ABSTRACT

STEED, LAURIE ELAINE. Development and Validation of Processes for Continuous Flow Microwave Processing of Foods Containing Sweetpotato Particulates. (Under the direction of Dr. Van-Den Truong and Dr. KP Sandeep.)

Continuous flow microwave processing has been successfully utilized in commercial production of homogenous foods, and now there is an opportunity to use this technology to enhance the quality of multiphase foods. However, due to the high temperatures and mechanical stress incurred by continuous processing, it is challenging to maintain food particulate shape, which would be necessary for consumer acceptance in products like soups. Covington, NC 413 and Oriental sweetpotato cubes (*Ipomoea batatas*), with orange, purple and white flesh, respectively, were subjected to various pretreatments in order to investigate their effects on reducing degradation of texture due to thermal processing. Control and treated samples were loaded into a stainless steel cell, heated in an oil bath to an internal temperature of 125 °C and held for 30 sec. Among the pretreatments examined a 2-step pretreatment that included soaking in 0.3M Na_2CO_3 at 25 °C for 1 h followed by low temperature blanching at 62 °C in 1% (w/v) $CaCl_2$ provided the greatest firmness retention for all sweetpotato cultivars. Covington cubes, which are the most sensitive to thermal degradation, were pretreated by the 2-step process and evaluated at temperatures ranging from 115-130 °C for 0-12.22 min. Across all temperatures and times, peak force to fracture of the tested cubes showed no significant difference ($p<0.05$) illustrating the robustness of the pretreatment. The Hunter L* a* b* color values showed that the pretreatment application and

subsequent processing caused significant decreases in all components, but the values stayed within what has been commonly reported for sweetpotatoes.

Since texture measurements indicated sweetpotatoes prepared by the 2-step pretreatment can maintain firmness at a level adequate to survive commercial processes, potential for use in high temperature thermal applications such as microwave processing was investigated. Dielectric property measurements showed that while dielectric constant did not change due to pretreatment, dielectric loss factor increased for pretreated Oriental and NC 413 sweetpotatoes when compared to raw samples and this was attributed to the application of $CaCl_2$ during the pretreatment. Five microwave runs with the target temperatures of 115, 121, and 125 °C were conducted utilizing a pilot scale 100 kW system. Pretreated cubes were inserted into a carrier fluid of orange-fleshed sweetpotato puree and received microwave application at three points. After passing through the hold tube they were cooled, and collected in a pressurized tank outfitted with a sieve. All inserted sweetpotato cubes were recovered, and subjected to firmness and color measurements. For all cultivars, microwave processing caused a significant decrease in firmness as measured by peak compression force (N), however texture was firm enough for all cubes to stay intact after going through the microwave heating process. Microwave processing also caused a significant decrease in color components, but not to a level outside of what has been previously reported.

After creating a food particulate that could withstand continuous flow microwave processing, microbiological validation of a pilot scale 100 kW microwave

system was attempted utilizing immobilized spore beads of *Geobacillus stearothermophilus.* Immobilized beads were placed in prefabricated cube-shaped particles made of polymethylpentene, which has been proven to heat more conservatively than food particles made from various vegetables. Prefabricated particles also contained magnets, which tracked their movement throughout the system and allowed for calculation of residence times. The prefabricated particles were inserted into a stream of orange-fleshed sweetpotato puree utilized as the carrier fluid and subjected to microwave application. At the end of the process they were collected and the immobilized spore beads were enumerated to determine surviving populations. Magnetic tracking showed that each particle was accounted for as it moved throughout the system unobstructed and spent 78±1 sec in the hold tube. Hold tube exit temperatures ranged from 96.9-129.9 °C due to variable microwave power. Log inactivation of *G. stearothermophilus* spores ranged from 0.20 – 2.05 and was most consistent when temperatures at the hold tube exit were stable. Based on the success of achieving free particle flow and utilization of immobilized spore beads as bioindicators, this study shows promise in achieving microbiological validation for a continuous flow microwave system.

Development and Validation of Processes for Continuous Flow Microwave Processing of Foods Containing Sweetpotato Particulates

by
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DEDICATION

To those who believed I was capable of great things, before I could even conceive of them.

BIOGRAPHY

Laurie Elaine Steed was born on February 12, 1983 to Monty and Sandy Steed. She grew up in Wilmington, North Carolina and spent her childhood playing with her younger brothers Aaron and Trevor. At a young age she enjoyed dancing, but in middle school she found her first true love in the sport of saddleseat equitation and rode horses competitively through high school, until her academic career became too rigorous. Laurie graduated in 2001 from New Hanover High School 8th in her class and with 21 credit hours accumulated from taking AP courses.

That fall Laurie moved to Raleigh to attend North Carolina State University and was admitted to the College of Engineering. Due to her early interest in chemistry, she thought that Chemical Engineering held the key to her future and matriculated into the program at the end of her freshman year. However, in the spring of her sophomore year she realized that the major was ill fitting for her and dropped out of her chemical engineering classes. She considered a major change of pace and contemplated not only transferring to several other schools, but also looked at the College of Design, specifically for architecture or interior design. However, when two of her Chi Omega sorority sisters, Melody Milroy and Tiffany Brinley, heard about her dilemma they were quick to suggest their own program - food science. Two weeks later Laurie had toured Schaub hall, filled out paperwork, and successfully switched her major to Food Science. When she started her coursework the fall of her junior year it was obvious that she had finally found her place at NC State.

In May 2005 Laurie graduated with her B.S. degree in Food Science and

accepted a research assistantship with Dr. Van-Den Truong of the USDA-ARS sweetpotato lab. She became involved in the Food Science club and served as Dairy Bar co-chair and Trip/Tour chair while working on her M.S. Thesis: Nutraceutical and rheological properties of purple-fleshed sweetpotato purees as affected by continuous flow microwave-assisted aseptic processing. Laurie originally had no interest in pursuing a degree higher than her M.S., but as she continued her studies and learned more about the industry, she became aware that what she would like to accomplish in her career would require a Ph.D. As luck would have it, there was a Ph.D. project that was granted funding but had no graduate student. When it was offered to Laurie, she accepted. In May 2007 she completed her M.S. degree and started her Ph.D. program in June 2007.

Laurie continued her involvement in the Food Science club and served as Vice President for the 2007-2008 school year. She coordinated campus visits with all of the visiting company representatives and formally interviewed with several as well. In the fall of 2007 she was offered an internship to General Mills for the summer of 2008, which she accepted. Laurie spent the summer working on reformulations of several Hamburger Helper products. The project was successful and later her formulas were implemented in commercial products. Her experience working in the food industry, and specifically with General Mills, was one of the highlights of her graduate career.

Today, Laurie has spent nine and a half years at NC State, and is a proud product of their wonderful Food, Bioprocessing and Nutrition Sciences department.

She will complete her Ph.D. and spend one last holiday season at home before moving to Minneapolis in January to start her professional career as a Research Scientist II at General Mills. Her academic career has taught her many things, but mostly has instilled her with the confidence in herself necessary to succeed at whatever the future holds.

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Dr. Jaykus – I have deeply respected you since I took your Food Micro class where you taught me how to apply knowledge, rather than just memorize facts. I believe that getting to work closely with you and experience an area of food science outside of my comfort zone has made me a better scientist and appreciate your support and guidance.

For my department

I am so proud to be a graduate from the FBNS Department at NC State, and I can only hope that the feeling is mutual. I would not be the student I am today if it were not for the guidance and mentorship of Dr. Farkas who I have considered a

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For my friends

To those that have come, conquered, and moved on: Jessica Childs, Josh Evans, Drew Watson, Megan Whitson, Maegan Olsen, Nick Kuhlman, Craig Koskiniemi, Christine Yen, Iris Liaw & (almost) Adam and Kristin Croissant – There is not a day

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Kelsey Ryan – Our friendship is new, and yet has been so dear to me as I finish this process. The summer of 2010 will never be forgotten for so many reasons and you are a part of all of them. I'm so glad that we finally got to be roommates, even if it did involve an air mattress, and love that we will be fellow Minnesotans!

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houses, your marriage, and now into your about-to-be family of 4. I cannot wait to see you as parents and to meet the little Monkey Man who will be lucky enough to know the endless love that you are both capable of giving.

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You never told me who I should be, or what I should do, but instead taught me how to figure it out for myself, all while providing unconditional love and support. Wherever you are is “home” and that is where my heart will always lie.

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CHAPTER 1

LITERATURE REVIEW

1. SWEETPOTATOES

1.1. Sweetpotato Origin and Worldwide Production

Sweetpotatoes (*Ipomoea* batatas) originated in the region between the Yucatan Peninsula of Mexico and the Orinoco River in Venezula and were domesticated in Central America at least 5000 years ago. In 1492, Columbus took sweetpotatoes to Europe from his first voyage, allowing Portuguese explorers to take them to Africa, India, Southeast Asia and the East Indies in the 16th century. Spanish ships brought sweetpotatoes from Mexico to the Philippines, which allowed other explorers to spread them to Vietnam, India, Burma and finally China in the 16th century (Loebenstein 2009). Due to their broad adaptability, hardiness and ability to multiply planting material rapidly from few roots, sweetpotatoes extended through Asia, Africa and Latin America in the 17th and 18th centuries (CIP 1999; Woolfe 1992).

Today sweetpotatoes are the seventh most important crop following wheat, rice, maize, potato, barley, and cassava (CIP 1999; Woolfe 1992). China is the leading producer of sweetpotatoes with production of about 100 million tons, or 70% of the total world production. Vietnam is the second largest producer, and both of these countries devote a majority of the crop to animal feed. However, they also incorporate multiple processed forms for human consumption, such as noodles, starch and alcohol (Loebenstein 2009).

Sweetpotatoes play an important role in many other countries as well. In Brazil sweetpotato is the fourth most consumed vegetable, while in Africa it is considered a "poor person's crop" and "women's work" to produce. The United States produces about 720 thousand metric tons and utilizes sweetpotatoes in fewer ways than other countries, predominantly as a fresh crop with canned chunks, frozen French fry and potato chip products becoming increasingly available. Consumption usually peaks during the winter months between the holidays of Thanksgiving and Christmas. With the exception of California, sweetpotatoes are mostly grown in southern states. North Carolina is the largest producer of sweetpotatoes and accounts for 38% of the annual United States sweetpotato production, while Mississippi and Louisiana account for 35% (Loebenstein 2009, Estes 2009).

1.2. Nutritional and Health Advantages of Sweetpotatoes

The nutritional superiority of sweetpotatoes has led this food commodity to a recent surge in popularity. The Center for Science in the Public Interest and the Nutrition Action Health Letter awarded the sweetpotato first place rankings when compared to the nutritional characteristics of other vegetables (NCSPC 2007). Developing countries have long been dependent upon sweetpotatoes as a valuable source of energy. Sweetpotatoes produce more biomass and nutrients per hectare than any other food crop in the world with carbohydrates accounting for 80-90% of the total dry matter of a sweetpotato root, and several types are present, including starch, cellulose, hemicelluloses, pectins and sugars (Loebenstein 2009; Padmaja 2009). Furthermore, they are a nutritionally rich crop, complete with vitamins (B1,

B2, C and E), minerals (calcium, magnesium, potassium and zinc), and dietary fiber (Suda and others 2003). These nutrient levels coupled with their non-specific growing conditions, make sweetpotatoes valuable to many regions in times of civil crisis and natural disasters (CIP 1999).

Orange sweetpotatoes derive their color from carotenoids and depending on the carotene content, the flesh color can range in intensity from white or cream to light or dark orange (Padmaja 2009). Beta-carotene represents 86.4 - 89.0% of the carotenoids in yellow and orange-fleshed sweetpotatoes. This pigment is considered important because of its role as a vitamin A precursor, which maintains and protects eye tissues, and has been linked to enhanced immune response and suppressed cancer development (Woolfe 1992). Since beta-carotene is the most abundant pigment in orange-fleshed varieties, which can contain up to 16 mg/100 g fresh weight (fw), sweetpotatoes are recognized as one of the best dietary sources of Vitamin A (Padmaja 2009).

In the United States orange-fleshed cultivars dominate sweetpotato production. Approximately 60-65% of the production area is devoted to Beauregard, a variety released by the Louisiana State University Agricultural Center in 1987 that gained popularity due to its resistance to several horticultural diseases and its good baking and canning qualities. Another cultivar, Covington, was released by the North Carolina Agricultural Research Station in 2005 and has grown to occupy over 30% of the sweetpotato production area. It has similar advantages to Beauregard, but also is high yielding and sizes its storage roots more evenly, resulting in less

jumbo roots (Yencho and others 2008; Carpena 2009). Other cultivars in production include Jewel and Evangeline, which could become important to the United States due to its darker orange color and more consistent root shape (Carpena 2009). Recently, researchers worldwide have developed many new cultivars of sweetpotatoes with varying flesh colors that are finding market success because they have the same nutritious benefits as orange sweetpotatoes, but also contain additional functional pigments including flavones, beta-carotene, phenolic acids and anthocyanins (Suda and others 2003).

Purple-fleshed sweetpotatoes have intense purple color in the skins and flesh of the storage root due to the accumulation of anthocyanins (Philpott and others 2003; Terahara and others 2004). These cultivars were developed in breeding programs for use as natural food colorants, but are now gaining popularity as a dietary source for anthocyanins. A prominent example is the Japanese cultivar, Ayamurasaki, which was developed at the National Agricultural Research Center for Kyushu Okinawa Region. Extracts have been used to make commercial products including natural colorants, food dies, juices, and fermented beverages while pastes and flours have been utilized in breads, noodles, jams and sweetpotato chips available in eastern Asia (Suda and others 2003; Yamakawa and Yoshimoto 2002; Oki and others 2002).

Furthermore, anthocyanins isolated from purple-fleshed sweetpotatoes show a great amount of promise in relation to their physiological function. Researchers have proven anthocyanins to have anti-mutation effects in *Salmonella typhimurium*

TA 98, the ability to suppress glucose metabolism by α-glucosidase inhibitory action, and play an active role in memory enhancement (Yoshimoto and others 1999, 2001; Matsui and others 2002; Cho and others 2003). In all of these experiments, rats were used to show in vivo physiological function of anthocyanins. Suda and others (2002) proved that acylated anthocyanins from purple-fleshed sweetpotato were directly absorbed and isolated intact from plasma. The peonidin-type anthocyanin examined had a larger molecular weight than other anthocyanins reported to be absorbed in rats or humans indicating that acylated anthocyanins from sweetpotatoes could also be absorbed (Suda and others 2002).

In the United States purple-fleshed sweetpotatoes are not commonly commercially produced or consumed. The Sweetpotato Breeding Program at North Carolina State University has grown a cultivar coded NC 414 for research purposes and a similar purple cultivar has found limited success in North Carolina markets and as a novelty crop sold by individual farmers. Steed and Truong (2008a) found that the peels of NC 414 roots have the highest phenolic and anthocyanin content, along with antioxidant activity but overall contribute only 10-15% of the total weight of the roots. Phenolic content of the whole and steamed roots were 469.9±13.8 and 401.6±24.1 chlorogenic acid equivalents (CAE)/100 g fw, respectively. These values were not significantly different, but as expected, were much higher than those reported by Truong and others (2007) for orange-fleshed sweetpotatoes which ranged from 78.6-181.4 CAE/100 g fw.

Anthocyanin content ranged from 80.2±5.5 to 107.8±1.8 mg cyanidin-3-glucoside/100 g fw for raw and steamed roots, respectively. This placed purple fleshed sweetpotatoes in the middle of the spectrum of high anthocyanin fruits and vegetables as their content compared well with cherries, grapes, plums, raspberries, eggplant and red radishes (Steed and others 2008a; Wu and others 2006). Antioxidant activity measured by the DPPH method (Brand-Williams and others 1995) showed that NC 414 had higher radical scavenging activity than 16 purple-fleshed cultivars examined by Oki and others (2002). Radical scavenging activity as measured by the ORAC assay (Prior and others 2003) placed them in a group of fruits with high antioxidant activities like blackberries, cultivated blueberries and sweet cherries, illustrating the potential of purple fleshed sweetpotatoes as a functional food ingredient (Wu and others 2004; Steed and Truong 2008a).

1.3. Commercially Available Processed Products

Despite the 42% increase in sweetpotato production over the last 20 years, per capita consumption has remained constant around 2 kg and was estimated to be 2.36 kg for 2008. Only about 1.5% of Americans eat fresh sweetpotatoes regularly, and even fewer (0.5%) consume processed products. Since few restaurants offer sweetpotatoes as a vegetable choice and only a small selection of processed forms are available to institutional suppliers, 89% of sweetpotato consumption occurs at home, which is more than any other vegetable purchased. This lack in diversity of processed products available in the marketplace presents an opportunity for growth and stimulation of the sweetpotato industry in the United States (Estes 2009).

Many commercial sweetpotato growers view the process of curing as in indispensable first step in providing a year-round supply of quality sweetpotatoes. Fresh roots are cured at 30 °C, 85-90% relative humidity, for 3-7 d immediately after harvest. This process provides several benefits such as enhancing culinary and eating quality and aiding wound healing, which reduces loss due to shrinkage and disease. After proper curing roots can be stored in climate-controlled facilities at 13 °C, 85-90% relative humidity with adequate ventilation and sweetpotatoes that are free from disease or other physiological problems can be stored for up to 13 mo and remain sellable (Smith and others 2009).

To guarantee a constant supply of sweetpotatoes, they are often canned. This process beings with grading, cleaning, pre-heating, peeling either manually or with 7-10% (w/w) boiling lye, and trimming. Sweetpotatoes are then halved, quartered or cut into cubes to improve consumer acceptability and facilitate filling of the cans. These pieces are blanched at 77 °C for 1-3 min and then packed tightly into cans. The cans are filled with a sugar syrup solution, (20-40% w/w concentration) salt water or water depending on the product. Size 303 cans are retorted at 121 °C for 35 min then cooled and stored at room temperature (Truong and others 1998; Padmaja 2009).

Frying thinly sliced sweetpotatoes into chips or rectangular strips into French fries is one way to transform them into more stable edible products. Cultivars with high amino acid and sugar content will encounter heavy browning due to the maillard reaction, which may be to some degree, undesirable. Depending on the region,

finished chips will be left as is, coated with sugar, salted, or spiced. French fries can be fried, frozen, and then oven baked before consumption or fried prior to consumption (Padmaja 2009).

Sweetpotato purees can be used directly as baby food or serve as the base for other food products such as dehydrated flakes, patties, breads, beverages and candies (Padmaja 2009; Kays 1985). Purees have many production advantages because a high quality puree can be made from any size or shape of roots (Fasina and others 2003). Since approximately 40% of the crop is left in the field due to inadequate size, the production of puree alleviates this lack of utilization (Kays 1985). To obtain a consistent product despite handling and storage differences, α- and β-amylase were added to achieve a consistent level of starch conversion, but this involved the introduction of a food additive. To bypass this problem an enzyme activation technique that utilizes native amylotlytic enzymes was developed, and this method is still used today (Kays 1985; Truong and Avula 2010).

Recently a method for converting purple sweetpotatoes into puree was published. The addition of water is imperative to create a flowable puree due to the higher dry matter content of this cultivar, which makes it naturally more viscous. Sweetpotatoes were washed, sliced, steam cooked, adjusted to a dry matter content of 18% and pushed through a 0.15 cm screen. This puree could then be subjected to different forms of thermal processing including continuous flow microwave processing and aseptic packaging (Steed and Truong 2008a).

There are two main forms of commercially available sweetpotato puree - canned and frozen. Due to the thickness of sweetpotato purees, the Natl. Food Products Assn. recommends that a size 307 x 409 can with an initial temperature of 87 °C be retorted for 84 min at 121 °C, to ensure adequate heating of the cold spot (NFPA 1996). This long processing time at retort temperature produces a poor quality product. Furthermore, the quality of the puree in the can varies depending on its location in relation to the can wall, where over-processing creates puree with dark color and burnt flavor. To improve the quality, it is best to limit the can size to a no. 10, however this reduces the applications in the food industry (Coronel and others 2005; Steed and others 2008b).

Due to the poor quality product created by canning, frozen packaging has become increasingly popular. While this offers a lower degradation of the nutritional and aesthetic properties of the puree, the resources needed by industry for storage and distribution are substantial. Also, thawing is a poorly controlled process that is time consuming and lengthened with bigger package (Coronel and others 2005; Kays 1985).

For all processed products, texture plays a key role in consumer acceptance. As sweetpotatoes are stored, naturally present enzyme systems decrease starch content to make the roots softer and more susceptible to disintegration and sloughing during processing (Walter and others 1992; Walter and others 2003). Sweetpotatoes canned shortly after harvest are firmer than those canned several weeks or months after harvest, and it has been a challenge to food processers to

maintain a consistent product year round (Walter and others 1992). Also, fried products made from roots that have been stored for long periods of time excessively retain oil and result in an unacceptable finished product. Taking this into consideration, it is imperative that the processes are adapted to contain techniques that will increase firmness (Padmaja 2009).

2. THE CHEMISTRY OF SWEETPOTATO TEXTURE

Maintaining the texture of fruits and vegetables during storage and processing is a key component to consumer acceptance and product success (Van Buren 1979). In sweetpotatoes specifically, the inability to control the textural properties of processed roots has been a major obstacle in the development of all of the commercially available products previously discussed (Walter and others 1993). The compounds that contribute to texture are found largely in the cell wall and middle lamella region, which plays a role in intercellular adhesion. These compounds include cellulose, which gives rigidity and resistance to tearing, hemicellulose and pectic substances, which confer plasticity and the ability to stretch (Van Buren 1979).

Pectic substances are composed of chains of galacturonic acid residues linked by α1->4 glycosidic bonds (Figure 1). They can be heavily crosslinked and act as "glue" between adjacent plant cells therefore contributing to the mechanical strength of the wall (Ainsworth 1994). About 1/3 of the dry substance of the primary cell wall and a greater proportion of the dry substance in the middle lamella are

pectic substances. They are chemically reactive and brought into solution more easily than other cell wall components, so changes in pectic substances are often related to textural changes that result from ripening, storage and cooking (Van Buren 1979).

Pectic substances can undergo a wide range of chemical reactions in conditions similar to those associated with food processing. One of the most important reactions, β-elimination, is catalyzed by hydroxyl ions readily found in cooking or canning environments (Figure 2). In this reaction depolymerization results from β-elimination splitting of the glycosidic bonds and requires that the carboxyl group of the residue undergoing β-elimination be esterified since that increases the electron deficit at C5. Hydroxyl ions speed the reaction by aiding in the removal of H+ from the C5 position, and heat is also shown to increase reaction time. Once the glycosidic bond is split, the pectic substances become more soluble, which results in softening of fruit and vegetable texture (Van Buren 1979).

When pectic substances undergo demethoxylation of the esterified carboxyl group, β-elimination cannot occur. There are several enzymes present within fruits and vegetables that are capable of catalyzing this reaction. Pectin methylesterases (PMEs) are such enzymes and they have been found in a wide range of produce. In cauliflower, carrots, potatoes, sweetpotatoes, peas and beans, endogenous PME is believed to be responsible for a firming effect when activated at a low blanching temperature (Benen and others 2003). PME demethylates the carboxymethyl groups, and this decrease in the degree of methylation of the galacturonic chains

has a two-fold affect on texture (Canet and others 2005). The resulting free carboxylic acid groups can bind calcium which can cross-link pectin chains creating greater cell-to-cell adhesion and firmer texture. But also, reducing the level of methylesterification of the pectic substances reduces the tendency of β-elimination to occur at higher temperatures, which also prevents softening (Anthon and Barrett 2006; Ni and others 2004).

