fluids flowing through a UV-SPR unit determined by KI-KIO_3 actinometry increased from 97 J/m^2 at 0 rpm to above 700 J/m^2 at operation speeds above 2400 rpm. Highly transparent fluid such as apple juice yielded log reductions of *E. coli* 25922 greater than 4 log cycles at operational speeds of 1200 rpm when fluid exit temperature was below 45˚C. Processing opaque fluids such as skim milk through the UV-SPR unit yielded inactivation values of *E. coli* 25922 greater than 3 log cycles at operational speeds of 2400 rpm or greater at temperatures not exceeding 45˚C. The UV-SPR system proved to be effective in enhancing the UV dose delivered to photochemical reactions in both transparent and opaque fluids.

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Table 4.1: Calculated values for formation of triiodide (*M*) and ultraviolet dose, H, delivered to 0.6 *M* KI, 0.1 *M* KIO_3 and 0.01 *M* $Na_2B_4O_7*10H_2O$ dosimetric solution due to varying rotational speeds during processing in an Ultraviolet-Shockwave Power™ Reactor (n = 6; mean values)[a].

Treatment (rpm)	UV (253.7 nm)	T_{in} (˚C)	T_{out} (˚C)	Abs[b] (352 nm)	I_3^- (*M* x 10^5)	Flow Rate (L/min)	H (J/m^2)
0 - Control	No	37.8	37.8	0^h	0^h	1.49	0^h
0 - Control	Yes	37.8	37.8	0.017^g	0.657^g	1.48	97.22^g
600	Yes	37.9	37.8	0.073^f	2.75^f	1.51	407.24^f
1200	Yes	38.1	38.5	0.088^e	3.31^e	1.49	490.73^{fe}
1800	Yes	38.6	39.4	0.11^d	3.95^d	1.50	584.42^d
2400	Yes	39.1	44.6	0.13^c	5.01^c	1.50	742.13^c
3000	Yes	39.3	53.0	0.14^c	5.26^c	1.50	779.23^c
3600	Yes	39.4	61.9	0.13^c	4.76^c	1.49	705.02^c
3600	No	39.6	66.3	0^h	0^h	1.50	0^g

[a] n = 6; means followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis.
[b] Abs = Absorbance resulting from serial dilutions of processed samples.

Table 4.2: Inactivation of *E. coli* ATCC 25922 in calcium fortified apple juice (pH = 3.9) processed in an Ultraviolet-Shockwave Power™ Reactor at an initial temperature of 18 ± 1˚C with varying operational speeds (n = 4; mean values)[a].

Treatment (rpm)	UV (253.7 nm)	T_{in} (˚C)	T_{out} (˚C)	Flow Rate (L/min)	N_0 (log_{10})	N (log_{10})	Log Reduction (log_{10})
0 - Control	No	18.6	18.6	1.5	7.17	7.17	0^f
0 - Control	Yes	18.6	18.6	1.5	7.17	7.08	0.09^e
600	Yes	18.6	19.2	1.5	7.17	3.72	3.45^d
1200	Yes	18.6	19.2	1.5	7.17	2.97	4.20^c
1800	Yes	18.6	20.8	1.5	7.17	2.84	4.34^c
2400	Yes	18.6	24.7	1.5	7.17	2.56	4.71^b
3000	Yes	18.6	33.1	1.5	7.17	2.21	4.96^b
3600	Yes	18.6	44.2	1.5	7.17	2.45	4.72^b
3600	No	18.6	44.2	1.5	7.17	7.03	0.14^e

[a] n = 4; means followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis (detection ≥ 10 CFU/mL).

Table 4.3: Inactivation of *E. coli* ATCC 25922 in skim milk (pH = 6.0) processed in an Ultraviolet-Shockwave Power™ Reactor at an initial temperature of 15 ± 1˚C with varying operational speeds (n = 4; mean values)[a]

Treatment (rpm)	UV (253.7 nm)	T_{in} (˚C)	T_{out} (˚C)	Flow Rate (L/min)	N_0 (log_{10})	N (log_{10})	Log Reduction (log_{10})
0 - Control	No	15.8	15.8	1.5	7.11	7.11	0[f]
0 - Control	Yes	15.8	15.8	1.5	7.11	7.03	0.08[e]
600	Yes	15.8	15.8	1.5	7.11	6.79	0.33[d]
1200	Yes	15.8	16.4	1.5	7.11	6.57	0.54[d]
1800	Yes	15.8	18.6	1.5	7.11	5.78	1.33[c]
2400	Yes	15.8	23.1	1.5	7.11	3.86	3.25[b]
3000	Yes	15.8	29.7	1.5	7.11	3.84	3.24[b]
3600	Yes	15.8	40.8	1.5	7.11	3.88	3.24[b]
3600	No	15.8	44.4	1.5	7.11	6.97	0.15[e]

[a] n = 4; means followed by same letter in column do not differ significantly (α = 0.05) according to ANOVA and SNK analysis (detection ≥10 CFU/mL).