2.1. Methods to Increase Firmness in Processed Products

2.1.1. *Low temperature blanching*

Low Temperature Blanching (LTB) takes place at temperatures ranging from 55-80 °C and has been effective for a range of vegetables including carrots, potatoes, beans, cauliflower and tomatoes (Truong and others 1998). This low temperature was first thought to activate PME by Bartolome and Hoff in 1972 when they noted that potato tissue preheated at 60-70 °C was firmer and sloughed less when fully processed. Based on their study of the cell wall constituents in preheated potatoes, they hypothesized that cell membranes are disrupted at temperatures greater than 50 °C which allows for solutes such as mineral ions from the cytoplasm and vacuoles to diffuse in and activate PME. The enzyme interacts with accessible methyl ester groups to produce free carboxyl groups which can cross link with diffusing magnesium or calcium ions to produce tissue that is more resistant to further degradation (Bartolome and Hoff 1972). LTB at 60-65 °C for 30-45 min was also shown to increase firmness, hardness, cohesiveness and chewiness for potatoes that were made into French fries. The LTB treatment reduced limpness and oil

absorption to overall improve the quality of the finished product when compared to the control (Aguilar and others 1997).

For white potato PME has been found to be most active after the roots have been kept in refrigerated storage at 4 °C for 35 d. This led to firmer cooked samples when blanched at 58-60 °C for 66-75 min before cooking (Canet and others 2005). This is especially important to note for processed sweetpotatoes. Usually sweetpotatoes are processed for only a short time every year because those that are stored for too long tend to soften and disintegrate. Truong and others (1998) performed LTB on cylindrical sweetpotato samples stored for 9-12 mo at temperatures ranging from 50-80 °C for 15-274 min. Samples un-blanched and blanched in boiling water for 2 min then cooled were used as controls. After LTB half of the sample was steamed at atmospheric pressure, and both halves were subjected to instrumental texture measurements utilizing compression analysis. In addition, samples of selected blanching treatments were canned in syrup for textural and sensory evaluations (Truong and others 1998). Results indicated that for sweetpotatoes an optimum LTB temperature is around 62 °C, with the highest peak forces from compression found at 60 and 90 min. Canned samples that were blanched at this temperature were also found to be 2-3 times more intact and 2-7 times firmer than controls, with sensory data showing greatest acceptance for samples blanched for 30-45 min. A study published in 2003 on sweetpotatoes subjected to LTB showed that PME activity decreased 82% after 20 min of blanching at 62 °C. However sample firmness continued to increase with blanching time up to

90 min, showing that firmness due to pectin demethylation only explains part of the observed increased firmness and the rest is due to unknown factors that have still not been elucidated (Walter and others 2003).

2.1.2. *pH modification*

The effect of pH on texture has been described as low pH leading to enhanced softening due to hydrolytic cleavage of glycosidic bonds of sugar components in the cell wall, while at neutral pH's enhanced softening is a result of the β-elimination reaction (Van Buren 1979). However, in a research note, carrots with 0.77-3.13% gluconic acid included in the brine before canning resulted in a firmer texture (Heil and McCarthy 1989). This was most likely a result of the lower processing temperature required for the lowered pH. Sweetpotatoes treated with citric, hydrochloric, acetic, lactic, and malic acids were found to decrease heat-mediated softening. It was proposed that the mechanism by which acidulants increase firmness was due to the least amount of pectin solubilizing and partial inactivation of endogenous amyloloytic enzymes (Walter and others 1992).

Acidulants have limited applications because of resulting flavor changes, and the firmness retention was lost when tissue acidification was readjusted to a normal pH, so base-mediated firmness retention was explored (Walter and others 1992; Walter and others 1993). Sweetpotato strips vacuum infiltrated with Na_3PO_4, Na_2CO_3, NH_4OH_2 or NaOH prior to heat processing were found to be firmer than untreated strips, and Na_3PO_4 and Na_2CO_3 were the most effective at increasing firmness. Unlike acid-mediated firmness retention, when base-treated strips were

readjusted to the normal pH range of 5.9-6.2, firmness did not decrease (Walter and others 1993). It was also found that vacuum infiltration with Na_3PO_4 followed by blanching, adjustment of tissue pH to approximately 6.0, and canning in sucrose syrup led to increased firmness retention and decreased disintegration when compared to sweetpotatoes without phosphate treatment. This firming effect was also found in sweetpotatoes that had been stored for up to 10 mo, therefore prolonging the processing season (Walter and others 1997). This type of treatment could easily be adapted for other types of processed sweetpotato products.

2.1.3. *Calcium chloride addition*

When heat processing causes an undesirable degree of softness calcium salts are commonly added before cooking, although rarely at a level over 0.1% of the fruit or vegetable weight in order to avoid off flavors. While calcium can cause softening by increasing the rate of β-elimination, it can also enhance firmness through complexes with pectic substances in a mechanism known as the "egg-box model" and overall the net result has been to increase firmness (Grant and others 1973). Calcium and potassium have a greater influence on texture of vegetables because their pH is commonly in a range where free carboxyl groups of pectins are dissociated and can interact with the ions (Van Buren 1979).

Smout and others (2005) found that a calcium pretreatment of soaking for 1 h in 0.5% $CaCl_2$ alone or before or after a heat step of 70 °C for 30 min caused a decrease in texture degradation kinetics, and therefore resulted in a significant improvement of carrot texture. Another study found that calcium addition with high-

pressure pretreatment or with LTB also lead to a pronounced improvement in carrot texture. This effect was greater than calcium treatment or LTB alone (Sila and others 2005). Furthermore, LTB in a $CaCl_2$ solution with a concentration of 0.17-0.21 M at 63.3-66 °C for 11.6-14.4 min was reported to provide an optimum protective effect in maintaining cell wall integrity for frozen jalapeno peppers (Perez-Aleman and others 2005).

In sweetpotatoes the effect of calcium has been coupled with base treated tissue. Sweetpotato strips treated with 0.03 M Na_2CO_3, blanched, vacuum infiltrated with 0.6% $CaCl_2$ in an acetate buffer resulted in a 3-fold increase in shear force over the control strips and could easily be applied in other processing applications (Walter and others 1993).

3. MICROWAVE PROCESSING OF FOOD

While thermal processing of food products is the most widely used method of food preservation, conventional methods for processing low acid foods to achieve commercial sterility and shelf-stability often cause a degradation of color, flavor, texture, and nutrients (Kumar and others 2007a; Wang and others 2003). An emerging technology that shows promise as an alternative method of thermal processing is microwave heating. Industry has already adapted the process to temper frozen foods, pre-cook bacon, pasteurize packaged food, and provide the final drying of pasta products (Sumnu and Sahin 2005).

3.1. Microwave Heating

Microwaves are non-ionizing radiation that fall within frequency bands of 300 MHz to 300 GHz. Because this frequency range adjoins the range of radio frequencies used for broadcasting, mobile phones, and radar transmissions, special frequency bands are reserved for microwave applications. The Federal Communications Commission permits 2450 MHz for home microwave ovens while 915 MHz is utilized mostly in industrial applications (Tang 2005). Microwaves are similar to visible light in that they can be focused into beams and transmitted through hollow tubes. Materials that come into contact with microwaves can absorb, reflect, or transmit the electromagnetic waves, the outcome of which is determined by the dielectric properties of the material (Singh and Heldman 2001; Coronel and others 2005). Heating occurs when materials convert the electromagnetic energy into thermal energy and occurs through ionic polarization or dipole rotation (Singh and Heldman 2001).

Microwave heating offers a way to overcome the problems associated with food preservation by canning and freezing methods, especially when considering viscous products. In contrast to conventional heating, which relies on heat transfer to the product from direct or indirect contact with a hot or to a cold medium, microwaves interact directly with the food to generate heat volumetrically (Sumnu and Sahin 2005). Heat is generated by the absorption of microwaves and conversion into thermal energy, which is then transferred through the food by conduction and convection to cause a rise in temperature (Singh and Heldman,

2001; Sumnu and Sahin, 2005). Because heat is generated volumetrically, microwave heating avoids overcooking of the surface of more viscous food products such as purees, and undercooking of the center which occurs with conventional heating methods, such as canning (Coronel and others 2005). Also, the heat generated by microwaves can significantly reduce the time required for pasteurization and sterilization resulting in a better quality product (Sumnu and Sahin, 2005).

3.2. Dielectric Properties of Food Materials

The dielectric properties of foods are primarily responsible for determining the way the material will heat when exposed to electromagnetic energy created by microwaves. Knowledge of the dielectric properties is essential to understanding the heating behavior of a food in microwave systems and they are characterized by two main components: the dielectric constant (ε') and the dielectric loss factor (ε'').

The dielectric constant is the ability of the food to store energy when placed in an electromagnetic field while the loss factor is an imaginary quantity for the ability of a material to convert electromagnetic energy into heat (Tang 2005; Sumnu and Sahin 2005). Loss tangent ($\tan \delta$) describes how well a product absorbs microwave energy and is a ratio of loss factor to the dielectric constant:

$$\tan \delta = \varepsilon''/\varepsilon'$$

When the dielectric constant changes with temperature, loss tangent is a better estimate of power dissipation. A product with a higher loss tangent will heat faster under microwave field as compared to a product with a lower loss factor when

exposed to microwave radiation at the same frequency (Gabriel and others 1998).

Power penetration depth is the distance in meters at which power drops to 1/e (37%) of its value at the surface of the material and is defined by the following equation (Nelson and Datta 2001; Kumar and others 2007a):

$$\delta_p = \frac{\lambda}{2\pi\sqrt{2\varepsilon'\left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right]}}$$

where λ is the wavelength of the microwave in free space in meters. This equation is valid for a plane wave incident upon a semi-infinite slab, and used to calculate the tube diameter for a continuous flow microwave heating system (Kumar and others 2007a). From the equation, penetration depth is related to the dielectric properties of the material and a reduced frequency or wavelength of the microwave, will result in a decreased penetration depth (Koskiniemi 2009).

3.3. Dielelectric Heating Mechanisms

3.3.1. *Ionic Conduction*

Applying an electric field to food materials that have ions causes the ions to move at an accelerated pace due to their inherent charge. As they move within the food matrix they collide with adjacent ions, causing a conversion of kinetic energy into thermal energy. Foods with higher concentrations of ions will have more collisions and therefore increase more in temperature (Singh and Heldman 2001). At lower frequencies ionic conductivity will be the major mechanism of heating the material (Tang 2005).

3.3.2. *Dipole Rotation*

Food materials contain polar molecules that have a random orientation. Water is a prevalent component of most food items, and is a known polar solvent. The application of an electric field causes the molecules to orient themselves to align with the polarity of the field. Microwaves create fields with rapidly alternating polarity and the polar molecules will rotate to maintain alignment with the changing electric field causing friction with the surrounding food matrix. This leads to the creation of heat, and higher temperatures cause faster rotation and therefore more heat generation (Singh and Heldman 2001). It is important to note that dipole rotation is a bulk phenomenon since it results from the movement of many molecules (Gabriel and others 1998).

Dipoles rapidly oscillate at a rate based on the microwave frequency, which can be millions to billions of times per sec. Every time the dipole re-orients to align itself with the electromagnetic field, the field has already changed again, resulting in a phase difference between the orientation of the field and the dipole. This phase difference produces dielectric heating due to the lost energy from random collisions of the dipole (Tang 2005).

3.4. Dielectric Properties of Sweetpotato Products

Sweetpotatoes contain 52-85% moisture and it has been reported that foods with moisture content greater than 35% will have a substantial amount of free water dominating overall dielectric behavior (Sumnu and Sahin 2005). With the high

percentage of polar molecules in sweetpotatoes, dielectric heating will greatly be influenced by the rotation of polar molecules (Walter and others 2000).

Dielectric properties for orange-fleshed sweetpotato purees were measured at a frequency range of 900-2450 MHz and temperatures from 10-145 °C and the values were within the range for food materials with >60% moisture (Fasina and others 2003; Nelson and Datta 2001). For sweetpotato purees, both dielectric constant and loss factor decreased with increasing frequency. The frequency range examined is reflective of the common frequencies used in food processing, usually 915 or 2450 MHz, and at these frequencies both ionic conduction and dipole rotation are involved. As frequency increases the ability of the molecules to orient with the rapidly changing field decreases. This causes a decrease in both dielectric constant and loss factor, which was illustrated with the sweetpotato puree. Also, dielectric properties were highest at 900 MHz due to the occurrence of more dipole rotation (Tang 2005; Fasina and others 2003).

Dielectric constant decreased with increasing temperature and dielectric loss factor increased for temperatures 35 °C and greater. It has been established that dielectric constant decreases with increasing temperature due to a decrease in dielectric relaxation time. Relaxation time is associated with time for the dipoles to revert to random orientation when the electromagnetic field is removed (Sumnu and Sahin 2005; Steed and others 2008b). Temperature increases also cause dipole rotation to decrease, because they increase thermal agitation so that fewer dipoles can re-orient with the changing electromagnetic field. This means that at higher

temperatures ionic conduction is predominantly responsible for the resulting loss factor of sweetpotato puree. Furthermore, increasing temperature leads to a reduction in viscosity of the puree, which led to increased mobility of the ions and higher conductivity (Tang 2005; Fasina and others 2003).

In another study, dielectric constant and loss factor were measured for orange-fleshed sweetpotato purees at frequencies 915 and 2450 MHz over a range of temperatures from 10-145 °C (Coronel and others 2005). The values were similar to those reported by Fasina and others (2003) and therefore also fell within the range reported for other food materials (Nelson and Datta 2001). For sweetpotatoes with high starch content such as the cultivars with white, yellow and purple-fleshed colors, the flow-ability of the purees needs to be adjusted by starch hydrolysis or water addition. When the viscosity of cooked purple-fleshed sweetpotatoes was adjusted by water addition to match that of orange-fleshed sweetpotato purees, the resulting dielectric properties corresponded well with what had been previously reported (Steed and others 2008b).

3.5. Measurement of dielectric properties

Of the available methods to measure dielectric properties, using an open-ended coaxial probe is the most preferred because it can measure properties over a wide frequency range, is easy to use, and can be used for solids or liquids. The tip of the probe is placed in contact with the food material and emits a sinusoidal wave at a specific frequency. The phase and amplitude of the reflected signal are read by a network analyzer and coincide with the dielectric properties (Ryynanen 1995;

Koskiniemi 2009). In this method the food material is placed in a sample cell and heated over a range of temperatures by heating in a water or oil bath. Measuring dielectric properties in these static conditions has limitations in that only a small portion of the sample is measured and only in a small area around the probe. In a multiphase food product this may not be representative of what is happening with the bulk of the food product.

Kumar and others placed a coaxial probe a continuous flow microwave system to measure the dielectric properties of food products that were circulated with temperature raising from 20-130 °C (2007b). The results showed that for homogeneous products like skim milk, pea puree, and carrot puree, dielectric properties measured in the continuous flow system were similar to those obtained by a static coaxial probe across a temperature range. However, for salsa con queso there were numerous discrepancies between the measurements taken at static and dynamic conditions. Static conditions were shown to under predict dielectric loss factor and over predict dielectric constant resulting in a lower loss tangent. This means that for salsa con queso measurement of dielectric properties under static conditions will predict a slower rate of microwave heating than will occur experimentally (Kumar and others 2007b).

3.6. Successful Microwave Application to Food Products

3.6.1. *Homogeneous food products*

In the past five years there has been a lot of work done with industrial scale continuous flow microwave systems; an emerging technology in food processing due

to the fast and efficient heating it offers. Most of this work has focused on homogeneous food products. In 2003 a research team processed milk with the microwave system and found that the overall temperature distribution was even with a less than 4 °C difference between the highest and lowest temperatures at the exit of the 5 kW applicator (Coronel and others 2003).

The system was scaled up to 60 kW capacity and has been used to process fruit and vegetable purees, where high viscosity often causes quality degradation by other thermal processing methods. In 2005, the first report of a vegetable puree processed this way was published. Orange-fleshed sweetpotato puree was subjected to continuous flow microwave processing and it was feasible to produce a shelf-stable product with no detectable microbial populations after 90 d of storage at room temperature. Also, the finished product had an apparent viscosity and color comparable to the untreated puree, illustrating high quality retention (Coronel and others 2005). This proved microwave heating as a viable technology that can be employed by the sweetpotato industry as a way to convert raw roots into a high quality shelf stable product, which can be utilized as a functional food ingredient.

Based on the successful development of a microwave process for orange-fleshed sweetpotato puree, the process was investigated for application to purple-fleshed sweetpotato purees, where color retention would be of utmost importance for a finished product. The process was again found successful and led to a 5.9% increase in total phenolic content and a 14.5% decrease in anthocyanin content, but processed purees maintained their antioxidant activity as measured by DPPH and

ORAC assays. Overall, color change measured by ΔE was not significant for the microwave processed puree, although there was a slight loss of saturation in blueish purple color (Steed and others 2008b). This product was compared to a purple-fleshed sweetpotato puree that was thermally processed by canning and found to be superior in all aspects, especially in the area of color retention, where the canned sample had high degradation of anthocyanins due to long retort times necessary to heat the cold spot. Consequently, the color of the canned puree was reddish- brown (Steed and others 2008c).

3.6.2. *Multiphase food systems*

With the success in homogeneous products, it is imperative from a processing standpoint to extend the technology to foods that contain particulates. A multiphase food product, salsa con queso, has been microwave processed. It was found that as processing temperature increased the temperature differences in the salsa con queso at the center and wall of the heating tube narrowed. Thus, continuous flow microwave processing is a feasible alternative method of processing for multiphase food products and could overcome the problems associated with degradation of color, flavor, texture, and nutrients that occurs with conventional heating methods (Kumar and others 2007a). However, in the United States processes for multiphase foods must be validated before a product can be marketed commercially. This is a large hurdle in the commercialization process because accepted validation techniques are costly and time intensive (Jasrotia and others 2008).

In a particulate food, the geometric center of a food particle is the critical point of interest, since in conventional heating this will be the slowest heating point. This is difficult to quantify because each particle flows with a different velocity resulting in a variation of achieved lethality and making it necessary to know particle residence time. Also, in a continuous flow system it is challenging to monitor the temperature distribution within the particles as they flow through the system, which dictates the need for mathematical modeling (Cacace and others 1994; Sandeep and Zuritz 1995).

One alternative to this dilemma is the use of biological validation techniques, and they are necessary when time-temperature history of the product is unavailable. The first validation of a multiphase aseptic process was for a diced potato soup and prepared by a joint workshop of industry professionals. When it was duplicated by Tetra Pak Inc. and filed with the FDA it received a no-objection letter. However, these filings have to be done for every particulate product, and the cost to food manufacturers with a broad portfolio is considerable (Jasrotia and others 2008; CAPPS and NCFST Workshop 1995-1996).

In order to make microbial validation more affordable, simulated food particles are often utilized. The fabricated particles contain an interior chamber that can be used to hold magnetic implants and sensors that can give temperature data or provide tracking through the system to obtain time-temperature data. Jasrotia and others (2008) developed a method for fabrication and validation of simulated products made from polypropylene and polymethylpentene. These materials had

thermal properties that showed they may heat slower than food particles and the study confirmed this expectation. Data also showed that despite the simulated food particles having a hollowed out cavity, they still heated slower than solid food particles and accumulated less F_0 values as well, proving that they exhibit conservative thermal properties. This information could be useful for future validation studies since the interior cavity allows for use of biological indicators and other validation tools (Jasrotia and others 2008).

4. MICROBIOLOGICAL VALIDATION

There are three main methods for assessing sterilization processes. Thermocouple techniques provide a time-temperature history by placing a thermocouple within the food product. This type of monitoring does not take into account environmental factors or convective movements during heating. Additionally, it is not applicable to food particles that move while being processed (Serp and others 2002). Chemical indicators use enzymes, sugar inversion, or color changes to show that a certain time-temperature history has been achieved, but this method is usually viewed as not precise enough and also has the shortcomings of the thermocouple method (Stam 2008; Serp and others 2002). Recently, a heat resistant enzyme, beta-glucosidase from *Pyrococcus furiosus* was used to develop a time-temperature indicator. It was found to be more resistant than other biological indicators and a convenient tool for fast, easy, and more robust assessment of the food safety of a thermal process (Yen 2009).

The use of biological indicators is considered to be the most advantageous method. Microorganisms are directly or indirectly incorporated into the material, which means that they will undergo the same thermal process as the native microflora (Serp and others 2002). Microbiological validation has been widely used for various thermal processes, including retorting, high-temperature short time processes and microwave heating (Brinley and others 2007; Guan and others 2003).

4.1. Bioindicators

To ensure food safety, when low acid foods are thermally processed to produce shelf-stable products the goal is commercial sterility which is defined as a 12D inactivation of *Clostridium botulinum*. This is the most important spore-forming pathogen because it produces a potent neurotoxin with an LD_{50} of 20-50 ng. Since spores are much more resistant to heat than their vegetative counterparts, it is important from a public health standpoint to guarantee that a process eradicates all threat of *C. botulinum* from occurring. The D-value is the time required at a given temperature to produce a 1 log or 90% reduction in the target bacterial population. Depending on the strain of *C. botulinum* the D-value at 121.1 °C (sterilization temperature) ranges from 0.05-0.22 min. This means that a 12D inactivation is about 2.64 min, but it is a common practice in industry to round this up to 3.0 min, since an overestimation will only guarantee further that all *C. botulinum* has been eliminated (Brown 2000).

Due to the inherent risk of working directly with *C. botulinum,* it is a common practice to utilize other microorganisms as bioindicators. Sweetpotato puree

processed by steam flash sterilization and aseptic filling was validated using inoculated packs of *Clostridium sporogenes, Bacillus subtilis,* and *Bacillus stearothermophilus* (Smith and Kopelman 1982). *C. sporogenes* is often chosen due to the genetic and cultural similarity to *C. botulinum*. It causes anaerobic spoilage, which leads to bloated containers and therefore allows for visual assessment. It has a D-value at 121.1 °C of 1.5 min which means that it is more resistant and a 5D inactivation will be equivalent to a 12D inactivation for *C. botulinum*. However researchers lately have had trouble cultivating spore stocks with thermal kinetics of these levels, and due to its anaerobic nature it is difficult to enumerate without the proper equipment.

B. subtilis is a gram-positive spore former. Spores from the *Bacillus* genus are distinguished by their aerobic nature (mostly strict, some facultative) and ability to produce catalase. Most Bacilli spores are environmentally ubiquitous and can survive for years in their dormant state (Slepecky 1992; Paidhungat and Setlow 2002). These spores are easy to produce and widely available for purchase. The D-value at 121.1 °C is 0.4 min, so they are more resistant that *C. botulinum* but are not pathogenic (Rawsthorne and others 2009).

B. stearothermophilus has since be reassigned to the genus *Geobacillus* and is nonpathogenic but is a common source of thermophilic spoilage when containers are temperature abused and held at 50-55 °C. It is the most heat resistant of the organims discussed and has a D-value of 2.0 min at 121.1 °C but has been reported as high as 16.7 min (Rawsthorne and others 2009; Stam 2008).

4.2. Spore Characteristics and Germination

Endospores are highly refractile structures formed within the vegetative cells. They have an unusual resistance to chemical and physical agents as first found in *B. subtilis* spores. Spores that survive thermal processing are of concern because they can germinate into vegetative cells and lead to sickness (Slepecky 1992; Paidhungat and Setlow 2002).

There are three fundamentally different processes that are responsible for changing a dormant bacterial spore into a vegetative cell: activation, germination and outgrowth. Activation is the process that conditions the spore for germination. It is usually achieved by a sublethal heat treatment, but exposure to low pH, thiol compounds, and strong oxidizing agents can also be responsible for activation (Keynan and Evenchik 1969). In Bacilli, pretreatment, or activation, has been shown to dramatically increase a subsequent germination response, but *B. subitilis* does not require prior treatment to germinate (Moir 1992; Paidhungat and Setlow 2002). An activated spore will maintain most of its spore properties, and an activated spore can undergo germination or revert to a fully dormant spore (Keynan and Evenchik 1969).

When a spore is committed, the irreversible process of germination ensues when activated spores are exposed to germinants. These can be metabolizable (amino acids, ribosides, and sugars), non-metabolizable (ions, surfactants, and chelates), enzymatic (lysozyme, spore lytic enzyme, and protease), physical in nature (cracking surface layers) or environmental such as changes in pH, temperature, water activity, and ionic strength. The earliest defined germinant for

Bacilli was L-alanine, but valine and isoleucine can also be effective, especially for *B. subtilis* and *G. stearothermophilus*. An alternative germinant for *B. subtilis* is a combination of asparagine, glucose, fructose, and KCl (AGFK) (Moir 1992).

Germination can be inhibited by exposure to D-alanine, which is a competive inhibitor to L-alanine. Alcohols behave non-competitvely to inhibit germination, and azide has been shown to inhibit *B. subtilis* germination by means of AGFK. This trigger is also sensitive to the presence of low concentrations of mercuric salts or tosyl arginine methyl ester, which will prevent germination by AGFK and L-alanine (Moir, 1992).

During germination the characteristic resistance of spores to heat, desiccation, pressure, vacuum, ultraviolet and ionizing radiation, antibiotics and other chemicals, and extremes of pH, are completely lost. However, the germinated spore is cytologically distinct from a vegetative cell because it lacks a full complement of typical vegetative macromolecules and enzymatic activities (Gould 1969). Germination is considered to be a degradative process, and therefore does not involve extensive synthesis of new macromolecules. In fact, during germination spores excrete up to 30% of their dry weight, which comes from the collapse of the core and cortex material (Moir 1992). The exudate is composed mostly of calcium, dipicolonic acid (DPA) and fragments of depolymerized murein. Changes in the cortex make up the principal cytological change during germination. It either disappears completely, swells, or becomes spongy or fibrillar without much change in volume (Gould 1969).

Detection of germinating spores can be accomplished by dark phase contrast optics. When the heat resistance of the endospore is lost during germination, so is the refractive index, causing the spores to turn from bright to dark contrast (Gould 1969). An easier method to quantitatively monitor germination is to measure the optical density. It will decrease by approximately 50% if all of the spores in the suspension germinate (Moir 1992). Germinated spores also become stainable due to an increase in permeability. Spores that have begun germination will approximately double in volume before the new cells emerge from the cell coats due to the production of new cell material. The enlargement can be detected microscopically and measured as another means of detecting germination (Gould 1969). New cell material is generated using energy produced by the metabolism of energy reserves stored in the dormant spore and the amino acids generated by protein degradation. While these endogenous energy reserves will support ATP production and some macromolecular synthesis early in germination, further growth will have to be supplemented with exogenous metabolites (Setlow 1984).

Dormant spores do not contain any stored functional mRNA and synthesis of functional mRNA begins in the first min of germination and precedes the initiation of protein synthesis by several min (Setlow 1984). Once a spore has completed the germination process it will enter the outgrowth stage, where the spore protoplast resumes growth, emerges from the coat remnants and RNA, protein and DNA synthesis begin (Moir 1992).

4.2.1. *Quantifying RNA in germinating spores*

Moeller and others (2006) published a method for extracting RNA from both dormant and germinating *B. subtilis* endospores that utilizes a rapid rupture step followed by acid-phenol extraction. This allowed for the extraction of a highly pure RNA sample (determined by the Nanodrop method) and increasing amounts of RNA were extracted as germination progressed over a 2 h time span. Being able to rapidly and simply extract a high quality sample of RNA allows for significant and reproducible analysis by DNA or RNA methods (Moeller and others 2006).

4.3. Spores Utilized for Microbial Validation of Microwave Processing

Macaroni and cheese packaged into trays was heated in a microwave-circulated water combination heating system operating at 915 MHz. The trays were inoculated with 1.1×10^8 spores/ml of *C. sporogenes* and processed at under-target, target and over-target conditions based on F_0 value. Temperature was measured throughout the process by fiber optic process and surviving spores were enumerated by the count-reduction method and the end-point method. No viable spores were found at or above-target processing conditions. However, all of the trays processed at under-target conditions presented visible swelling and odor characteristic of spoilage by *C. sporogenes* (Guan and others 2003).

Bioindicators produced by SGM Biotech Inc. (Bozeman MT USA) were tested in microwave processed sweetpotato puree to assess their feasibility for microbial validation. The indicators consisted of 0.1 ml spore suspension of *G. stearothermophilus* (1.8×10^6) or *B. subtilis* (4.6×10^6) enclosed in polypropylene

tubing. The spore suspension also contained a chemical indicator so that if spores grew post processing it would cause a change in pH that would lead to a color change from purple to yellow. These pouches were then inserted into the hopper of a 60kW microwave system and processed at under-target (126 °C), target (132 °C) and over-target (138 °C) conditions (Brinley and others 2007).

For *B. subtilis* there was no evidence of spore survival at and over-target conditions. However, the *G. stearothermophilus* results were conflicting. Like *B. subtilis* there was no indication of spore survival by enumeration for target and over-target processes, however 50% of the pouches developed a color change. For the under-target process, the results were inconsistent in that there was a color change in 29 of 37 pouches and some showed a log reduction greater than what was expected. This was attributed to the fact that within the system the pouches were getting caught in mixers and easily adhered to the sides of pipes leading to overall low recovery and an inability to guarantee normal, uninhibited flow within the system (Brinley and others 2007).

Validation of a continuous microwave system was also attempted utilizing immobilized spores as biological indicators for a multiphase food product. Spores were mixed with a 3% sodium alginate solution and dropped into 100 mM $CaCl_2$ to form the immobilized beads based on the methodology of Serp and others (2002). *B. subtilis* spore solutions had a concentration of approximately 10^9 spores/ml and were stained using FD&C Blue No. 1 lake so that they would be visually distinguished from *G. stearothermophilus* spores (10^8 spores/ml). The immobilized

beads were then placed in the inner cavity of polymethylpentene cubes with an outer diameter of 1.3 mm. Cubes were inserted into a 60 kW continuous flow microwave system utilizing salsa con queso as a carrier fluid and processed at 128, 132, and 138 °C (Stam 2008).

Cubes were found lodged in the hold tube and some reported *B. subtilis* populations≥10^8 CFU/bead indicating that minimal processing was achieved. In a second attempt a CMC solution was used as the carrier fluid, but mechanical problems still prevailed. This resulted in no detectable surviving spores of *B. subtilis* or *G. stearothermophilus* from any of the particles. In a 3rd and final attempt, CMC was again used as the heating medium and all 30 particles inserted were retrieved. Two particles had surviving *G. stearothermophilus* at 105 spores/particle and one particle produced a plate with positive growth of *B. subtilis* (Stam 2008).

4.3.1. *Molecular Based Detection Methods*

The "gold standard" for enumeration of spore survivors from a thermal process is standard plating methods. However, these methods are labor intensive and often require several days of incubation to the detriment of the food processer. Molecular amplification approaches like quantitative real-time polymerase chain reaction (qPCR) have shown promise but have not been applied due to two main factors: release of nucleic acid form spores is difficult and the detection of DNA does not necessarily equate with the presence of viable spores. A recent study solved this problem by utilizing a DNA-intercalating agent, propidium monozide (PMA), which can only penetrate the membranes of dead cells to bind with DNA and prevent

it from being amplified during PCR. The results show that samples treated with PMA had qPCR results comparable to those obtained by cultural enumeration, while those not treated with PMA were overestimated by qPCR (Rawsthorne and others 2009). This technology could show promise in the development of rapid methods of detection of surviving microbial populations but has yet to be tested in a pilot scale thermal process.

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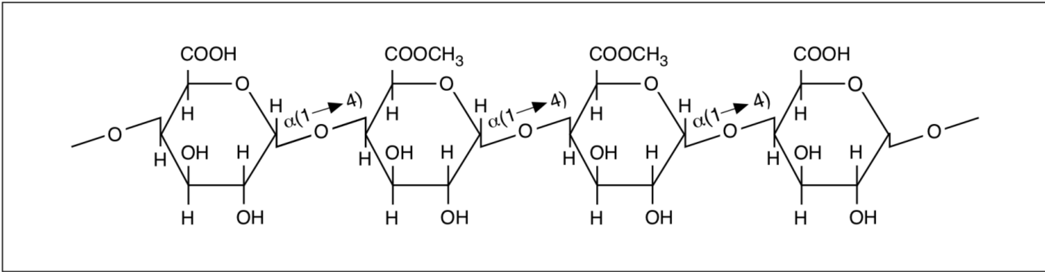

FIGURE 1: The Structure of Pectin
(Ainsworth 1994)

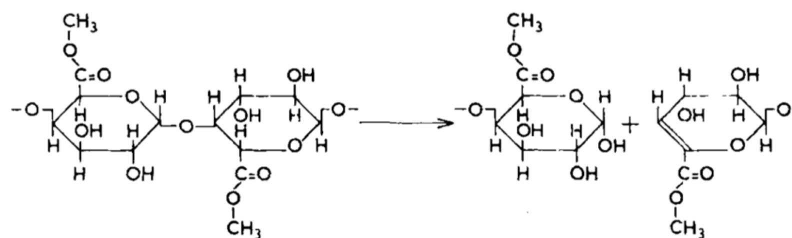

FIGURE 2: Splitting of the Glycosidic Bond by Beta-Elimination
(Van Buren 1979)

CHAPTER 2

INFLUENCE OF PRETREATMENT CONDITIONS ON TEXTURE AND COLOR RETENTION OF THERMALLY PROCESSED SWEETPOTATOES

1. ABSTRACT

Covington, Oriental and NC 413 sweetpotato cubes (*Ipomoea batatas*), which respectively have orange, white and purple flesh, were subjected to various pretreatments in order to investigate their effects on reducing texture degradation due to thermal processing. Control and treated samples were loaded into a stainless steel cell, heated in an oil bath to an internal temperature of 125 °C and held for 30 sec. Among the pretreatments examined, a 2-step process that included soaking in 0.3 M Na_2CO_3 at 25 °C for 1 h followed by low temperature blanching in 1% (w/v) $CaCl_2$ at 62 °C, provided the greatest firmness retention for all sweetpotato cultivars. Covington cubes, which are the most sensitive to thermal degradation, were pretreated by the 2-step process and evaluated at temperatures ranging from 115-130 °C for 0-12.22 min. Across all temperatures and times, peak force to fracture of the tested cubes showed no significant difference ($p<0.05$) illustrating the robustness of the pretreatment. The Hunter L* a* b* color values showed that the pretreatment application and subsequent thermal process caused significant changes in all components, but the values stayed within what has been commonly reported for sweetpotatoes. Results indicated that this 2-step pretreatment can maintain firmness of sweetpotato cubes to a level adequate to survive commercial processes, and especially has potential for use in high temperature thermal applications such as microwave processing.

2. INTRODUCTION

Maintaining the texture of fruits and vegetables during storage and processing is a key component to consumer acceptance and product success. In sweetpotatoes specifically, the inability to control the textural properties has been a major obstacle in the development of commercially available products such as canned chunks, French fries, and purees (Walter and others 2003).

One of the first methods investigated to prevent softening in vegetable processing was the addition of $CaCl_2$ to cross-link with pectic substances present in the cell walls, and result in increased tissue firmness. $CaCl_2$ addition has been successfully applied in processing a wide range of vegetables including potatoes, cucumbers, carrots, bell peppers and sweetpotatoes (Bartolome and Hoff 1972; Van Buren 1979; Walter and others 2003; Castro and others 2007; Sila and others 2004). Since sweetpotatoes soften during storage, $CaCl_2$ treatment has been used as a soaking step or directly added to the syrups of canned sweetpotatoes to improve the product texture and therefore extend the available window for processing (Walter and others 2003; Walter and others 1992). With $CaCl_2$ vacuum infiltration followed by alkaline treatment, sweetpotato French fries increased their firmness retention as compared to control samples or French fries infiltrated only with base (Walter and others 1993).

Another technique that has been studied for improving the texture of processed vegetables such as canned snap beans, canned or frozen cauliflower, tomatoes, potatoes and carrots, is low temperature blanching (LTB) (Truong and

others 1998, Van Buren 1979). In 1972, Bartolome and Hoff studied the effect of LTB on white potatoes and hypothesized that temperatures greater than 50 °C disrupted cell membranes, which allowed solutes to diffuse into cells and activate pectin methyl esterase (PME). The enzyme interacts with accessible methyl ester groups to produce free carboxyl groups which can cross link with diffusing magnesium or calcium ions to enhance tissue resistance to further degradation (Bartolome and Hoff 1972). LTB at 60-65 °C for 30-45 min was also shown to increase firmness, hardness, cohesiveness and chewiness for potatoes that were made into French fries (Aguilar and others 1997). Truong and others (1998) found that the optimum conditions for LTB sweetpotatoes were at 62 °C, for 60 and 90 min. Sweetpotatoes blanched at 62 °C prior to canning were found to be 2-3 times more intact and 2-7 times firmer than the controls, with sensory data showing greatest acceptance for samples blanched for 30-45 min.

While all of these pretreatments have found commercial success, there has been limited application to thermal processes involving high temperatures (>121 °C) or continuous movement of the food particulates. The recent emergence of microwave assisted aseptic technology has shown promise for foods that tend to be degraded by the long retort schedules necessary for canning and it is desirable to extend this technology to food with particulates (Kumar and others 2007; Steed and others 2008a). Since hold tube temperatures are ≥125 °C, and the food is continuously pumped through an extensive system, it is imperative that food particles made from vegetables withstand the mechanical forces and thermal treatments

throughout the process. Our objectives were to test a variety of pretreatments on food particulates made from sweetpotatoes and determine which would offer the greatest firmness retention in a continuous flow and high temperature environment for an extended time period.

3. MATERIALS AND METHODS

Sample preparation of sweetpotatoes

Three sweetpotato cultivars were utilized; Covington, Oriental, and NC 413, which have orange, white and purple flesh, respectively. All sweetpotatoes were grown at the Clinton Research Station of the Sweetpotato Breeding Program, North Carolina State University. They were harvested in October of 2009 and cured at 30 °C, 85-90% relative humidity for 7 d then stored at 13 °C 85-90% relative humidity until experiments were conducted. Duplicate batches of roots (n=20) were taken from each cultivar and cut into 9.52 mm (3/8 inch) cubes by putting the whole washed roots through a French fry press with a 9.52 mm screen. The rectangular strips were hand diced into cubes, which were divided into sample sets for the various pretreatments or processed quickly after cutting to serve as control samples.

Effect of pretreatment on firmness retention of cubes

The pretreatments examined included: 1) LTB in de-ionized H_2O held at 62 °C for 30 and 60 min, 2) soaking in 0.5 and 1.0% (w/v) $CaCl_2$ solutions at 25 °C for 30 and 60 min, and 3) application of a 2-step pretreatment that involved soaking cubes in 0.03 M Na_2CO_3 at 25 °C for 1 h, followed by a 1 h soak in 1% $CaCl_2$ at 62 °C.

After pretreatment all samples were removed from the solutions, blotted dry with paper towels, placed in Ziploc® bags and held at 4 °C overnight until thermal processing and analysis.

Application of thermal process

To evaluate the effect of the pretreatment process, sweetpotato cubes were placed in a stainless steel cell (ID = 0.022 m) sealed with tri-clamps tightened on the end caps. One end cap was outfitted with a thermocouple that protruded into the cell and was attached to a data acquisition system. The thermocouple was pushed into the center of a sweetpotato cube so that recorded measurements were based on the internal temperature of the cube. On top of this cube, eight cubes were stacked into the cell in groups of two. The rest of the cell was filled with orange sweetpotato puree produced by steaming Covington roots for 20 min (100 °C, atmospheric pressure) in a 7.5 L pot outfitted with a steam basket (Home Essentials, Kmart, Troy, MI, USA) and homogenizing in a Robotcoupe grinder (Model RSI 2YI Ridgeland, MS, USA).

Duplicate samples (n=8 cubes) from each batch were heated in a high temperature silicon oil bath (Model EX111 Neslab Thermo Scientific, Waltham MA USA) to 125 °C and then immediately removed and placed in an ice slurry until the internal temperature cooled to approximately 25 °C. These samples were considered a 0 sec time point and accounted for the effect of the come up time, which averaged 12 min. This process was repeated and samples were held for 30

sec once the internal temperature reached 125 °C. All samples were kept at 25 °C until texture and color analysis.

Effect of 2-step pretreatment on firmness retention of cubes exposed to a range of temperatures

Cubes subjected to the 2-step pretreatment were heated in an oil bath at 115 °C for 0, 4, 8 and 12.22 min, 120 °C for 0, 1.25, 2.5 and 3.86 min, 125 °C for 0, 0.5, and 1.22 min, and 130 °C for 0 and 0.5 min in order to test the robustness of the pretreatment on cube firmness. End times for each temperature were solved for based on an equivalent 12D inactivation for *Clostridium botulinum* using:

$$F_0 = 10^{(T-Tref)/z}\Delta t$$

where F_0 = 3.0 min, T_{ref} = 121.1 °C and z = 10 °C. This experiment was carried out on Covington samples, except at 130 °C where cubes of Oriental and NC 413 were also tested. Samples were processed in the oil bath as previously discussed and held at 25 °C until texture and color analysis.

Firmness measurements

Compression tests on sweetpotato cubes were measured using a TA-XT2 texture analyzer (Texture Technology Corp., Scarsdale, NY, USA.) equipped with a 50-kg load cell and 50 mm cylindrical probe. Data acquisition and peak force at fracture were obtained using the Texture Expert software (Texture Expert Exceed v. 2.56, Stable Micro Systems, London, UK). The following operating parameters were used: pre-test speed, 2 mm/s, test speed, 1.6mm/x; post-test speed, 10.0 mm/s; distance 9 mm; acquisition rate, 200 point/s; force units in Newtons. Thirty-two

cubes from each treatment were analyzed. For samples that were too soft to exhibit distinct peak force at fracture (<1.0 N) due to complete cooking during the thermal process, inflection point of the curve was reported.

Color Measurements

Hunter L* a* and b* color values of sweetpotato cubes was measured using a Minolta CR-300 Chroma Meter (Konica Minolta, Inc., Ramsey, NJ). The instrument was calibrated with D65 light source and a white tile. Color measurements were taken for all samples at each stage of processing: control, unprocessed (after pretreatment) and after processing in an oil bath after denoted time/temperature combinations. Ten samples from each treatment were measured at three different locations on each sample. Hue angle (H°) was calculated using these equations followed by conversion from radians into degrees:

$$H^\circ = \tan^{-1}(b^*/a^*) \text{ when } a^*>0 \text{ and } b^*>0$$
$$H^\circ = 180^\circ + \tan^{-1}(b^*/a^*) \text{ when } a^*<0$$
$$H^\circ = 360^\circ + \tan^{-1}(b^*/a^*) \text{ when } a^*>0 \text{ and } b^*<0$$

Chroma (C*) was calculated as $[a^{*2}+b^{*2}]^{1/2}$ and $\Delta E = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$ was calculated using the values of the controls as references.

Statistics

The experiments were performed with 2 replications and multiple samples (n=10 for color and n=16 for firmness) were taken from each replicate for all analyses. Group differences were evaluated using analysis of variance (ANOVA) *F*-tests using SAS version 9.1.3 (SAS Inst. Inc., Cary NC, USA) with $p<0.05$ considered to be a statistically significant difference. Group means were separated

using Tukey's studentized range.

4. RESULTS AND DISCUSSION

Effect of pretreatment on firmness retention of cubes

The firmness of sweetpotatoes cubes subjected to thermal processing in an oil bath was measured by peak compression force (N) and the results are presented in Table 1. Force deformation curves and resulting peak forces obtained by compression tests were in agreement with what has been previously reported for sweetpotatoes (Truong and others 1998). Even though samples were also removed at 0 sec of processing and analyzed, these data are not shown because the values were not significantly different ($p<0.05$) from the 30 sec values for any treatment or cultivar. For sweetpotato cubes made from Covington, the control samples were fractured at 207.5 N, which was not significantly different ($p<0.05$) from the values reported for most of the treated samples. Two samples, LTB-60 min and 0.5% $CaCl_2$-60 min, did fracture at significantly lower forces, 140.5 and 158.8 N, respectively. This could be due to the longer soak time, which could have had a negative effect, or just inherent variation within the sweetpotato samples. It is interesting that neither LTB pretreatments provided a significant level of firmness retention for the orange-fleshed Covington cultivar after thermal processing at 125 °C for 30 sec. Calcium chloride soaking increased firmness retention and by doubling the concentration, the peak force at fracture increased as well, however soaking for 60 min provided no significant increase when compared to the 30 min

values. Covington cubes subjected to the 2-step pretreatment had the greatest firmness retention and fractured at a peak force of 5.2 N which was much higher than a 0.7 N value of the control samples (Table 1). Control samples were completely cooked after heating in the oil bath and had to be handled with great care so as not to destroy them, especially when removing them from the test cell.

The results for Oriental cubes were similar to those of Covington, in that the 2-step pretreated cubes had the greatest increase in force needed to fracture at 20.2 N, while control samples fractured at 4.5 N.   NC 413 is the only cultivar that does not show a significantly greater amount of firmness retention for the 2-step pretreatment. Instead it is considered statistically similar to the peak force at fracture provided by the 0.5% $CaCl_2$-60 min pretreatment. This is the only instance where an increased soak time in $CaCl_2$ results in an increased firmness of the cubes and peak forces nearly double from 10.7 N for 30 min to 20.2 for 60 min.

Overall, peak force values were higher for Oriental and NC 413 cultivars, and this is most likely due to the high dry matter content present in these sweetpotatoes. Various purple-fleshed cultivars have been shown to contain 30-37% dry matter. The yellow and white-fleshed cultivars such as Oriental, have been reported to have dry matter contents of 34.4% (Steed and others 2008b; Brinley and others 2008). In contrast, Covington only contains about 20% dry matter, which most likely leads to its overall softer texture (Yencho and others 2008).

The lack of a significant increase in firmness of samples undergoing LTB is in contrast with what has previously been reported. Truong and others (1998) found

that when the cylinders of Jewel sweetpotatoes (an orange cultivar) were blanched at 60°C for 45 min then steam cooked, the peak forces of fracture were greater than 5 N. Furthermore, after canning these LTB samples retained their intactness with significantly higher fracturability and hardness values than the controls (Truong and others 1998). Another study on Jewel sweetpotatoes also found that cylinders blanched at 62 °C for 45 min then cooked in boiling water for 20 min had a compression force of 11.04 N which was significantly greater than 3.51 N exhibited by the untreated control (Walter and others 2003).