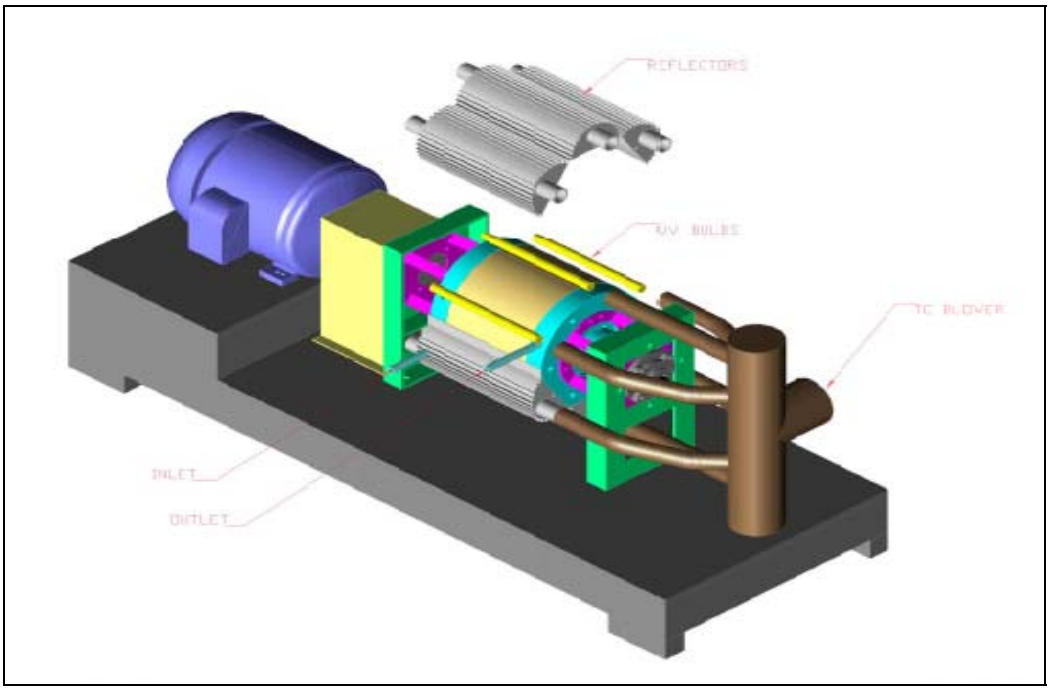

Fig.4.1 - Schematic of the Ultraviolet-Shockwave Power™ Reactor (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