LTB at 62 °C is believed to effectively increase firmness due to the activation of pectin methyl esterase (PME). PME hydrolyses the carboxymethyl groups of pectin, and the demethylation of the galacturonic chains has a two-fold affect on texture (Canet and others 2005). The resulting free carboxylic acid groups can firstly bind calcium ions which cross-link with pectic chains in a mechanism known as the "egg-box model" (Grant and others 1973), resulting in greater cell-to-cell adhesion and firmer texture. Secondly, decreasing the level of methylesterification of the pectic substances reduces the tendency of β-elimination to occur when the LTB samples are subjected to further processing at higher temperatures. Beta-elimination is catalyzed by hydroxyl ions readily available in cooking or canning environments and results in splitting of the α1->4 glycosidic bonds between the galacturonic acid residues that make up pectin. Once the glycosidic bond is split, the pectic substances become more soluble, which results in softening of fruit and vegetable texture (Van Buren 1979). Since PME reduces the occurrence of β-

elimination, activation of PME has been applied in fruit and vegetable processing to improve the firmness of thermally processed products (Anthon and Barrett 2006; Ni and others 2004).

The lack of increased firmness for sweetpotato samples treated by LTB in this study could be due to a number of factors. Previous studies are reported for Jewel sweetpotatoes, an older orange cultivar that was mostly replaced in commercial farms by Beauregard, which is now being phased out by Covington (Carpena 2009). Cultivar and differences in growing conditions can account for a wide range of variation within sweetpotatoes. In addition, the thermal process utilized in this study involved heating the sweetpotatoes to an internal temperature of 125 °C which is greater than any temperatures incurred at steaming and boiling as previously reported. It could be that any benefits of LTB were simply not great enough to overcome the effects of the higher temperature and a long come up time of 12 min. Furthermore, while PME is usually attributable to the increased firmness in LTB samples, Walter and others (2003) found that PME activity decreased by 82% in sweetpotatoes subjected to LTB for 20 min at 62 °C. However sample firmness continued to increase with blanching time up to 90 min, illustrating that firmness due to pectin demethylation only explains part of the observed increased firmness and the rest is due to unknown factors that have not been elucidated (Walter and others 2003).

As shown in Table 1, $CaCl_2$ addition did increase fracture forces for all cultivars as compared to the control samples. Carrot pieces soaked for 1 h in 0.5%

$CaCl_2$ alone or in combination with a heat step of 70 °C for 30 min caused a decrease in texture degradation kinetics, and therefore resulted in a significant improvement of texture (Smout and others 2005). Also, when $CaCl_2$ addition is coupled with LTB it leads to a pronounced improvement in carrot texture and has a protective effect in maintaining cell wall integrity for frozen jalapeno peppers (Sila and others 2004; Perez-Aleman and others 2005).

In sweetpotatoes the effect of calcium has been coupled with base treated tissue. Walter and others (1993) found that sweetpotato strips treated with 0.03 M Na_2CO_3, blanched and vacuum infiltrated with 0.6% $CaCl_2$ in an acetate buffer resulted in a 3-fold increase in shear force over the control strips. These findings were the basis for the application of the 2-step pretreatment in this study. Preliminary data (not shown) showed little promise for samples only treated with Na_2CO_3. As mentioned above, samples treated with LTB and $CaCl_2$ alone were also not showing the degree of firmness believed to be sufficient for Covington sweetpotato cubes to retain intactness in a high temperature thermal process. Thus, a 2-step process including these pretreatments was developed and evaluated. A 1 h soak in 0.03 M Na_2CO_3 at 25 °C can improve texture by enzymatic de-esterification of methyl esters of pectin (Walter and others 1993). Then, a 1 h LTB step in 1% $CaCl_2$ at 62 °C provides the optimum temperature for PME activity which can further increase the amount of calcium ions for crosslinking with pectin (Truong and others 1998; Walter and others 2003; Van Buren 1979). The 2-step pretreatment resulted in the highest fracture forces for cubes made from all cultivars. For this reason, this

pretreatment was considered to be the most appropriate and was subjected to a more extensive experiment examining its performance over a range of high temperatures and times.

Effect of 2-step pretreatment on firmness retention of cubes exposed to high temperatures

The results of the sweetpotato cubes subjected to the 2-step pretreatment followed by heating at high temperatures are summarized in Table 2 and Figure 1. Since Covington cubes were the softest, they were utilized for the entire experiment to assess the effect of high temperature processing on firmness retention of the 2-step pretreated samples based on the assumption that fracture forces for Oriental and NC 413 cubes would be greater. To prove this, cubes from these cultivars were subjected to the highest temperature, 130 °C for 0 and 0.5 min. Fracture forces for thermally processed cubes were not significantly different when compared to one another despite the temperature or time the cubes were subjected to it, and ranged from 5.2-16.4 N. Even though a fracture force of 5.2 N for Covington cubes after heating at 130 °C may seem low, informal observation and assessment showed that the cubes were strong enough to withstand handling during removal from the test cell, and they remained intact for compression tests.

As expected, Oriental and NC 413 cubes subjected to 130 °C for 0.5 min exhibited fracture forces at 19.1 and 20.4 N, respectively, which were greater than those for Covington cubes (Figure 1). The results illustrated the robustness of the 2-step pretreatment and its potential to be utilized in thermal processes where

temperatures are commonly greater than 121 °C. This is especially important for processes wherein texture should be maintained throughout the time necessary for a 12D inactivation of *C. botulinum*, the major pathogen of concern in thermally processed low acid foods.

Color changes

The Hunter color values for samples subjected to the 2-step pretreatment and subsequent thermal processing are shown in Table 3. The application of any pretreatment caused significant changes to L*, a*, and b* values as compared to those of the control (data not shown). Therefore the values for the 2-step pretreatment were chosen for assessing color changes, especially since this was the only pretreatment to be evaluated further and the technique deemed to show the greatest promise in future applications. In almost all cases each step in the process led to a significant change in L*, a*, and b*.

Covington sweetpotatoes darkened with processing treatments as shown by a decrease in L*, which is a lightness index and ranges from 0 (black) to 100 (white). The intensity of red color, indicated by a*, decreased with the treatments while yellow intensity, or b*, increased. This changed the color from the vibrant orange of fresh cut sweetpotatoes to a yellow-orange color, which was illustrated by a sharp decrease in hue angle (H°) and chroma (C*) (Table 3). All reported values fell within the color changes reported for the orange-fleshed sweetpotatoes subjected to acidification and pasteurization (Koskiniemi 2009). Overall change in color as represented by ΔE showed that the pretreatment and the thermal process each

contributed about 50% to the color change. Beta-carotene, the pigment responsible for orange color in sweetpotatoes, can go through many changes throughout this process, including de-compartmentalization of the pigment in the cells, isomerization and oxidation, all of which can affect the resulting color (Purcell and others 1969; Koskiniemi 2009). Despite the changes to individual color components, overall color change was comparable to that of cooked sweetpotato color and no brown pigments were observed indicating that the changes would be acceptable.

Oriental sweetpotatoes also showed significant changes to all color parameters. Visually, the most obvious change was the increased darkening represented by a large decrease in L* and the decrease in b*, or loss of yellow color intensity. After processing the sweetpotatoes changed from a white/cream color to more of grayish color. However, color values after processing compared well with those reported for puree made from another white cultivar, Picadito, indicating that the change in color components due to high temperature heating in this study were not abnormal but more a result of what is naturally seen when the roots undergo cooking (Brinley and others 2008).

Purple-fleshed sweetpotatoes had the lowest overall change in color when compared to the control as shown by ΔE. However, NC 413 also showed a greater departure in color values commonly reported for purple cultivars. Purple color is the result of red and blue components and therefore is evidenced by a positive a* and a negative b*. For purple sweetpotatoes a* and b* have been reported to range from 11.7-26.2 and -3.7 to-13.3, respectively, (Steed and others 2008b; Brinley and others

2008). However, in this experiment both red and blue intensity fell beneath what has been previously reported and had values more indicative of a brown color, rather than purple (Table 3). Since control samples were initially low in these color components, this could be explained by the activity of polyphenol oxidase, which results in enzymatic browning of fruit and vegetables cut pieces exposed to oxygen. Jang and others (2005) isolated polyphenoloxidase (PPO) in purple-fleshed potatoes and found that the enzyme is most active at room temperature and degraded at temperatures >70 °C. Also, anthocyanins are known to be water soluble and the loss of anthocyanins was visually evident in the deep purple color of the soak solutions after application of the pretreatments. This was further evident by the significant decreases in the color values of the samples after the 2-step pretreatment as shown in Table 3.

5. CONCLUSIONS

A 2-step pretreatment that combined the firmness retention effects of pH modification, low-temperature blanching, and $CaCl_2$ addition was effective in increasing firmness of sweetpotato cubes from the orange-, white- and purple-fleshed sweetpotatoes. The pretreated cubes showed the highest firmness retention after subjecting to thermal processing at high temperature ranging from 115-130 °C. It is of significant importance to industry that sweetpotato cubes can be made to maintain their shape and strength at temperatures as high as 130 °C, and to our knowledge no textural study has evaluated firmness at such a high temperature.

Furthermore, this study provides data for a prevalent new orange cultivar, Covington, along with data for purple and white fleshed sweetpotatoes and demonstrates the potential for pretreated sweetpotato particulates to maintain quality during high temperature thermal processing such as continuous-flow microwave processing.

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TABLE 1: Average Peak Compression Force[1] (N) of Pretreated Sweetpotato Cubes Before and After Thermal Processing[2]

Treatment	Cultivar					
	Covington		Oriental		NC 413	
	Before	After	Before	After	Before	After
Control	207.5 ± 7.6^{ab}	0.7 ± 0.1^{d}	155.0 ± 8.5^{bc}	4.5 ± 0.3^{d}	146.8 ± 4.7^{b}	3.6 ± 0.4^{f}
2-step Pretreatment	204.3 ± 13.5^{ab}	5.2 ± 0.2^{a}	197.5 ± 12.4^{ab}	20.2 ± 1.1^{a}	162.2 ± 5.2^{ab}	22.3 ± 1.2^{a}
LTB 30 min	209.6 ± 11.8^{ab}	0.7 ± 0.1^{d}	196.2 ± 9.7^{ab}	7.4 ± 0.5^{cd}	148.1 ± 7.1^{b}	8.9 ± 0.5^{e}
LTB 60 min	140.5 ± 17.0^{bc}	0.6 ± 0.0^{d}	141.7 ± 18.8^{c}	9.9 ± 0.7^{bcd}	118.7 ± 7.5^{c}	12.1 ± 0.7^{d}
0.5%* $CaCl_2$ 30 min	216.3 ± 12.3^{a}	1.8 ± 0.1^{c}	194.6 ± 9.2^{ab}	13.2 ± 3.7^{b}	178.7 ± 7.8^{a}	10.7 ± 0.5^{de}
0.5% $CaCl_2$ 60 min	158.8 ± 8.2^{bc}	1.4 ± 0.1^{c}	197.7 ± 12.0^{ab}	14.9 ± 1.0^{ab}	146.5 ± 3.9^{b}	20.4 ± 1.1^{ab}
1% $CaCl_2$ 60 min	229.1 ± 15.1^{a}	2.3 ± 0.2^{b}	196.5 ± 9.7^{ab}	12.8 ± 1.0^{bc}	158.6 ± 6.4^{ab}	15.7 ± 0.8^{c}
1% $CaCl_2$ 60 min	225.6 ± 15.9^{a}	2.4 ± 0.1^{b}	228.3 ± 15.4^{a}	13.3 ± 0.7^{b}	144.3 ± 4.0^{b}	$17.4\pm1.0b^{c}$

[1] - Values reported are the means ± the standard error (n=32) and different superscripts within a column denote significance ($p<0.05$).
[2] – Thermally processed cubes were heated at 125 °C for 30 sec.
[*] – Percentages of $CaCl_2$ on weight/volume basis.

TABLE 2: Average Peak Compression Force[1] (N) of Covington Cubes Subjected to 2-Step Pretreatment and High Temperature Processing

Processing Temperature (°C)	Time (min)	Peak Compression Force (N)
Pretreatment[2]		240.6 ± 11.9^{a}
130	0	5.2 ± 0.4^{b}
	0.5	6.1 ± 0.4^{b}
125	0	8.3 ± 0.5^{b}
	0.5	6.7 ± 0.4^{b}
	1.22	6.6 ± 0.4^{b}
120	0	10.1 ± 0.7^{b}
	1.25	8.4 ± 0.5^{b}
	2.5	8.4 ± 0.5^{b}
	3.86	6.8 ± 0.2^{b}
115	0	16.4 ± 0.8^{b}
	4	11.0 ± 0.5^{b}
	8	7.7 ± 0.4^{b}
	12.22	6.3 ± 0.2^{b}

[1] - Values are the means ± the standard error (n=32) and different superscripts denote significance ($p<0.05$) between treatments.
[2] - Pretreatment value was the peak compression force for samples that went through the 2-step pretreatment but were not thermally processed.

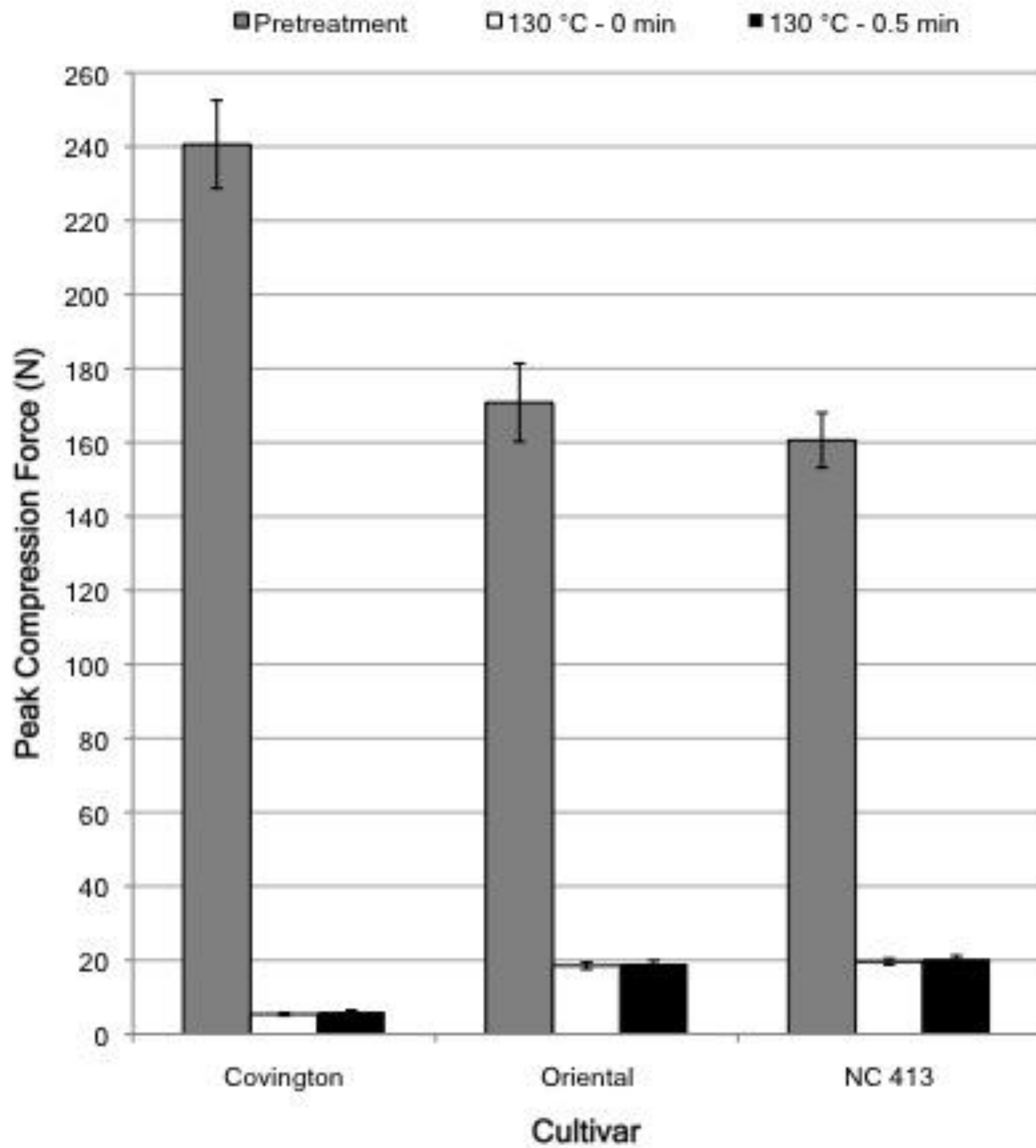

FIGURE 1: Peak Compression Force (N) of 2-step Pretreated Cubes After Thermally Processing at 130 °C

TABLE 3: Hunter Color Values[1] for Sweetpotato Cubes Subjected to a 2-step Pretreatment and Subsequent Thermal Processing at 125 °C for 30 sec

Cultivar	Treatment	L*	a*	b*	H°	C*	ΔE
Covington	Control[2]	62.7±0.3[a]	23.0±0.2[a]	32.6±0.3[a]	35.1±0.1[a]	39.9±0.3[a]	Ref[3]
	Treated	58.6±0.4[b]	18.7±0.4[b]	31.3±0.4[b]	30.8±0.3[b]	36.5±0.5[b]	6.1
	Processed	55.9±0.3[c]	10.6±0.2[c]	35.7±0.9[b]	16.3±0.2[c]	37.3±0.8[b]	14.5
Oriental	Control	75.9±0.3[a]	-0.4±0.2[a]	21.9±0.3[a]	179.1±0.5[a]	21.9±0.3[a]	Ref
	Treated	74.0±0.6[b]	-1.6±0.1[b]	17.1±0.4[b]	174.7±0.4[b]	17.2±0.4[b]	5.3
	Processed	57.5±0.6[c]	-2.1±0.1[c]	13.4±0.3[c]	171.0±0.2[c]	13.5±0.3[c]	20.3
NC 413	Control	37.7±0.2[a]	11.1±0.3[a]	1.9±0.1[a]	80.4±0.8[a]	11.2±0.3[a]	Ref
	Treated	38.6±0.2[b]	6.9±0.2[b]	2.7±0.1[b]	68.4±0.7[b]	7.5±0.2[b]	4.4
	Processed	35.5±0.1[c]	4.3±0.1[c]	2.0±0.1[b]	65.4±0.6[c]	4.7±0.1[c]	7.1

[1] - Values reported are the means ± the standard error (n=10). For each color component different superscripts denote significance ($p<0.05$) between treatments.
[2] - Control cubes are raw while treated cubes were subjected to the 2-step pretreatment but are not thermally processed. Processed cubes were thermally processed at 125 °C for 30 sec.
[3] - Ref denotes that these values are used as the reference values for the ΔE calculation.

CHAPTER 3

CONTINUOUS FLOW MICROWAVE PROCESSING OF FOODS CONTAINING SWEETPOTATO PARTICULATES

1. ABSTRACT

Continuous flow microwave processing has been successfully utilized in commercial production of homogenous foods, and it is imperative to extend this technology to processing of multiphase food products. However, due to the high temperatures and mechanical stress incurred by continuous processing it is challenging to maintain particle shape, which would be necessary for consumer acceptance of food products such as soups. A 2-step pretreatment has been developed and applied to cubes of Covington, Oriental, and NC 413 sweetpotatoes that resulted in firmness retention at a level deemed suitable to survive microwave processing. Dielectric property measurements showed that while dielectric constant did not change due to pretreatment, dielectric loss factor did increase for pretreated Oriental and NC 413 sweetpotatoes when compared to control samples. Five microwave test runs with the target temperatures of 115, 121, and 125 °C were conducted utilizing a pilot scale 100 kW system. Pretreated cubes were inserted into a carrier fluid of orange-fleshed sweetpotato puree and received microwave application at three points. After passing through the hold tube they were cooled, and collected in a pressurized tank outfitted with a sieve. All inserted sweetpotato cubes were recovered, and subjected to firmness and color measurements. For all sweetpotato cultivars, microwave processing caused a significant decrease in

firmness as measured by peak compression force (N), however texture was firm enough for all cubes to stay intact after going through the microwave heating process. Microwave processing also caused a significant decrease in color components, but not to a level outside of what has been previously reported. Based on the quality retention of microwave processed sweetpotato particulates, this process laid an important foundation for application of aseptic processing to particulates from other foodstuffs.

2. INTRODUCTION

While thermal processing of food products is the most widely used method of food preservation, conventional methods for processing low acid foods to achieve commercial sterility and shelf-stability often cause a degradation of color, flavor, texture, and nutrients (Kumar and others 2007; Wang and others 2003). Microwave heating is an emerging technology that shows promise as an alternative method of thermal processing with rapid heating and high product quality retention. Industry has already adapted the process to temper frozen foods, pre-cook bacon, pasteurize packaged food, and provide the final drying of pasta products (Sumnu and Sahin 2005).

Due to the fast and efficient heating it offers, several studies have been conducted on the applications of continuous-flow microwave systems to process homogeneous food products such as milk and vegetable purees (Coronel and others 2003, 2005; Kumar and others 2007; Steed and others 2008a). High viscosity and

low thermal conductivities of fruit and vegetable purees often causes quality degradation by conventional thermal processing methods but these problems can be overcome by microwave heating. Orange-fleshed sweetpotato puree was subjected to continuous flow microwave processing and packaged in flexible containers to produce a shelf-stable product with no detectable microbial populations after 90 d of storage at 25 °C. The finished product had an apparent viscosity and color comparable to the untreated puree, illustrating high quality retention (Coronel and others 2005). Microwave processed purple-fleshed sweetpotato purees increased 5.9% in total phenolic content and decreased 14.5% in anthocyanin content, but maintained their antioxidant activity. Total color change measured by ΔE was not significant for the microwave processed puree, although there was a slight loss of saturation in blueish-purple color (Steed and others 2008a). When compared to a purple-fleshed sweetpotato puree thermally processed by canning, microwave processed puree was superior in all aspects, especially in color and anthocyanin retention. Due to the long retort times necessary to heat the cold spot, the canned sample had high degradation of anthocyanins, which resulted in a reddish-brown color (Steed and others 2008b).

Microwave heating has proved to be a viable and valuable technology, especially to the sweetpotato industry where it can be employed as a method to convert the roots into a high quality shelf stable product for utilization as a functional food ingredient. Now, it is imperative from a processing standpoint to extend the technology to foods that contain particulates. A multiphase food product, salsa con

queso, has been microwave processed. As processing temperature increased, the temperature differences in the salsa con queso at the center and wall of the heating tube narrowed, and microwave processing was deemed suitable for multiphase food products (Kumar and others 2007). However, the food particulates in salsa con queso are small (approximately 1 cm at widest point) and it is not necessary to maintain particulate integrity. For future applications, like aseptic processing of soups, larger food particles will be necessary, but this causes a dilemma. In the United States processes for multiphase foods must be validated before a product can be marketed commercially (Jasrotia and others 2008). The FDA requires the processors to demonstrate by means of experiments and mathematical modeling that every portion of the food product receives adequate heat treatment to ensure commercial sterility (Kumar and others 2007). Previous attempts to do so in microwave systems using larger, rigid particles that held bioindicators, showed a number of difficulties in guaranteeing uninhibited particle flow and resulted in an overestimation of microbiological inactivation (Brinley and others 2007; Stam 2008).

Recently, UltrAseptics (Raleigh, NC, USA) developed a 100 kW microwave system that was designed to accommodate particulate processing. This study explored the feasibility of processing sweetpotato particulates prepared by a 2-step pretreatment (Chapter 2) in the 100 kW continuous flow microwave system. An additional objective was to determine the future potential of the technology in processing of multiphase food products and process validation studies.

3. MATERIALS AND METHODS

Sample preparation of sweetpotato particulates

Three cultivars namely Covington, Oriental, and NC 413, which have orange, white and purple flesh, respectively, were utilized. All sweetpotatoes were grown at the Clinton Research Station of the Sweetpotato Breeding Program, North Carolina State University. Sweetpotatoes harvested in October 2008 were utilized for the August 2009 microwave run, while sweetpotatoes harvested in October 2009 were used in the February 2010 run. All sweetpotatoes were cured at 30 °C, 85-90% relative humidity for 7 d then stored at 13 °C 85-90% relative humidity until utilization. A batch of roots (n=20) were taken from each cultivar and cut into 9.52 mm (3/8 inch) cubes by passing the whole washed root through a French fry press with a 9.52 mm screen. The rectangular strips were hand diced into cubes which underwent a 2-step pretreatment process that involved a 1 h soak in 0.03 M Na_2CO_3 at 25 °C, followed by a 1 h soak in 1% $CaCl_2$ at 62 °C. After the pretreatments, all samples were removed from the solutions, blotted dry, placed in Ziploc® bags and held at 4 °C overnight until microwave processing. After microwave processing cubes were collected, rinsed off in tap water, blotted dry, and subjected to texture analysis and color measurement.

Measurement of dielectric properties

Dielectric properties were measured for two replicates of raw and 2-step pretreated sweetpotato cubes. Sweetpotato cubes were made from two batches of roots (n=10) and then half of each batch was pretreated. Control and pretreated

cubes were separately homogenized in a Robotcoupe mixer (Model RSI 2YI Ridgeland, MS, USA) before analysis. Dielectric constants and loss factors were measured at 915 and 2450 MHz with an open-ended coaxial probe (HP 85078B, Agilent Technologies, Palo Alto, CA, USA) placed in a pressurized test cell that was filled with the homogenized sample. The test cell was submerged in an oil bath (Model RTE111, Neslab Thermo Scientific, Waltham, MA, USA) and the dielectric properties were measured using a network analyzer (HP 8753C, Agilent Technologies, Palo Alto, CA, USA).

100 kW microwave processing

A pilot scale 100 kW microwave heating unit (UltrAseptics, Raleigh, NC, USA) operating at 915 MHz was used for continuous flow thermal processing (Figure 1). There were 5 separate test runs conducted, 3 in August 2009 and 2 in February 2010. The carrier fluid for the experiments was microwave processed and aseptically packaged orange-fleshed sweetpotato puree donated by Yamco LLC (Snow Hill, NC, USA). It was loaded into the hopper and pumped through the microwave unit using a dual-piston positive displacement pump (Model A7000, Marlen Research Corp, Overland Park, KS, USA) at a flow rate of 5.7 L/min. The puree was pre-heated to 50-55 °C by running hot water (85 °C) through the cooling section which acted as a tubular heat exchanger. Then, the microwave generator was turned on and the target temperatures of the puree at the hold tube exit were maintained at 118, 121 and 125 °C by adjusting the microwave power from 30-40 kW. Temperature was measured throughout the system at noted locations (Figure

1) every 0.5 sec by the in-line type T thermocouples connected to a computer-based data acquisition system (TempScan v.4, TempScan/1100, IOTech, Cleveland, OH, USA).

Sweetpotato particles were incorporated into the puree flow by closing valves on the main line which diverted the flow of the puree to the injection port, which carried the particles to the applicator section. The particles were inserted in groups of three, one cube of each cultivar, in between the prefabricated tracer particles. Cube-shaped tracer particles were made from polymethylpenetene (PMP) with a 1.3 mm outer dimension and cylindrical inner cavity of 1 ml volume. Two NdFeB-based magnets (0.1 g each) were placed in each tracer particle for tracking residence time of the particles throughout the microwave system. Magnets were tracked as they moved in and out of each section using an 8 channel Particle Flow Monitoring System equipped with 8 magnetic sensors per channel (ThermaLytics, Raleigh, NC, USA). Magnetic signals from the prefabricated particles were recorded and analyzed using the ParticleMon software (ThermaLytics, Raleigh, NC, USA) in order to determine residence time and approximate time/temperature history of the particles.

Microwaves were generated and delivered to the puree and sweetpotato cubes by an ess-shaped aluminum waveguide that ran parallel to 3 microwave applicators. In the applicator sections, puree and particles flowed in microwave transparent Teflon borosilica glass tubes and absorbed microwave energy. The minimum residence time in the hold tube was 88 sec and the puree was cooled using

a tubular heat exchanger with chilled water (4 °C) as the cooling medium. Particle collection was achieved using a pressurized tank outfitted with a course stainless steel sieve, which allowed the puree to flow through but retained the sweetpotato cubes. Between each run at a target processing temperature, the microwave generator and pump were turned off for particle collection. When the pressure was released from the pressurized tank, the lid could be removed and the cubes were collected. Sweetpotato cubes were rinsed, blotted dry and kept in Ziploc® bags at 25 °C until firmness and color measurements were taken.

Firmness measurements

Compression tests on sweetpotato cubes were measured using a TA-XT2 texture analyzer (Texture Technology Corp, Scarsdale, NY, USA) equipped with a 50-kg load cell and 50 mm cylindrical probe. Data acquisition and peak force at fracture were obtained using the Texture Expert software (Texture Expert Exceed v. 2.56, Stable Micro Systems Ltd, London, UK). The following operating parameters were used: pre-test speed, 2 mm/s, test speed, 1.6 mm/s; post-test speed, 10.0 mm/s; distance 9 mm; acquisition rate, 200 point/s; force units in Newtons. Thirty cubes from each microwave run were analyzed.

Color measurements

Hunter L* a* and b* color values of sweetpotato cubes was measured using a Minolta CR-300 Chroma Meter (Konica Minolta, Inc, Ramsey, NJ). The instrument was calibrated with D65 light source and a white tile. Color measurements were taken for all samples at each stage of processing: control, unprocessed (after

pretreatment) and after microwave processing. Ten samples from each treatment were measured at three different locations on the sample. Hue angle (H°) was calculated using theses equations followed by conversion from radians into degrees:

$$H° = \tan^{-1}(b^*/a^*) \text{ when } a^*>0 \text{ and } b^*>0$$
$$H° = 180° + \tan^{-1}(b^*/a^*) \text{ when } a^*<0$$
$$H° = 360° + \tan^{-1}(b^*/a^*) \text{ when } a^*>0 \text{ and } b^*<0$$

Chroma (C*) was calculated as $[a^{*2}+b^{*2}]^{1/2}$ and $\Delta E = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$ using the values of the raw sweetpotatoes as references.

Statistical analysis

Group differences were evaluated using analysis of variance (ANOVA) *F*-tests (SAS version 9.1.3, SAS Institute Inc, Cary, NC, USA) with $p<0.05$ considered to be a statistically significant difference. Group means were separated using Tukey's studentized range.

4. RESULTS AND DISCUSSION

Dielectric Properties

The dielectric properties measured at 915 MHz for Covington, Oriental and NC 413 are shown in Figures 2-4, and those measured at 2450 MHz are found in Appendix 1. All measured values were within the range for food materials with >60% moisture (Fasina and others 2003; Nelson and Datta 2001). For all cultivars dielectric constant decreased with increasing temperatures, which has been established as a result in a decrease in dielectric relaxation time. Relaxation time is associated with the time for dipoles to revert to random orientation when the electromagnetic field is removed (Sumnu and Sahin 2005; Steed and others 2008a).

Dielectric constant decreased from 67.5 at 12 °C to 51.4 at 131°C, from 55.5 at 20 °C to 48.1 at 131 °C, and from 55.3 at 17 °C to 45.3 at 131 °C for raw Covington, Oriental and NC 413 samples, respectively. These values are similar to what has been reported previously for sweetpotato purees of varying flesh colors (Fasina and others 2003; Brinley and others 2007; Steed and others 2008a). Dielectric constant values for Oriental and NC 413 cultivars were lower than those of Covington cultivar across the temperature range. The difference can be attributed to the lower moisture content of these cultivars. Covington sweetpotatoes have 80% moisture, while purple-fleshed cultivars have been shown to have 63-70% and yellow or white cultivars have around 66% (Yencho and others 2008; Brinley and others 2008; Steed and others 2008a). Since most of the water in sweetpotato exists as free water, sweetpotatoes with lower moisture content (Oriental and NC 413 cultivars) have less polar molecules to re-orient with the changes in electromagnetic polarity caused by the electromagnetic field, and therefore have lower dielectric constants. These results are in accordance with previous reports (Brinley and others 2008).

Dielectric loss factor decreased for all cultivars over the range of temperatures examined (Figures 2-4). Previous reports on the influence of temperature and moisture on the dielectric loss factor of different food products are in agreement with this trend (Nelson and Datta 2001; Brinley and others 2008). Raw NC 413 samples exhibited a noticeable variation in dielectric loss factor (ε”) between the two replicates. Since each replicate was performed on a sample made from a different

batch of roots, this difference is most likely the result of the inherent variation in sweetpotato roots, with respect to moisture content (Figure 4). The application of a 2-step pretreatment caused no notable departure from the dielectric properties reported for control Covington samples, but did cause changes for Oriental and NC 413. One replicate of pretreated Oriental roots reported higher dielectric loss factor values than the other (Figure 3), and pretreated NC 413 roots had higher dielectric loss factor values than both replicates of raw NC 413 sweetpotatoes (Figure 4). The addition of salt has been shown to increase the loss factor in sweetpotatoes due to an increase in ionic conductivity that can occur. It is possible that due to the lower moisture content of the white and purple-fleshed cultivars this effect is more pronounced than for Covington (Tang 2005; Koskiniemi 2009). Also, the inherent variation between sweetpotato roots previously mentioned could cause one batch to increase $CaCl_2$ uptake, which would lead to the higher dielectric loss factor seen in Figure 3.

Firmness measurements

It was attempted to pass untreated sweetpotato cubes through the microwave system at a target processing temperature of 118 °C. Due to the low percentage of intact cubes recovered at the end of processing (<5-20%, depending on cultivar) and the fact that the recovered cubes were easily mashed or broken, the control samples were deemed unsuitable for post processing analysis. This proved that pretreatment was necessary in order to maintain cube structure during microwave processing.

Also, 100% of inserted pretreated sweetpotato particles were collected at the end of each microwave test run and all particles maintained their cube shape.

Firmness measurements for all cultivars were taken throughout the stages of processing and the results are shown in Table 1. Application of the 2-step pretreatment caused no significant change in the firmness of sweetpotato cubes when compared to the control cubes, but there was a significant decrease in peak compression force at fracture of the samples from all cultivars as a result of the cooking that occurred during microwave processing. Firmness values for samples microwave processed at 125 °C fell within the ranges expected and reported for 2-step pretreated samples in Chapter 2 that were thermally processed in an oil bath.

Table 2 shows that the average processing temperature had no clear effect on the resulting firmness when examined alone. When coupled with the storage time of sweetpotatoes at processing there were some trends that emerged. For both 4 and 10 mo stored Covington sweetpotatoes, increasing the target processing temperature from 118 to 125 °C caused a significant decrease in cube firmness. This was also true for 4 mo stored NC 413 and 10 mo stored Oriental sweetpotatoes. But since this trend did not hold true in all cases, it suggests that stored sweetpotatoes responded differently to the firming pretreatment. It is also important to note that the sweetpotatoes stored for 4 and 10 mo were from different harvests, and therefore there is a great deal of naturally inherent variability in the roots due to completely different growing conditions.

Softening of sweetpotatoes during storage has been widely reported and firmness has been enhanced utilizing pretreatments including low temperature blanching (LTB), $CaCl_2$ addition and base infiltration (Walter and others 1993). The positive effect of LTB on firmness retention of Jewel sweetpotatoes that were pretreated before boiling and canning has been documented. LTB at an optimum temperature of 62 °C is believed to effectively increase firmness due to the activation of pectin methyl esterase (PME) (Truong and others 1998; Walter and others 2003). PME hydrolyses the carboxymethyl groups of pectin, and the demethylation of the galacturonic chains has a two-fold affect on texture (Canet and others 2005). The resulting free carboxylic acid groups can bind calcium ions, which cross-link with pectic chains to increase the firmness of samples. Also, decreasing the level of methylesterification of the pectic substances reduces the tendency of β-elimination; the splitting of the α1->4 glycosidic bonds between galacturonic acid residues that make up pectin. Since depolymerized pectin is more soluble, PME increases firmness by preventing the reaction from occurring (Van Buren 1979). $CaCl_2$ addition coupled with LTB leads to a pronounced improvement in carrot texture and has a protective effect in maintaining cell wall integrity for frozen jalapeno peppers (Sila and others 2004; Perez-Aleman and others 2005). In sweetpotatoes, $CaCl_2$ addition coupled with 0.03 M Na_2CO_3 sweetpotato strips resulted in a 3-fold increase in shear force over the control strips (Walter and others 1993).

Therefore, the 2-step pretreatment was designed to improve firmness based on all of these mechanisms. A 1 h soak in 0.03 M Na_2CO_3 at 25 °C can improve

texture by enzymatic de-esterification of methyl esters of pectin (Walter and others 1993). Then a 1 h LTB step in 1% $CaCl_2$ at 62 °C provides the optimum temperature for PME activity which can further increase the amount that calcium ions can crosslink with pectin (Truong and others 1998; Walter and others 2003; Van Buren 1979). However, the activity of PME on pectin does not completely explain the firming effects of pretreatments and there are unknown factors that have yet to be elucidated (Walter and others 2003). These unknown factors, and their changes during storage, could be responsible for the inconsistent response of sweetpotatoes to the pretreatment.

Color measurements

The values for color components, L*, a*, b*, hue angle (°H), chroma (C*) and ΔE, are shown in Table 3. For microwave processed samples at a target temperature of 125 °C, the presented results are the average values of the two microwave runs at this temperature. In most cases, each step of processing caused a significant decrease for all color components in all cultivars, which was expected based on the results from thermal processing in an oil bath (Chapter 2). Processed values for Covington fell within the range of color changes reported for the orange-fleshed sweetpotatoes subjected to acidification and pasteurization by continuous flow microwave processing (Koskiniemi 2009). Values for Oriental and NC 413 microwave processed sweetpotatoes also were comparable to previous reports (Steed and others 2008a; Brinley and others 2007).

The overall color change (ΔE) was greater for samples microwave processed at 125 °C than for those thermally processed in an oil bath at 125 °C (Chapter 2). This is most likely due to the fact that while the target temperature in the microwave was 125 °C, both runs at this temperature reached as high as 132 °C. In the oil bath the maximum temperature reached was 125 °C and samples were only held for 30 sec. Due to the large size of the microwave system, exposure times at the higher recorded temperatures are greater than 30 sec, as it can take anywhere from 6-8 min for particulates to travel through the applicator sections and hold tube.

100 kW microwave processing of particulates

Data from the ParticleMon monitoring system showed that tracer particles flowed through the system without inhibition (data not shown), and it is reasonably assumed that the sweetpotato cubes did so as well. There were no clogs during processing and all cubes were recovered in the pressurized tank designed for particle collection, which was a significant improvement over previous attempts (Brinley and others 2007; Stam 2008). Despite unobstructed particle flow, Table 2 shows that a wide range of temperatures were present during all microwave runs. Most runs had a 20 – 25 °C variation in temperature, but the first run at 118 °C had a 42.4 °C variation. This is believed to be a result of mechanical malfunctions. One piston of the Marlen dual piston pump was pumping at a slightly slower rate and the microwave generator fluctuated in power output. Together these factors caused different rates of heating within the puree and in the wide range of temperatures

reported. However, it is encouraging that this led to no noticeable trend in resulting firmness and the cubes maintained their structure despite the variable processing conditions.

5. CONCLUSIONS

Sweetpotato cubes subjected to a unique 2-step pretreatment maintained their shape and had unobstructed flow during processing in a 100kW continuous flow microwave system. Maintaining particulate integrity was considered a significant achievement and this process shows potential in aseptic processing of particulates from other foodstuffs. Furthermore, the successful advances in producing unobstructed particulate flow and monitoring of tracer particles lays a foundation for necessary process validation studies in the future.

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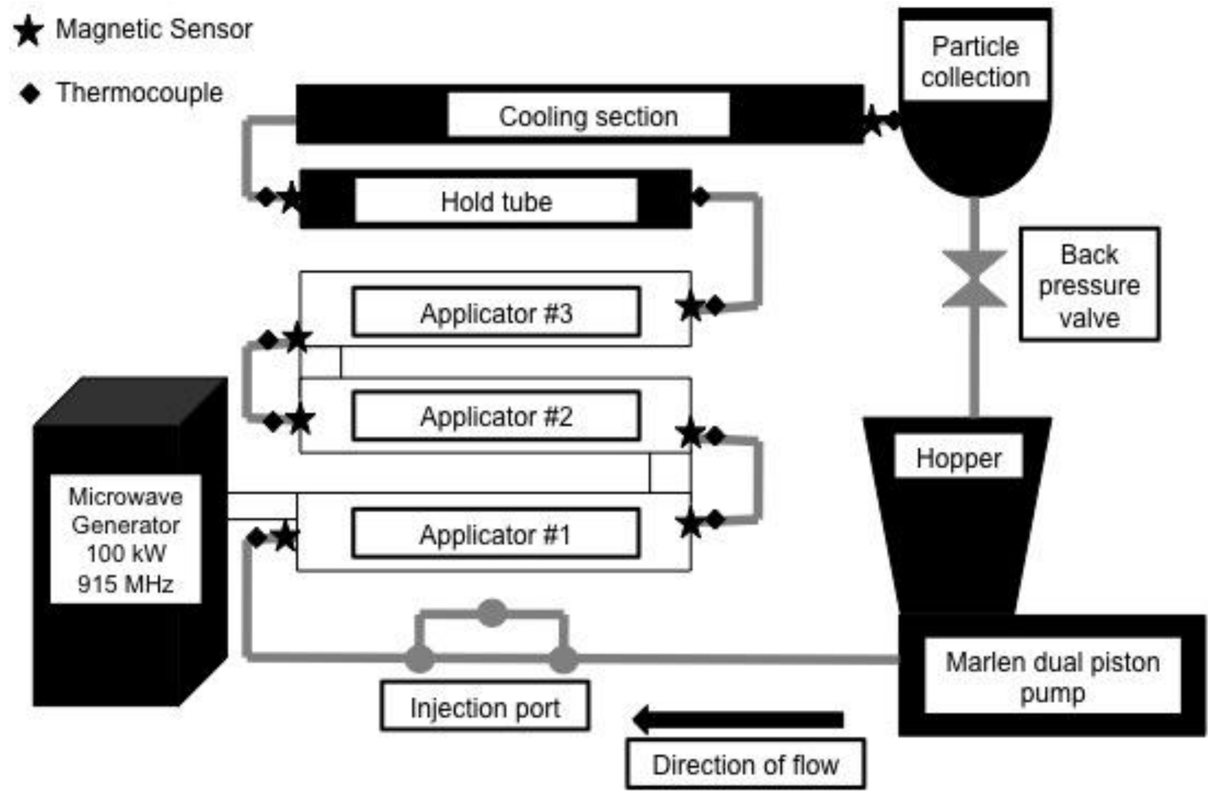
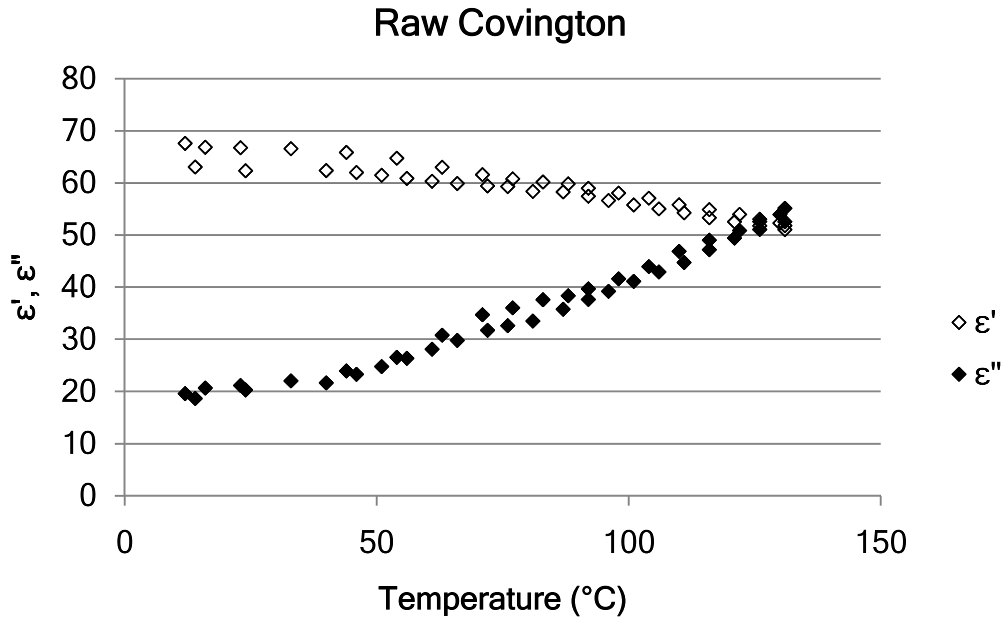


FIGURE 1: Schematic of 100 kW Continuous Flow Microwave System



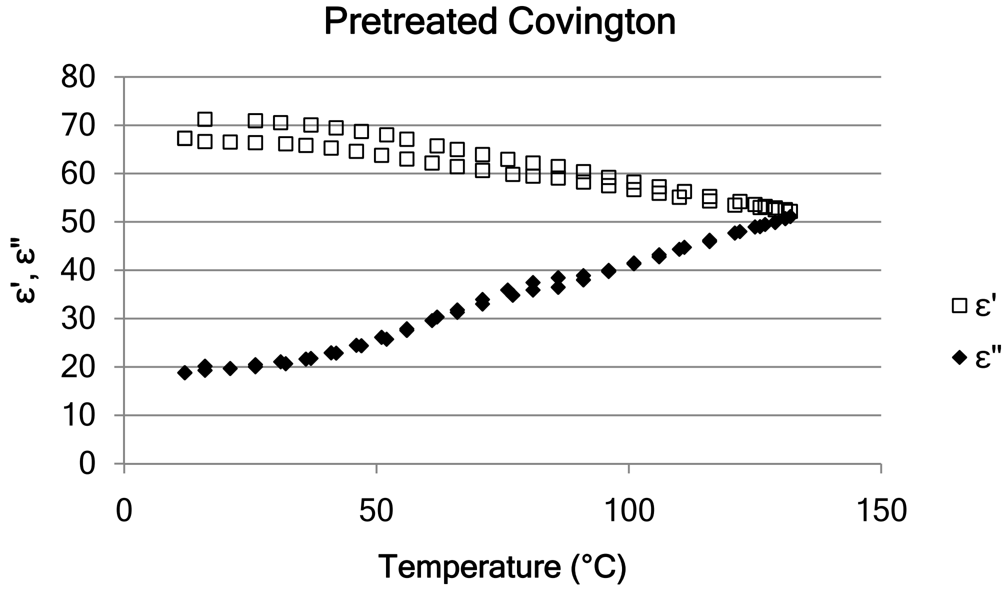


FIGURE 2: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 915 MHz for Raw and Pretreated Covington Samples From Two Batches