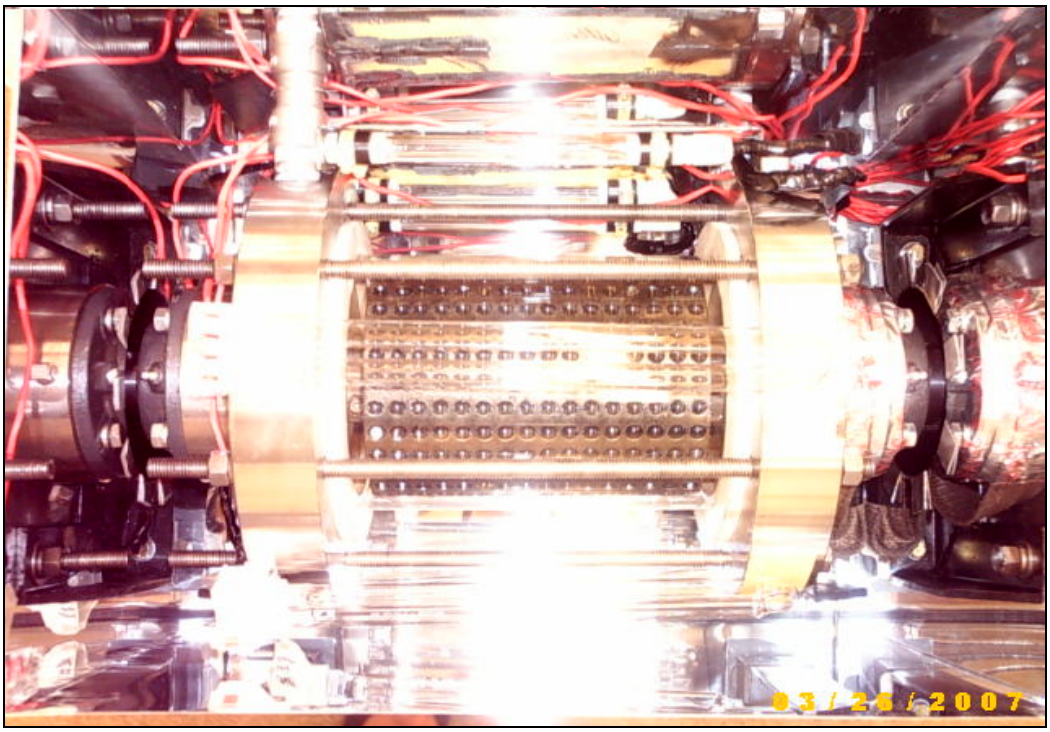

Fig. 4.2 - 12 HP UV-Shockwave Power™ Reactor (6" x 7.75" pilot scale unit) without UV illumination (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

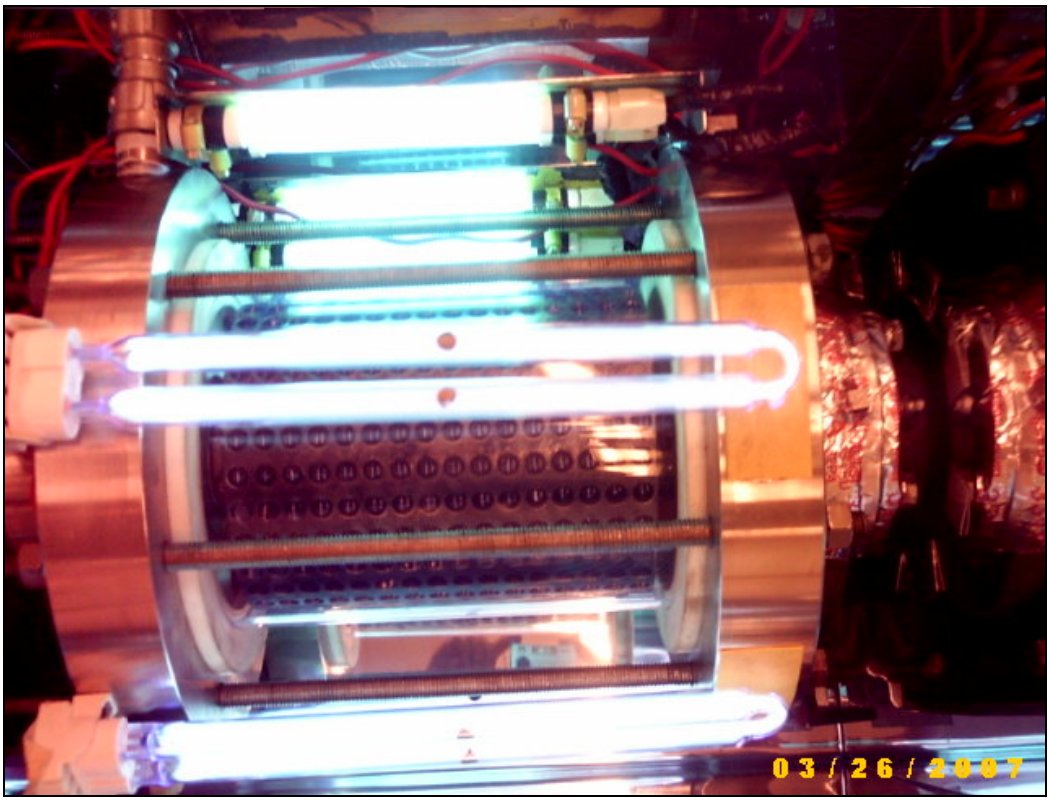

Fig. 4.3 - 12 HP UV-Shockwave Power™ Reactor (6” x 7.75” pilot scale unit) with UV illumination (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

Fig.4.4 - Hydrodynamic cavitation within inner rotor cavities (Reproduced with permission from Hydrodynamics Inc. Rome, GA).

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Minimal processing of fluid foods can be achieved using hydrodynamic cavitaion and UV radiation. Enhance microbial inactivation was achieved by hydrodynamic cavitation when coupled with a moderate temperature rise during the treatments. Bacterial and yeast vegetative cells were more susceptible to inactivation by hydrodynamic cavitation when compared to spores. Pasteurization of high-acid fluid foods was achieved with hydrodynamic cavitation at temperatures below that recommended for conventional thermal processing. Commercial sterilization of high-acid fluid foods was demonstrated with hydrodynamic cavitation but there is a need for further testing to optimize processing parameters. These parameters are associated with the geometry of the cavitation reactor. Reduced energy use, increased efficiency, enhanced mixing and capability for high product throughput are strong advantages for hydrodynamic cavitation processing. Combining hydrodynamic cavitation in a Taylor-Couette type reactor with UV processing increased the UV dose delivered to transparent and opaque fluids when compared to fluid flow through the annulus without cavitation. Transparent and opaque fluid food was pasteurized at near ambient temperature using hydrodynamic cavitation in conjunction with UV treatment.

Utilizing hydrodynamic cavitation as a processing technology allows processors to minimally heat treat fluid foods while extending shelf-life of perishable products such as apple juice. Enhanced inactivation of *S. cerevisiae* (6.27 log cycles) can be achieved at reduced processing temperatures (65.6˚C and 76.7˚C) when subjected to sufficient hydrodynamic cavitation compared to what can be predicted using heat inactivation parameters (D and z-values). The use of hydrodynamic cavitation in cellular disruption provides additional evidence of the antimicrobial effects of hydrodynamic cavitation applied in food processing applications. Energy consumption can be reduced significantly (173 and 215 kJ/kg versus 258 kJ/kg) and

process efficiency can be significantly increased by processing with hydrodynamic cavitation in fruit juice manufacturing. Hydrodynamic cavitation equipment may be scaled up for large product throughput with enhanced energy saving and efficiency (55 – 84%).

Hydrodynamic cavitation provides processors an effective method for pasteurizing low-acid and commercialy sterilizing high-acid foods. Hydrodynamic cavitation induced adequate destructive forces to inactivate vegetative cells of bacteria, yeast, yeast ascospores and heat resistant bacteria spores. Common spoilage microorganisms such as lactic acid bacteria and yeast were eliminated at reduced temperatures through the synergistic effect of temperature and hydrodynamic cavitation. Adequate lethality was obtained for commercial sterility of high-acid foods using the current SPR design. However, modification involving equipment geometry and operating procedures need to be implemented in order to achieve commercial sterility in high-acid foods. Differences in resistance to hydrodynamic cavitation were not observed between gram-positive bacteria and yeast since thermally induced lethality predominated under the conditions of the test. Bacteria spores proved to be the most resistant to both thermal effects and hydrodynamic cavitation effects. With hydrodynamic cavitation spore inactivation was achievable at reduced processing temperatures; however the total lethality was not adequate for commercial sterility. Modification of SPR geometry and consideration for in-process residence time in the SPR must be investigated in order to validate effectiveness of the technology for commercial sterilization of low-acid foods. Increased fluid feed temperature is an option for processors to improve total lethality at relatively low speeds and low exit temperature.

Hydrodynamic cavitation induced microbial lethality greater than that accounted for by thermal effects. Lethality from hydrodynamic cavitation is strongly dependent upon moderate processing temperatures (65.6˚C and above), elevated product exit temperature with maximal

SPR residence time, and rotational speed of the SPR rotor. Foods such as acidic fruit juices and milk can be safely processed at reduced processing temperatures. Reduction of processing temperature translates into superior products.

The UV-SPR unit increased the UV energy absorbed by fluids traveling in the annular space under an irradiated surface. The amount of UV energy absorbed is dependent on rotor speed and may also be dependent on the flow rate. Fluid is in turbulent flow in the UV-SPR caused fluid transfer from the fluid bulk to the irradiated surface. The UV dose received by fluids flowing through a UV-SPR unit determined by KI-KIO_3 actinometry increased from 97 J/m^2 at 0 rpm to above 700 J/m^2 at operation speeds above 2400 rpm. Highly transparent fluid such as apple juice yielded log reductions of *E. coli* 25922 greater than 4 log cycles at operational speeds of 1200 rpm when fluid exit temperature was below 45˚C. Processing opaque fluids such as skim milk through the UV-SPR unit yielded inactivation values of *E. coli* 25922 greater than 3 log cycles at operational speeds of 2400 rpm or greater at temperatures not exceeding 45˚C. The UV-SPR system proved to be effective in enhancing the UV dose delivered to photochemical reactions in both transparent and opaque fluids.