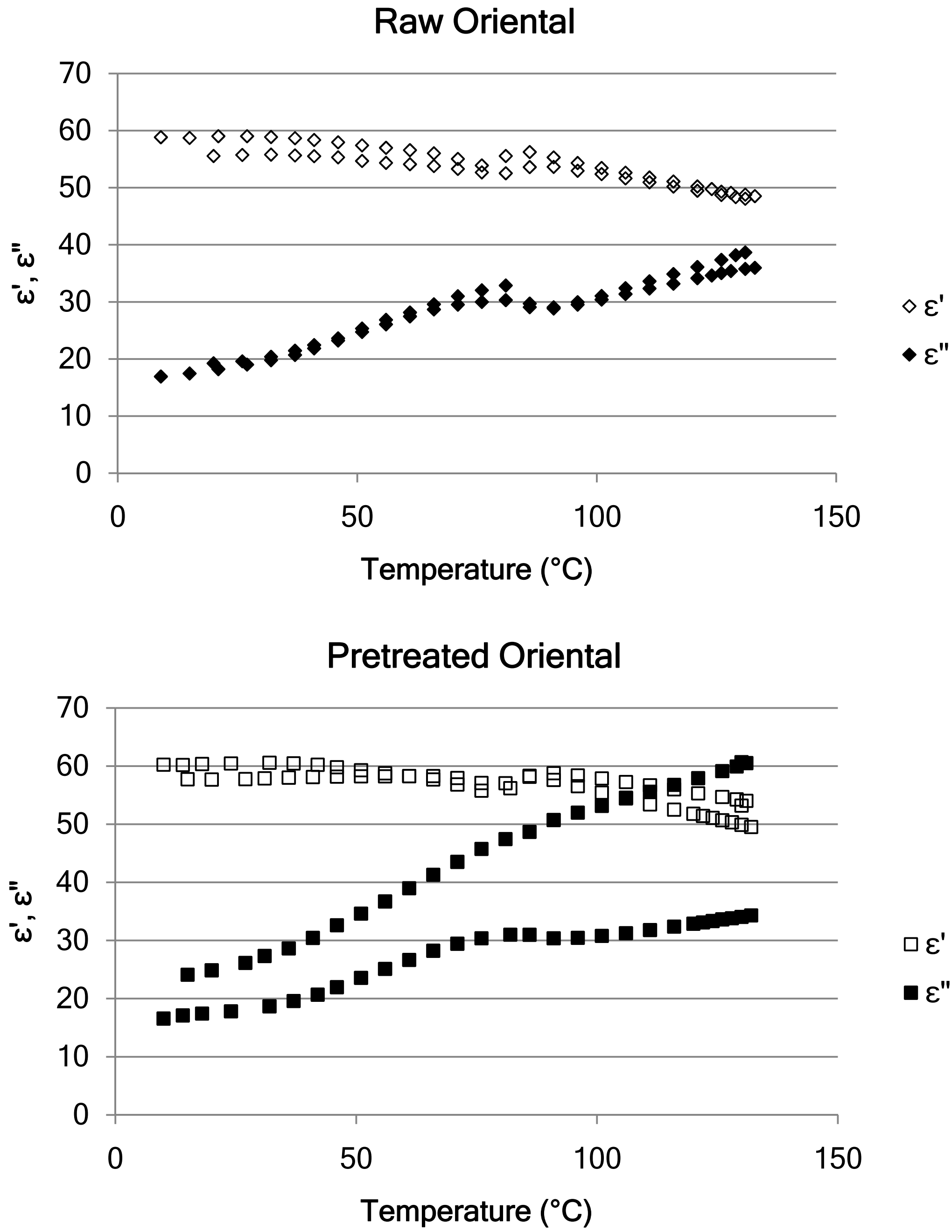


FIGURE 3: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 915 MHz for Raw and Pretreated Oriental Samples From Two Batches

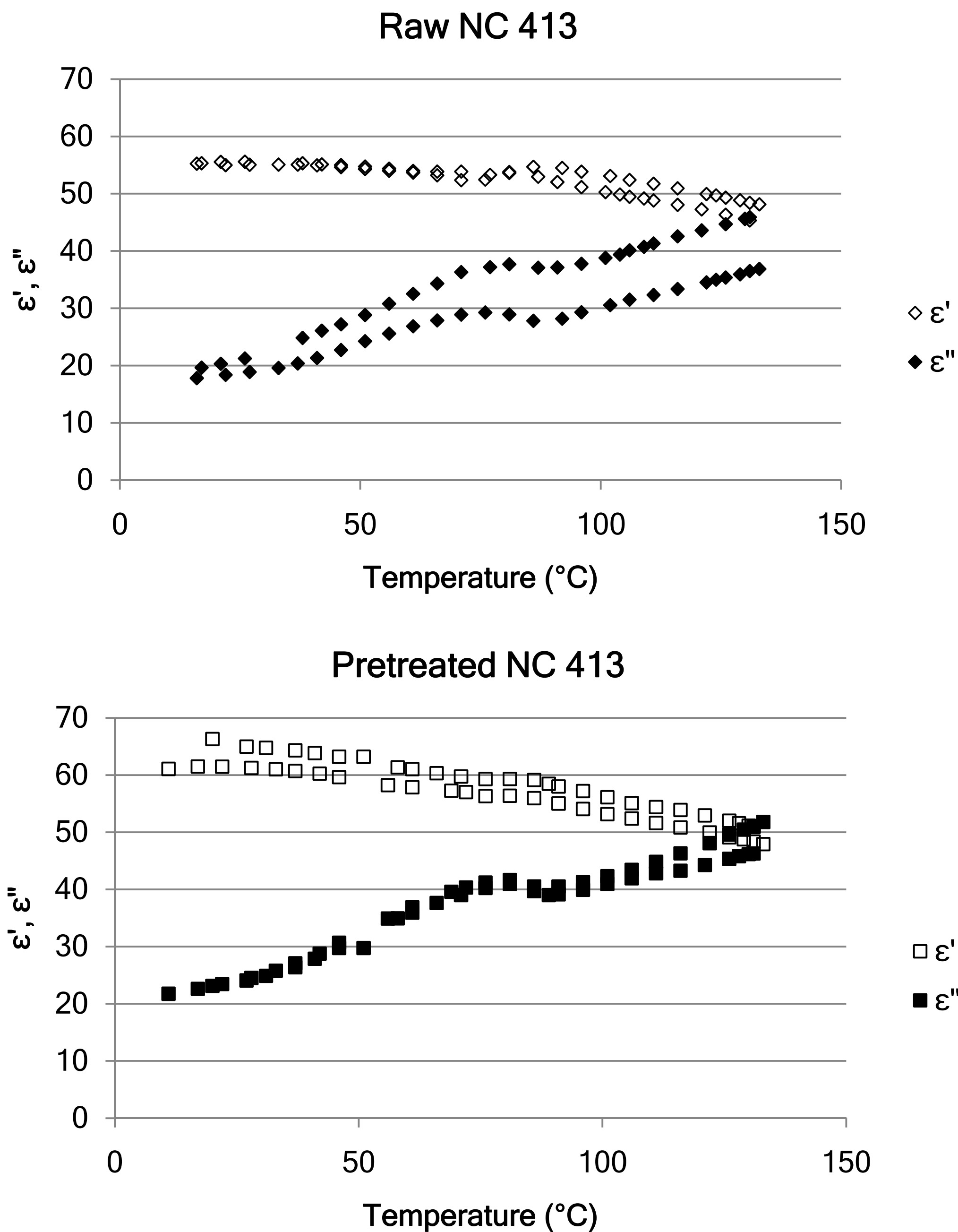


FIGURE 4: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 915 MHz for Raw and Pretreated NC 413 Samples From Two Batches

TABLE 1: Peak Compression Force[1] (N) of Sweetpotato Cubes at Different Stages of Microwave Processing

Sample	Cultivar		
	Covington	Oriental	NC 413
Control[2]	172.2±6.3[a]	155.1±3.8[a]	146.8±3.7[a]
Pretreated	182.4±7.4[a]	161.8±4.1[a]	145.0±3.7[a]
Microwave Processed at 125 °C	7.7±0.6[b]	20.4±1.4[b]	20.1±1.3[b]

[1] – Values reported are the means ± the standard error (n=30). For each cultivar different superscripts denote significance ($p<0.05$) between treatments.
[2] – Control samples were raw and received no treatment while pretreated samples were subjected to the 2-step pretreatment but received no thermal process.

TABLE 2: Microwave Processing Temperatures and Firmness of Sweetpotato Cubes

Target Temperature (°C)	Temperature Range at Hold Tube Exit (°C)	Average Temperature at Hold Tube Exit (°C)	Cultivar Firmness[1]			Sweetpotato Storage Time
			Covington	Oriental	NC 413	
118	92.1-134.5	116.3	16.2±2.2[ab]	39.6±3.1[a]	22.8±2.2[bc]	4 mo
118	106.4-126.1	117.1	18.0±2.6[a]	21.6±2.0[b]	41.7±4.7[a]	10 mo
121	105.1-131.5	177.8	17.2±3.0[ab]	23.5±2.1[b]	33.9±4.9[ab]	10 mo
125	108.9-132.4	122.8	9.1±1.0[bc]	16.8±1.8[b]	24.4±2.1[bc]	10 mo
125	107.9-132.9	123.7	6.3±0.7[c]	24.0±2.0[b]	15.8±1.2[c]	4 mo

[1] - Firmness is measured by the peak compression force (N). Values reported are the means ± the standard error (n=30). For each cultivar different superscripts denote significance ($p<0.05$) between treatments.

TABLE 3: Hunter Color Values[1] for Sweetpotato Cubes Subjected to a 2-step Pretreatment and Subsequent Microwave Processing at 125 °C

Cultivar	Treatment	L*	a*	b*	H°	C*	ΔE
Covington	Control[2]	63.4 ± 0.2^{a}	22.7 ± 0.2^{a}	33.3 ± 0.2^{a}	34.3 ± 0.1^{a}	40.3 ± 0.3^{a}	Ref[3]
	Pretreated	57.1 ± 0.3^{b}	20.0 ± 0.2^{b}	31.9 ± 0.3^{b}	32.1 ± 0.1^{b}	37.6 ± 0.3^{b}	7.0
	Processed	48.0 ± 0.4^{c}	8.2 ± 0.2^{c}	26.1 ± 0.5^{b}	17.8 ± 0.5^{c}	27.4 ± 0.5^{c}	22.3
Oriental	Control	75.6 ± 0.4^{a}	0.1 ± 0.1^{a}	21.6 ± 0.3^{a}	180.2 ± 0.2^{a}	21.1 ± 0.3^{a}	Ref
	Pretreated	73.2 ± 0.7^{b}	-1.0 ± 0.1^{b}	18.4 ± 0.4^{b}	177.2 ± 0.2^{b}	18.5 ± 0.4^{b}	4.1
	Processed	51.1 ± 0.4^{c}	-0.6 ± 0.1^{c}	13.0 ± 0.3^{c}	177.6 ± 0.4^{b}	13.0 ± 0.3^{b}	26.0
NC 413	Control	40.4 ± 0.3^{a}	11.8 ± 0.2^{a}	3.1 ± 0.2^{a}	74.9 ± 1.1^{a}	12.4 ± 0.2^{a}	Ref
	Pretreated	38.4 ± 0.1^{b}	6.0 ± 0.1^{b}	3.2 ± 0.1^{a}	62.2 ± 0.8^{b}	6.9 ± 0.1^{b}	6.2
	Processed	34.4 ± 0.1^{c}	3.2 ± 0.1^{c}	1.6 ± 0.1^{b}	64.1 ± 0.7^{c}	3.6 ± 0.1^{c}	10.6

[1] – Values reported are the means ± the standard error (n=60). For each color component different superscripts denote significance ($p<0.05$) between treatments.
[2] – Control samples were raw and received no treatment while pretreated samples were subjected to the 2-step pretreatment but received no thermal process. Processed cubes went through the microwave system at a target temperature of 125 °C.
[3] – Ref denotes that these values are used as the reference values for ΔE calculation.

CHAPTER 4

MICROBIOLOGICAL VALIDATION OF A CONTINUOUS FLOW INDUSTRIAL MICROWAVE SYSTEM

1. ABSTRACT

Microbiological validation of a pilot scale 100 kW microwave system was attempted utilizing immobilized spore beads of *Geobacillus stearothermophilus* placed in prefabricated cube shaped particles made of polymethylpentene, which has been shown to heat more conservatively than food particles made from various vegetables. Prefabricated particles contained magnets so their movement throughout the system could be tracked and residence times were calculated. The prefabricated particles were inserted into a stream of orange-fleshed sweetpotato puree utilized as the carrier fluid and subjected to microwave application. At the end of the process they were collected and immobilized spore beads were enumerated to determine surviving populations. Magnetic tracking showed that each particle was accounted for as it moved throughout the system unobstructed and spent approximately 39 sec in each applicator section and 78 sec in the hold tube. Hold tube exit temperatures ranged from 96.9-129.9 °C due to variable microwave power, which resulted in a variation of surviving spore populations. Based on the success of achieving free particle flow and utilization of immobilized spore beads as bioindicators, this study shows promise in achieving microbiological validation for a continuous flow microwave system.

2. INTRODUCTION

Continuous flow microwave heating is associated with improved color, flavor, texture, and nutrient retention of homogeneous food products like milk and fruit and vegetable purees (Kumar and others 2007; Coronel and others 2005; Steed and others 2008). A study on salsa con queso determined that microwave heating was a feasible aseptic process for this kind of low-acid multiphase food product, and may be applicable to other foodstuffs with particulates. However, the food particulates in salsa con queso are small (approximately 1 cm at widest point) and it is not necessary to maintain particulate integrity (Kumar and others 2007).

For future applications, like aseptic processing of soups, larger food particles will be necessary. In Chapter 3 it was shown that 9.52 mm sweetpotato cubes given a 2-step pretreatment were firm enough to survive continuous flow microwave processing in a 100 kW system. But, in the United States processes for multiphase foods containing particulates larger than 3.2 mm must be validated before a product can be marketed commercially (Kumar and others 2007; Jasrotia and others 2008). The FDA requires the processors to demonstrate by means of experiments and mathematical modeling that every portion of the food product receives adequate heat treatment to ensure commercial sterility (Kumar and others 2007). In aseptic processing, validation presents a unique challenge because it is necessary to obtain accurate time-temperature history at the center of food particles traveling through the system. At this time, such measurements are not practical without restricting the

free movement of food particles. Therefore, microbiological validation of the process is necessary in order to prove commercial sterility (Jasrotia and others 2008).

In low-acid foods commercial sterility is defined as a 12D inactivation of *Clostridium botulinum*, which is considered the most important spore-forming pathogen because it produces a potent neurotoxin with an LD_{50} of 20-50 ng. Since spores are much more resistant to heat than their vegetative counterparts, it is imperative from a public health standpoint to guarantee that a process eradicates all threat of *C. botulinum* from occurring. The D-value is the time required at a given temperature to produce a 1 log or 90% reduction in the target bacterial population. *C. botulinum* D-values at 121.1 °C can range from 0.05-0.22 min, depending on the strain. This means that a 12D inactivation is about 2.64 min, but it is a common practice in industry to round this up to 3.0 min, since an overestimation will only guarantee further that all *C. botulinum* has been eliminated (Brown 2000).

Due to the inherent risk of working directly with *C. botulinum,* it is a common practice to utilize other microorganisms as bioindicators. Sweetpotato puree processed by steam flash sterilization and aseptic filling was validated using inoculated packs of *Clostridium sporogenes, Bacillus subtilis,* and *Geobacillus stearothermophilus* (Smith and Kopelman 1982). Bioindicators of 0.1 ml spore suspension of *G. stearothermophilus* ($1.8x10^6$) or *B. subtilis* ($4.6x10^6$) enclosed in polypropylene tubing produced by SGM Biotech Inc. (Bozeman MT USA) were tested in microwave processed sweetpotato puree at under-target (126 °C), target (132 °C) and over-target (138 °C) conditions. Within the microwave system

bioindicators got caught in mixers and easily adhered to the sides of pipes, which led to overall low recovery. In addition, the inability to guarantee normal, uninhibited flow of the bioindicators within the system commonly led to over or under-estimation of inactivation (Brinley and others 2007).

Validation of a continuous microwave system was also attempted utilizing immobilized spore beads of *B. subtilis* and *G. stearothermophilus* as biological indicators for a multiphase food product. The immobilized beads were placed in the inner cavity of polymethylpentene cubes with an outer diameter of 1.3 mm proven to heat more conservatively than food products (Jasrotia and others 2008). Cubes were inserted into a 60 kW continuous flow microwave system utilizing salsa con queso or CMC as a carrier fluid and processed at 128, 132, and 138 °C. Again, mechanical problems prevailed and particles clogged in the system or became stuck in the hold tube, which resulted in an overestimation of microbiological inactivation (Stam 2008).

This study utilized a 100 kW microwave system (UltrAseptics, Raleigh, NC, USA) that was designed to accommodate particulate processing in order to achieve unobstructed particulate flow and established a method for microbiological validation of aseptic processes.

3. MATERIALS AND METHODS

Geobacillus stearothermophilus spores and immobilization

Geobacillus stearothermophilus spore stock was purchased from NAMSA (Northwood, OH, USA) at a concentration of 1.8 x 10^6/0.1 ml and kept refrigerated at 4 °C until use. This spore stock came with a reported D-value at 121.1 °C in steam of 2.1 min. Spores were immobilized by mixing in a 1:1 ratio with a 3% sodium alginate (Fluka, Switzerland) solution. The sodium alginate solution was prepared by constant vigorous mixing for 1-2 h over low heat and followed by autoclaving at 121 °C for 20 min. The mixture of spores and alginate was pipetted using a Finnpipette repeater (Thermo Fisher Scientific, Waltham, MA, USA) outfitted with a 500 µl tip and set to dispense 20 µl volumes into filter sterilized 100 mM $CaCl_2$. Beads were removed from the $CaCl_2$ solution and kept in sterile dH_2O at 4 °C until use.

Consistency of *G. stearothermophilus* spore population throughout process of bead formation

An experiment was conducted to assess the spore population throughout the process of immobilized bead formation. Samples of spore stock, spore stock mixed 1:1 with sodium alginate, equivalent volumes of 20 µl beads not formed in $CaCl_2$, and immobilized spore beads were serially diluted and enumerated by pour plating with BHI agar. Plates were inverted and incubated at 55 °C for 48 h.

Simulated particle microbiological validation

Simulated food particles were developed and fabricated at North Carolina State University (Raleigh, NC, USA). The fabricated particles were made of polymethylpenetene (PMP) in the shape of a cube with a 1.3 mm outer dimensions and a cylindrical inner cavity capable of holding a 1 ml volume. Two NdFeB-based magnets (0.1 grams each) were placed in each particle to allow for residence time tracking throughout the microwave system. Three immobilized spore beads of *G. stearothermophilus* were added to the inner cavity of the particle along with 100 µl of sterile dH_2O. The lids were pressed on the particles to seal them and a code was etched into the top of the particle so it could be identified after processing.

Particles were collected after the microwave run and kept at 4 °C until enumeration. When they were opened, the spore beads were removed and placed in a 1:10 dilution of filter sterilized 50 mM sodium citrate for 30 min with continuous vortexing. Serial dilutions were completed in peptone water and samples were pour plated in duplicate in BHI agar. Plates were inverted and incubated at 55 °C for 48 h.

100 kW microwave processing

A pilot scale 100 kW microwave heating unit (UltrAseptics, Raleigh, NC, USA) operating at 915 MHz was used for continuous flow thermal processing (Chapter 3). The carrier fluid for the experiments was microwave processed and aseptically packaged orange-fleshed sweetpotato puree donated by Yamco LLC (Snow Hill, NC, USA). It was loaded into the hopper and pumped through the microwave unit using a dual-piston positive displacement pump (Model A7000, Marlen Research Corp,

Overland Park, KS, USA) at a flow rate of 5.7 L/min. The puree was pre-heated to 40-45 °C by running hot water (85 °C) through the cooling section which acted as a tubular heat exchanger. Then, the microwave generator was turned on and the target temperature of the puree at the hold tube exit was maintained at 121 °C by adjusting the microwave power from 30-40 kW. Temperature was measured throughout the system at noted locations (Chapter 3) every 2 sec by the in-line type T thermocouples connected to a computer-based data acquisition system (TempScan v.4, TempScan/1100, IOTech, Cleveland, OH, USA).

Prefabricated particles were incorporated into the puree flow by closing valves on the main line, which diverted the flow of the puree to the injection port and carried the particles to the applicator section. Magnets were tracked as they moved in and out of each section using an 8 channel Particle Flow Monitoring System equipped with 8 magnetic sensors per channel (ThermaLytics, Raleigh, NC, USA). Magnetic signals from the prefabricated particles were observed real time for each particle using the ParticleMon software (ThermaLytics, Raleigh, NC, USA). Prefabricated particles were inserted only after the previous particle had shown magnetic signals entering and exiting the first applicator section. Later, the recorded magnetic signals could be analyzed to determine residence time and approximate time/temperature history of the particles. Three groups of 35 particles were inserted into the system, with a 10-15 min break in between each set.

Microwaves were generated and delivered to the puree and pre-fabricated cubes by an ess-shaped aluminum waveguide that ran parallel to 3 microwave

applicators. In the applicator sections, puree and particles flowed in microwave transparent Teflon borosilica glass tubes and absorbed microwave energy. The puree was cooled using a tubular heat exchanger with chilled water (4 °C) as the cooling medium. Particle collection was achieved using a pressurized tank outfitted with a course stainless steel sieve, which allowed the puree to flow through but retained the prefabricated cubes. After particle collection, prefabricated cubes were kept at 4 °C until enumeration.

4. RESULTS AND DISCUSSION

Consistency of *G. stearothermophilus* spore population throughout process of bead formation

The process of immobilizing the spore stock into beads was evaluated to assure that spore populations remained constant throughout the process and results are reported in Table 1. There was a significant decrease in the spore population after mixing 1:1 with alginate from 2.92×10^7 to 1.48×10^7 CFU/ml. This was expected based on the fact that mixing 1:1 is a dilution and is reflected by a significant decrease in log (CFU/ml) shown in Table 1. However, this is in disagreement with previous findings by Stam who had no significant changes in spore population (2008). This is most likely due to the fact that her bead equivalent reported a higher spore population, which probably played a role in the statistics. The rest of the process of creating immobilized spore beads causes no significant changes to the spore populations, which remain consistent. Immobilization recovered 99.7% of the

spore population present when an equivalent amount of the 1:1 alginate mixture was plated, demonstrating that immobilizing *G. stearothermophilus* spores is an effective way to utilize them in process validation (Stam 2008; Serp and others 2002).