The force behind innovative product development and research in today's market can be attributed to technology (Moskowitz and others 2005). New technologies offer companies and researchers alternative paths to successful introduction of products in the market place. To validate claims of minimally processed product superiority, instrumental and sensory analyses need to be executed. Instrumental analysis should include color, apparent viscosity and particle size reduction measurements. All these attributes contribute to the consumers' overall eating experience. These attributes should be statistically compared to traditionally processed products. Following comparison of traditionally processed products, marketplace products may be

assessed to determine the range of acceptable attributes with regard to alternatively processed products. In addition to instrumental analysis of processed products using controlled cavitation and UV radiation, sensory analysis of products should be evaluated.

Several categories of sensory testing exist and each type of evaluation offers the researcher unique and valuable information about the product in question. Three most common types of sensory testing are commonly referred to as affective, descriptive and difference testing. Affective testing is the most commonly used sensory tool in industry and is spilt into acceptance and preference testing. Affective testing shows useful to the researcher for learning how to better develop and evaluate the quality of newly proposed products. Acceptance testing relies on a panel of consumer judges to evaluate the acceptability of individual product samples. In preference testing, the panel of judges is asked to select one sample that best exemplifies the most desired or accepted sample among a series of products. Affective testing can also be used in conjunction with a ranking system for products allowing the researcher to determine the relative order of product acceptability. Interval scales allow the degree of acceptability to be determined. Hedonic, a specific interval scale, is employed to cover a range of acceptance responses, from like extremely to dislike extremely.

As new food products are developed, it is of key importance to be able to describe specific characteristics of the product. Descriptive sensory is intended to yield feedback on specific attributes of newly developed food products. This type of sensory testing employs a panel of judges that have been well trained to identify and rate sensory attributes of the food, allowing the novel food to be compared to existing products. It also allows a sensory profile of the innovative product to be established. Commonly, a lexicon of descriptors for various product characteristics is used in communicating perceived deviations from standardized samples. This

type of testing is valuable in flavor research, allowing a company to tract changes in product flavor so changes can be made in order to maintain product flavor and texture consistency.

Difference or discrimination testing allows researchers to assess whether an inherent difference among various products can be detected. The test also allows for selective screening of judges based on their ability to detect certain differences between products. In addition to being able to identify whether differences exists among products, the test provides companies a useful tool for determining whether an expensive ingredient or a processing change is justified. Several versions of difference testing exist: paired comparison, duo-trio test, triangle test and rank order (McWilliams 2005).

Utilization of sensory testing can afford product development researchers and processors valuable tools for validating the use of new technologies and ingredients in product formulation and development. Future research pertaining to sensory studies is needed to validate the commercialization of hydrodynamic cavitation and UV radiation technology. Food grade hydrodynamic cavitation equipment with standard operating procedures for ensuring clean and sanitary processing equipment are currently being developed. This limitation precluded any sensory studies on products processed in the test unit. However, the following research is suggested when continuing research to validate this technology. Products processed with new technologies must be assesses for product quality and performance with respect to sensory research. Affective of acceptance testing will yield processors valuable results for launching newly developed products, however, additional sensory studies such as difference and descriptive testing must be done to strengthen the validation for implementing the technology into mainstream processing regimens. Difference testing must be done in order to justify technology implementation with regards to perceived taste difference compared to

conventionally processed products. Detectable differences in products, whether good or bad, must be accounted for and nature of the difference must be resolved. Saving energy and time may offer processors huge advantages, but product quality must be forefront in decision making processes. In addition to difference testing, descriptive testing must be performed also. Descriptive testing will allow researchers to evaluate what sensory property is responsible for any detectable difference and may present advantageous marketing strategies for successfully launching new products. For example, conventional heating regimens may damage nutrients in products, resulting in a loss of detectable quality. The detectable quality must be identified using descriptive sensory testing in order to justify how utilizing new processing technology can minimize that loss. Sensory testing is critical in successful product launch and validating implementation of new technologies.

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