Microwave processing of prefabricated particles

All 105 particles inserted were accounted for based on the analysis of the recorded magnetic signals in ParticleMon software and trigger times for each section are shown in Appendix II. For the most part movement was consistent for all particles and reflected by small standard deviations. Average residence times were 39±1, 39±1 and 38±4 sec for applicator sections 1, 2 and 3, respectively (Table 2). In between applicator 2 and 3 was the only time that residence times appeared inconsistent and were 49±15 sec. Machinated particles are not completely identical and small differences in density can cause large differences in flow behavior. Residence times in this section for particles 83-100 double from about 42 sec to 1:20 sec. However, this anomaly appears to be a result of the monitoring system since this same group of particles reversed channels 7 and 8 for the reported trigger times, which resulted in negative residence times for the hold tube. When these channels were switched back for calculations the average residence time for the hold tube was 78±1 sec, but the discrepancy remained for the area in between applicator 2 and 3. While there is heating happening in all of these sections, from a validation standpoint only the accumulated heat treatment in the hold tube is considered, and in this section particle movement was consistent.

Hold tube exit temperatures ranged from 96.9-127.9 °C for the first set of inserted particles (Table 3). A temperature range this great has been seen before with the microwave system and was believed to be due to mechanical issues (Chapter 3). However, in this experiment, tracking the microwave power input showed a large variation from 28.7-38.7 kW (Figure 1). Initially power increased, which is reflected in steadily rising temperatures at the hold tube exit temperatures over the course of the first particle set. Microwave power for the rest of the time particles were inserted was mostly level, but did have a few dips. The temperature ranges for particle sets 2 and 3 were less varied than for particle set 1 and average temperatures for both sets was approximately 123 °C (Figure 1, Table 3). Since dips in power did not last for extended periods of time, this is probably why the temperature variation is not as drastic for last two particle sets. The variation in generated power could be due to several factors. Since operating between 25 and 35 kW is on the low end of the capacity of the generator there is more variability in the power output. In subsequent runs on the same system, when power was kept between 40 and 50 kW, the power output was less variable (data not shown). In previous runs on a 60 kW system, power was kept between 25 and 30 kW and there was little observed variation of hold tube exit temperature (Steed and others 2008). A second possibility is that the magnetron and/or microwave generator could be failing due to age and level of use.

Microbiological data

Figures 2-4 show the results from immobilized spore beads enumerated after microwave processing. Any gaps in particle insertion times are due to samples that were lost in the enumeration process. For all particle sets the range of log inactivation ranged from 0.20-2.05. The first particle set was inserted while the microwave system was increasing in temperature as previously discussed. This is illustrated in Figure 2, and log inactivation values also have a loose positive correlation to this rise in temperature and present higher values towards the end of the insertion time. Particle sets 2 and 3 had more stable temperatures, and log inactivation values for particle set 3 are the most consistent, but still cover a range from 0.20-1.76. This could be a result of exposure to higher temperatures in the heating sections of the microwave. Not all particles will flow in the center of the pipe and temperatures at the wall are higher, especially after the first heating section where they can be as high as 130 °C. Due to the ess shaped heating section, there is less variation between the temperatures at the wall and center at the exit of applicator 3 and the start of the hold tube, because of mixing. Since all particles in set 2 and 3 were exposed to consistent temperatures in the hold tube, the range in log inactivation represents the inherent variation that will result from particles moving through the system differently. These trends are illustrated in the comparison of the log inactivation values for the three particle sets shown in Figure 5. Also, this visualization of the data shows that particle set 3 has the lowest log inactivation values when compared to the other two particle sets.

No particle showed complete microbial inactivation and log reduction values were relatively low when compared to previous validation studies. Brinley and others (2007) reported complete inactivation as low as the detection limit for all bioindicators of *B. subtilis* and *G. stearothermophilus* at target and over-target processing temperatures. Another study utilizing prefabricated cubes and had only 3 of 30 particles present any surviving spore populations (Stam 2008). In both of these studies the high level of inactivation was proposed to be a result of particles getting stuck in sections of the microwave system where they received continuous heating (Brinley and others 2007, Stam and others 2008). However, in this study the magnetic residence time data shows that particles free-flowed through the system and there was no evidence of clogs. Also, in this experiment the carrier fluid temperature only went as high as 129.9 °C. But in previous validation studies, temperature of the carrier fluid was kept at 126 °C for an under-target process, 132 °C for a target process and 138 °C for an over-target process, which could allow for a greater inactivation.

5. CONCLUSIONS

This study established a feasible method for microbiologically validating a continuous flow aseptic processing where time-temperature history is difficult to obtain. Immobilized spore beads were found to maintain spore populations after mixing with alginate and had nearly 100% recovery after bead formation. Prefabricated particles were proven to move through a continuous flow microwave

system unobstructed based on magnetic tracking data, and spore beads were recovered and enumerated. This study laid a foundation for future validation experiments on multiphase foods that could be filed with the FDA, like Tetra Pak Inc. did for diced potato soup.

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TABLE 1: Consistency (logCFU/ml)[1] of Alginate-Immobilized beads of *G. stearothermophilus*

Spore Stock	1:1 Alginate Mixture	Bead Equivalent	Immobilized Bead	% Recovery
7.45±0.08[a]	7.17±0.03[b]	7.17±0.08[b]	7.12±0.04[b]	99.7

[1] – Values reported are the means ± the standard deviation of 5 replicates. Different letters across the row denote significance ($p<0.05$).

TABLE 2: Average Residence Times (in sec)[1] of Prefabricated Particles in Different Sections of 100 kW Microwave System

App 1*	Between App 1 & 2	App 2	Between App 2 & 3	App 3	Hold Tube	Cooling Section
39±1	29±1	39±1	49±15	38±4	78±1	14:22#± 3:55

[1] – Values reported are the means ± the standard deviation (n=105).
* - "App" is short for Applicator and refers to a microwave application section.
# - The cooling section is much longer than other sections, the residence time is in min:sec.

TABLE 3: Temperatures at Hold Tube Exit during Processing of Three Particle Sets

Particle Set	Temperature Range at Hold Tube Exit (°C)	Average Temperature at Hold Tube Exit (°C)
1	96.9-127.9	115.9± 6.4
2	109.9-129.9	123.4±3.1
3	112.6-129.0	123.3±2.4

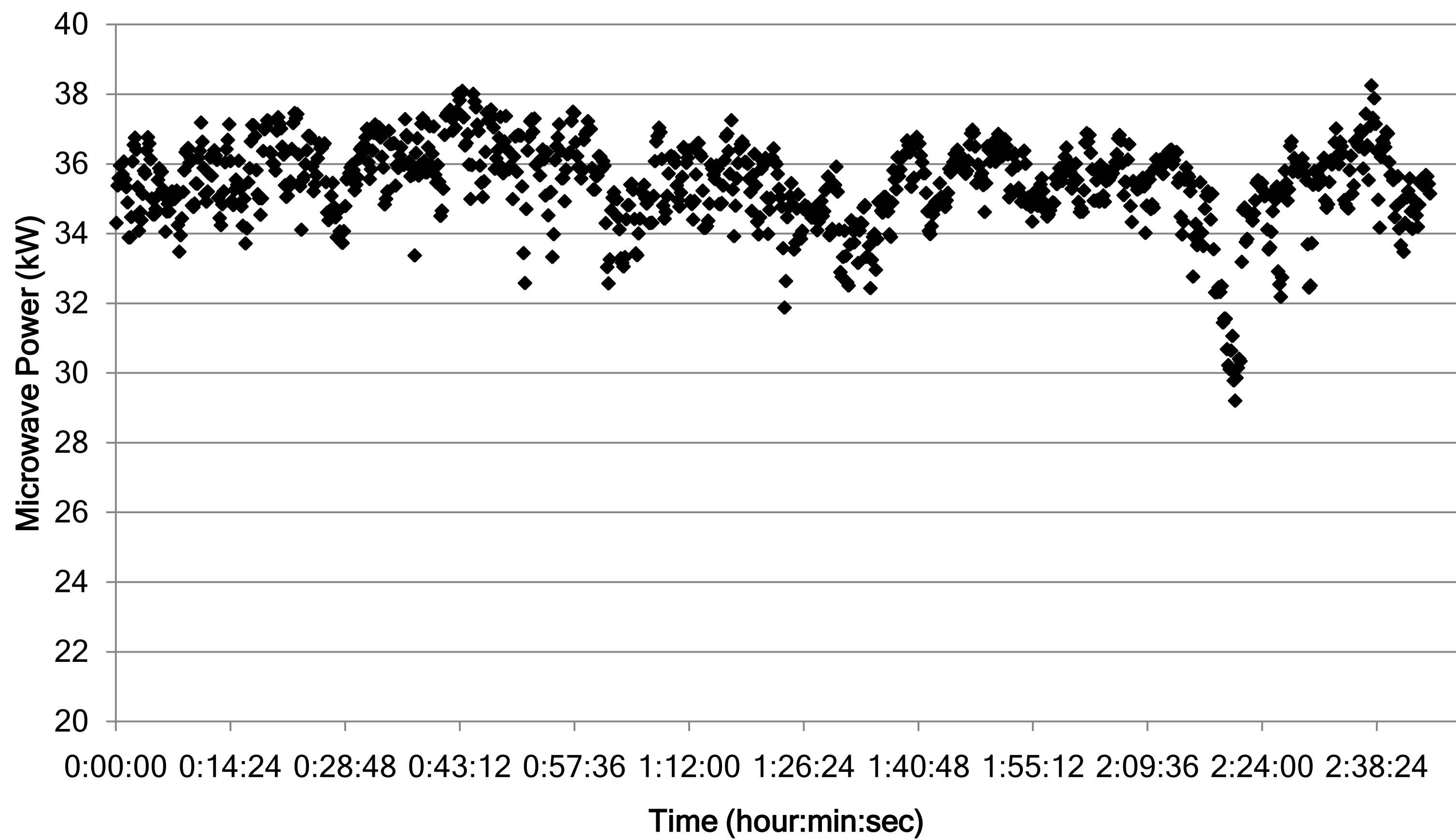

FIGURE 1: Microwave Power Fluctuation Throughout Particle Insertion

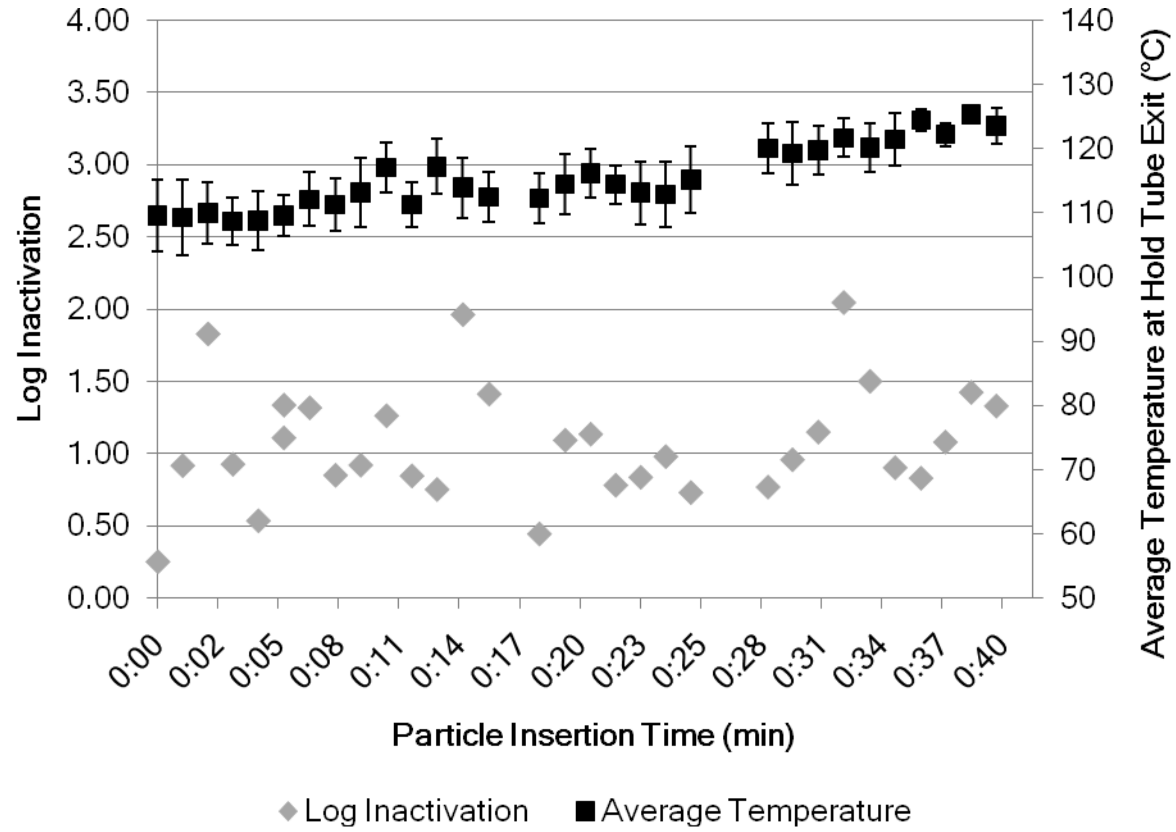

FIGURE 2: Log Inactivation and Average Temperature at Hold Tube Exit (°C) for First Set of Particles

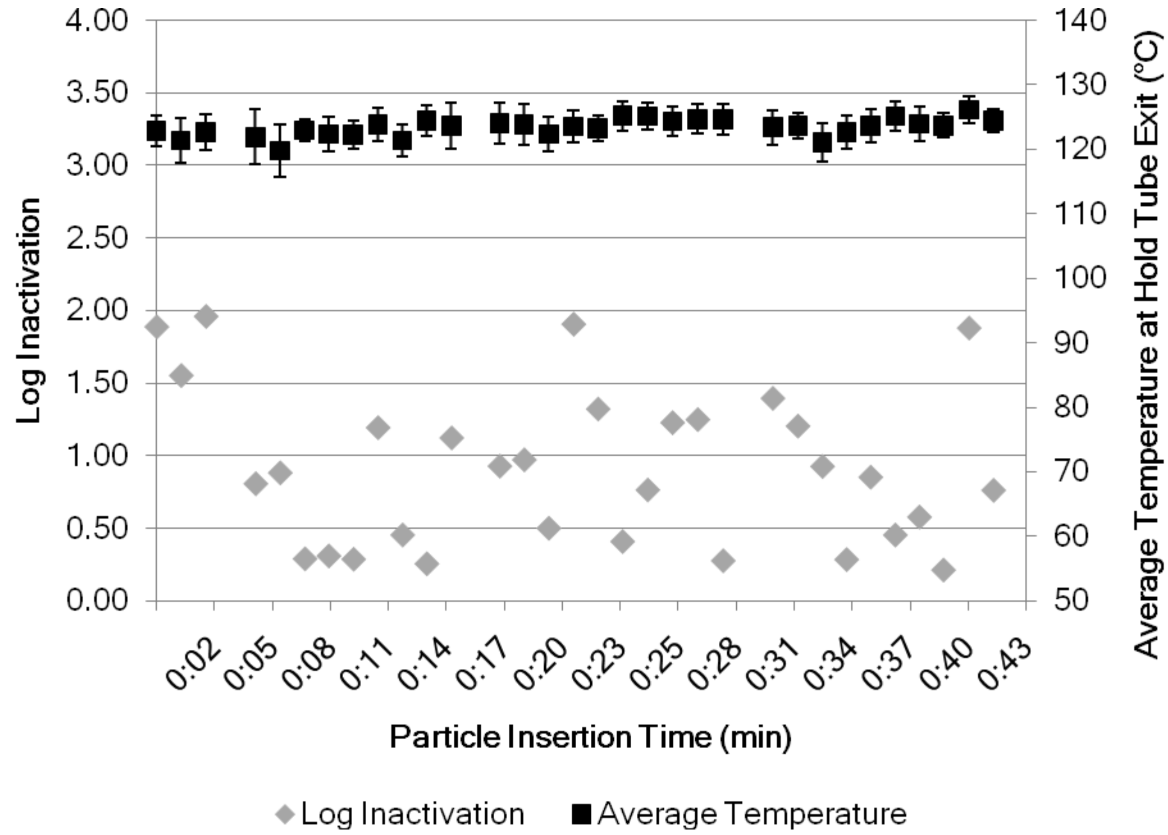

FIGURE 3: Log Inactivation and Average Temperature at Hold Tube Exit (°C) for Second Set of Particles

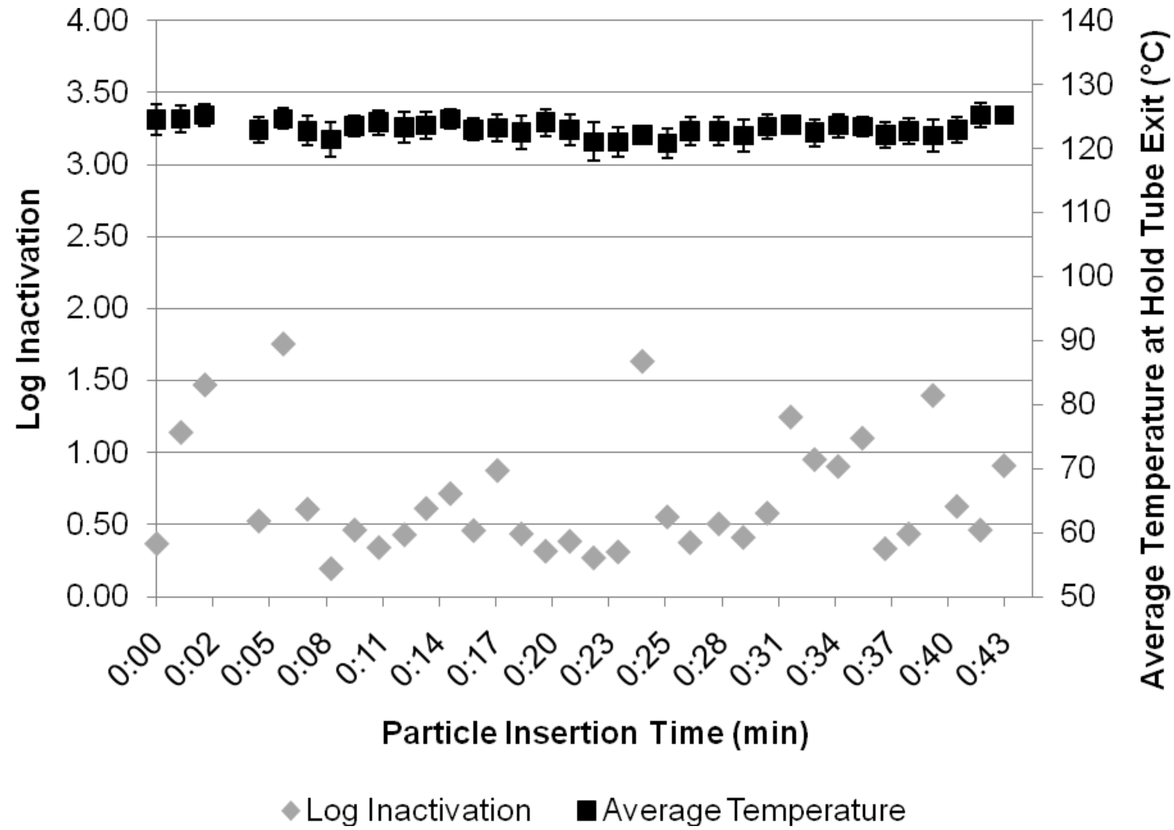

FIGURE 4: Log Inactivation and Average Temperature at Hold Tube Exit (°C) for Third Set of Particles

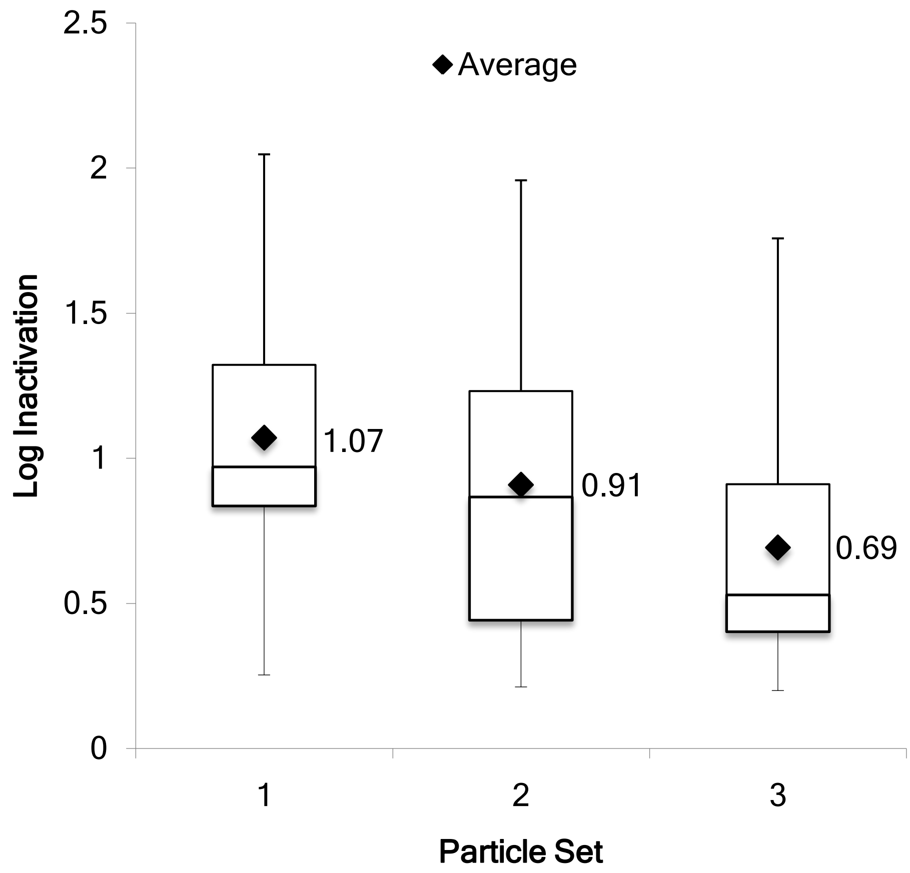

FIGURE 5: Comparison of Log Inactivation Data for Particle Sets

APPENDICIES

APPENDIX I

DIELECTRIC PROPERTIES FOR SWEETPOTATOES AT 2450 MHZ

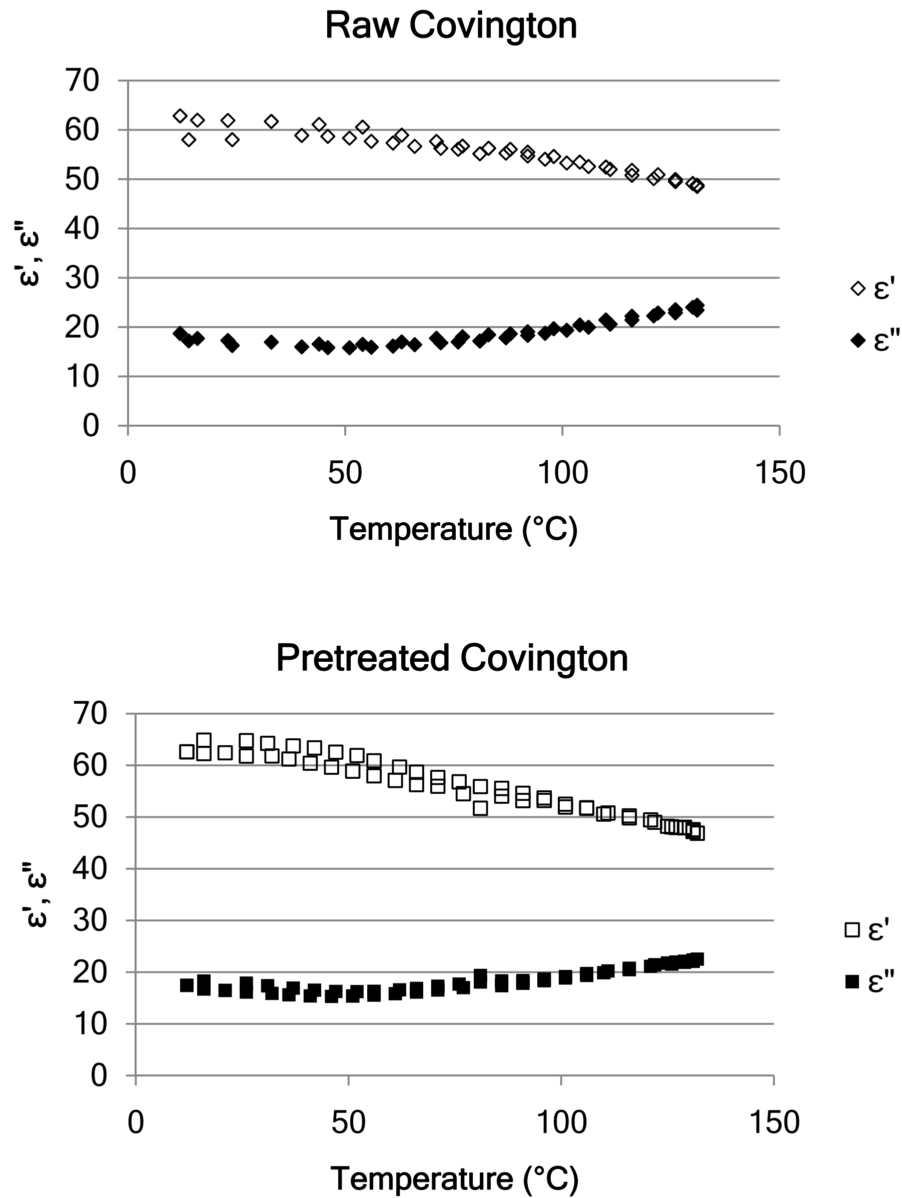

FIGURE 1: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 2450 MHz for Raw and Pretreated Covington Samples From Two Batches

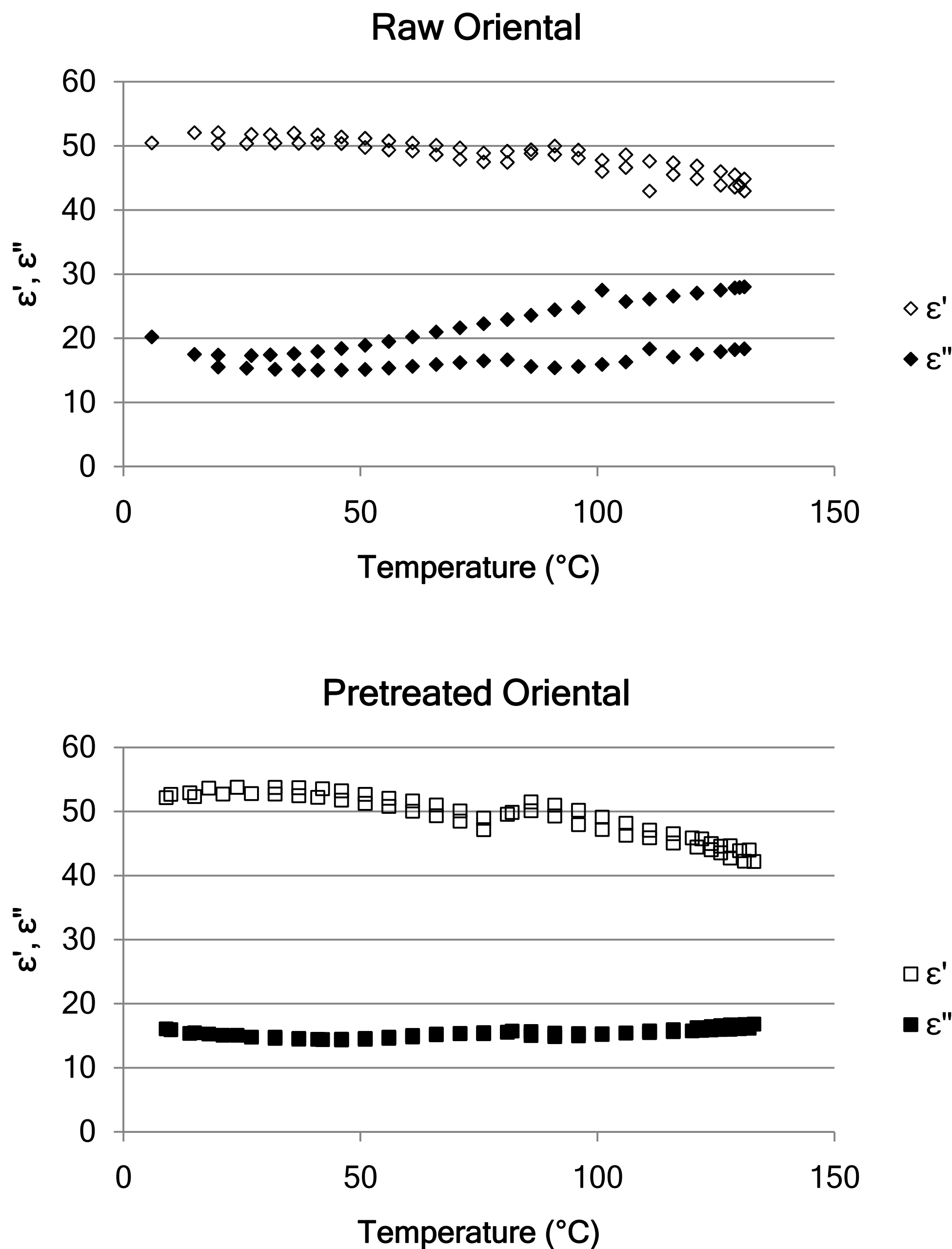

FIGURE 2: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 2450 MHz for Raw and Pretreated Oriental Samples From Two Batches

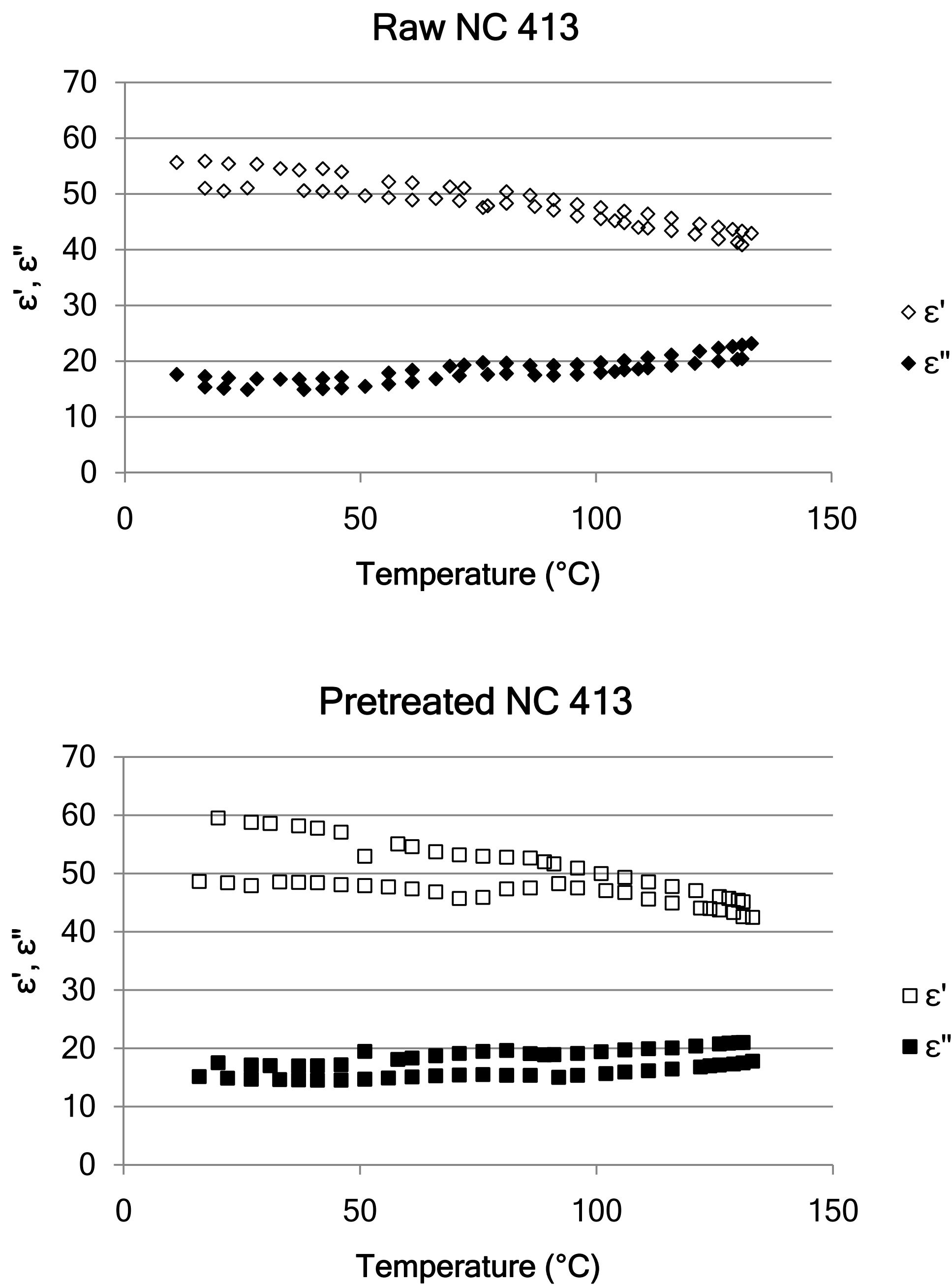

FIGURE 3: Dielectric Constants (open symbols) and Dielectric Loss Factors (closed symbols) Measured at 2450 MHz for Raw and Pretreated NC 413 Samples From Two Batches

APPENDIX II

TRIGGER TIMES FOR PARTICLES AS THEY MOVE THROUGH THE 100 kW MICROWAVE SYSTEM

Particle	App 1 In[1]	App 1 Out	App 2 In	App 2 Out	App 3 In	App 3 Out	Hold Tube Out	Cool Out
1	11:44:18	11:44:58	11:45:26	11:46:05	11:46:47	11:47:27	11:48:43	12:00:24
2	11:45:31	11:46:09	11:46:39	11:47:17	11:48:00	11:48:38	11:49:59	12:01:44
3	11:46:44	11:47:21	11:47:50	11:48:29	11:49:11	11:49:51	11:51:10	12:02:56
4	11:47:54	11:48:33	11:49:01	11:49:40	11:50:22	11:51:02	11:52:20	12:03:56
5	11:49:07	11:49:45	11:50:14	11:50:52	11:51:35	11:52:15	11:53:34	12:05:14
6	11:50:20	11:50:58	11:51:28	11:52:06	11:52:48	11:53:28	11:54:45	12:06:19
7	11:51:34	11:52:12	11:52:42	11:53:20	11:54:02	11:54:42	11:55:59	12:07:21
8	11:52:48	11:53:26	11:53:55	11:54:34	11:55:16	11:55:56	11:57:14	12:08:44
9	11:54:01	11:54:39	11:55:08	11:55:47	11:56:29	11:57:08	11:58:25	12:10:06
10	11:55:14	11:55:53	11:56:23	11:57:00	11:57:43	11:58:22	11:59:41	12:11:25
11	11:56:28	11:57:06	11:57:34	11:58:14	11:58:55	11:59:35	12:00:52	12:12:22
12	11:57:40	11:58:19	11:58:46	11:59:26	12:00:08	12:00:47	12:02:06	12:13:25
13	11:58:53	11:59:33	12:00:00	12:00:40	12:01:22	12:02:01	12:03:19	12:15:02
14	12:00:07	12:00:45	12:01:15	12:01:53	12:02:36	12:03:16	12:04:32	12:16:17
15	12:01:19	12:01:58	12:02:26	12:03:06	12:03:48	12:04:28	12:05:44	12:17:31
16	12:02:32	12:03:11	12:03:39	12:04:18	12:05:00	12:05:40	12:06:57	12:18:36
17	12:03:45	12:04:23	12:04:53	12:05:31	12:06:13	12:06:54	12:08:12	12:19:35
18	12:04:58	12:05:37	12:06:06	12:06:45	12:07:27	12:08:07	12:09:24	12:21:13
19	12:06:11	12:06:49	12:07:18	12:07:57	12:08:39	12:09:19	12:10:34	12:22:19
20	12:07:24	12:08:02	12:08:30	12:09:09	12:09:51	12:10:30	12:11:47	12:23:30
21	12:08:37	12:09:14	12:09:42	12:10:21	12:11:03	12:11:42	12:12:59	12:24:14
22	12:09:49	12:10:27	12:10:56	12:11:34	12:12:16	12:12:54	12:14:12	12:25:57
23	12:11:01	12:11:40	12:12:08	12:12:47	12:13:30	12:14:09	12:15:26	12:26:46

Particle	App 1 In	App 1 Out	App 2 In	App 2 Out	App 3 In	App 3 Out	Hold Tube Out	Cool Out
24	12:12:16	12:12:53	12:13:22	12:14:01	12:14:43	12:15:23	12:16:40	12:28:14
25	12:13:27	12:14:06	12:14:35	12:15:13	12:15:54	12:16:35	12:17:53	12:29:14
26	12:14:38	12:15:17	12:15:44	12:16:23	12:17:06	12:17:45	12:19:04	12:30:00
27	12:15:51	12:16:30	12:16:58	12:17:37	12:18:19	12:18:59	12:20:18	12:31:25
28	12:17:04	12:17:42	12:18:10	12:18:50	12:19:32	12:20:12	12:21:30	12:33:00
29	12:18:18	12:18:56	12:19:25	12:20:04	12:20:47	12:21:27	12:22:43	12:34:15
30	12:19:29	12:20:10	12:20:40	12:21:17	12:22:00	12:22:39	12:23:56	12:34:22
31	12:20:44	12:21:22	12:21:51	12:22:29	12:23:10	12:23:53	12:25:09	12:35:10
32	12:21:56	12:22:34	12:23:02	12:23:42	12:24:24	12:25:03	12:26:21	12:37:55
33	12:23:08	12:23:46	12:24:16	12:24:54	12:25:37	12:26:17	12:27:35	12:39:14
34	12:24:20	12:24:58	12:25:27	12:26:07	12:26:49	12:27:29	12:28:47	12:41:27
35	12:25:32	12:26:11	12:26:39	12:27:18	12:28:00	12:28:40	12:29:57	12:52:50
36	12:36:43	12:37:22	12:37:51	12:38:30	12:39:12	12:39:52	12:41:10	12:53:51
37	12:37:57	12:38:35	12:39:04	12:39:43	12:40:25	12:41:05	12:42:24	12:55:13
38	12:39:11	12:39:50	12:40:17	12:40:57	12:41:40	12:42:19	12:43:37	12:56:42
39	12:40:24	12:41:04	12:41:33	12:42:12	12:42:55	12:43:34	12:44:55	12:57:48
40	12:41:38	12:42:18	12:42:45	12:43:25	12:44:07	12:44:47	12:46:06	12:58:48
41	12:42:52	12:43:30	12:43:59	12:44:38	12:45:20	12:46:00	12:47:16	12:59:38
42	12:44:05	12:44:44	12:45:14	12:45:52	12:46:35	12:47:14	12:48:34	13:01:09
43	12:45:18	12:45:57	12:46:26	12:47:04	12:47:45	12:48:25	12:49:43	13:02:38
44	12:46:32	12:47:11	12:47:39	12:48:17	12:49:00	12:49:39	12:50:56	13:04:38
45	12:47:45	12:48:23	12:48:51	12:49:30	12:50:12	12:50:52	12:52:10	13:06:12
46	12:48:58	12:49:36	12:50:05	12:50:44	12:51:25	12:52:05	12:53:23	13:07:29
47	12:50:11	12:50:49	12:51:17	12:51:56	12:52:38	12:53:17	12:54:34	13:08:26
48	12:51:23	12:52:02	12:52:30	12:53:09	12:53:52	12:54:31	12:55:50	13:09:15
49	12:52:37	12:53:14	12:53:44	12:54:23	12:55:04	12:55:44	12:57:02	13:11:03
50	12:53:49	12:54:26	12:54:54	12:55:34	12:56:15	12:56:55	12:58:13	13:12:22

Particle	App 1 In	App 1 Out	App 2 In	App 2 Out	App 3 In	App 3 Out	Hold Tube Out	Cool Out
51	12:55:01	12:55:39	12:56:09	12:56:47	12:57:29	12:58:08	12:59:27	13:12:56
52	12:56:14	12:56:52	12:57:21	12:58:00	12:58:41	12:59:21	13:00:39	13:14:27
53	12:57:27	12:58:06	12:58:35	12:59:13	13:00:27	13:00:35	13:01:53	13:15:58
54	12:58:40	12:59:19	12:59:47	13:00:26	13:01:08	13:01:48	13:03:05	13:17:00
55	12:59:53	13:00:32	13:01:01	13:01:40	13:02:21	13:03:01	13:04:19	13:18:26
56	13:01:08	13:01:47	13:02:15	13:02:54	13:03:36	13:04:16	13:05:35	13:19:36
57	13:02:21	13:03:00	13:03:29	13:04:08	13:04:50	13:05:30	13:06:47	13:22:06
58	13:03:35	13:04:15	13:04:44	13:05:23	13:06:05	13:06:45	13:08:02	13:23:18
59	13:04:52	13:05:32	13:06:01	13:06:39	13:07:21	13:08:01	13:09:19	13:24:34
60	13:06:07	13:06:46	13:07:15	13:07:54	13:08:36	13:09:16	13:10:33	13:25:37
61	13:07:22	13:08:01	13:08:28	13:09:08	13:09:50	13:10:29	13:11:47	13:26:53
62	13:08:35	13:09:14	13:09:42	13:10:21	13:11:04	13:11:44	13:13:04	13:27:52
63	13:09:48	13:10:26	13:10:56	13:11:34	13:12:17	13:12:57	13:14:15	13:29:26
64	13:11:00	13:11:39	13:12:07	13:12:47	13:13:29	13:14:09	13:15:26	13:30:39
65	13:12:13	13:12:52	13:13:20	13:13:59	13:14:42	13:15:21	13:16:38	13:31:43
66	13:13:27	13:14:05	13:14:35	13:15:13	13:15:55	13:16:34	13:17:50	13:32:25
67	13:14:40	13:15:18	13:15:46	13:16:25	13:17:03	13:17:46	13:19:04	13:34:12
68	13:15:53	13:16:31	13:17:00	13:17:38	13:18:19	13:18:58	13:20:16	13:54:03
69	13:17:07	13:17:46	13:18:14	13:18:52	13:19:34	13:20:14	13:21:31	13:55:17
70	13:18:20	13:18:58	13:19:27	13:20:05	13:20:47	13:21:27	13:22:43	13:56:32
71	13:38:16	13:38:55	13:39:24	13:40:02	13:40:44	13:41:24	13:42:40	13:59:02
72	13:39:30	13:40:09	13:40:38	13:41:17	13:41:58	13:42:38	13:43:54	14:00:39
73	13:40:43	13:41:21	13:41:49	13:42:29	13:43:11	13:43:51	13:45:08	14:02:06
74	13:43:29	13:44:07	13:44:35	13:45:14	13:45:56	13:46:36	13:47:53	14:02:44
75	13:44:43	13:45:21	13:45:50	13:46:29	13:47:11	13:47:51	13:49:07	14:04:19
76	13:45:56	13:46:34	13:47:04	13:47:43	13:48:25	13:49:05	13:50:21	14:05:37
77	13:47:09	13:47:48	13:48:17	13:48:55	13:49:37	13:50:17	13:51:35	14:06:14

Particle	App 1 In	App 1 Out	App 2 In	App 2 Out	App 3 In	App 3 Out	Hold Tube Out	Cool Out
78	13:48:23	13:49:01	13:49:29	13:50:08	13:50:50	13:51:29	13:52:48	14:07:50
79	13:49:36	13:50:15	13:50:43	13:51:22	13:52:03	13:52:43	13:54:00	14:08:35
80	13:50:49	13:51:27	13:51:57	13:52:38	13:53:22	13:54:01	13:55:19	14:10:29
81	13:52:02	13:52:40	13:53:08	13:53:47	13:54:29	13:55:08	13:56:27	14:11:36
82	13:53:13	13:53:52	13:54:19	13:54:59	13:55:42	13:56:22	13:57:39	14:12:08
83	13:54:26	13:55:04	13:55:32	13:56:12	13:57:33	13:58:06	13:58:50	14:14:00
84	13:55:38	13:56:17	13:56:46	13:57:24	13:58:46	13:59:20	14:00:04	14:15:24
85	13:56:50	13:57:30	13:57:58	13:58:37	13:59:59	14:00:33	14:01:16	14:16:09
86	13:58:04	13:58:43	13:59:12	13:59:51	14:01:13	14:01:48	14:02:31	14:17:44
87	13:59:18	13:59:58	14:00:27	14:01:06	14:02:27	14:03:02	14:03:44	14:18:44
88	14:00:33	14:01:14	14:01:42	14:02:20	14:03:40	14:04:16	14:04:59	14:19:46
89	14:01:48	14:02:27	14:02:56	14:03:34	14:04:55	14:05:29	14:06:14	14:21:35
90	14:03:01	14:03:40	14:04:09	14:04:47	14:06:09	14:06:43	14:07:26	14:21:44
91	14:04:13	14:04:54	14:05:23	14:06:02	14:07:25	14:07:55	14:08:43	14:22:41
92	14:05:27	14:06:07	14:06:34	14:07:13	14:08:35	14:09:24	14:09:53	14:24:07
93	14:06:56	14:07:35	14:08:02	14:08:41	14:10:03	14:10:38	14:11:21	14:26:30
94	14:08:10	14:08:48	14:09:17	14:09:57	14:11:18	14:11:52	14:12:34	14:27:37
95	14:09:22	14:10:02	14:10:31	14:11:09	14:12:31	14:13:03	14:13:48	14:29:18
96	14:10:36	14:11:15	14:11:43	14:12:22	14:13:43	14:14:17	14:15:01	14:30:12
97	14:11:49	14:12:28	14:12:56	14:13:34	14:14:56	14:15:30	14:16:11	14:30:32
98	14:13:02	14:13:41	14:14:10	14:14:48	14:16:08	14:16:41	14:17:24	14:32:33
99	14:14:16	14:14:53	14:15:20	14:15:59	14:17:22	14:17:51	14:18:39	14:32:53
100	14:15:26	14:16:04	14:16:33	14:17:11	14:18:32	14:18:58	14:19:49	14:35:30
101	14:16:37	14:17:15	14:17:43	14:18:22	14:19:04	14:19:44	14:21:02	14:36:34
102	14:17:49	14:18:27	14:18:56	14:19:35	14:20:18	14:20:58	14:22:15	14:37:56
103	14:19:02	14:19:41	14:20:11	14:20:49	14:21:31	14:22:11	14:23:31	*
104	14:20:15	14:20:54	14:21:23	14:22:01	14:22:43	14:23:23	14:24:42	*

Particle	App 1 In	App 1 Out	App 2 In	App 2 Out	App 3 In	App 3 Out	Hold Tube Out	Cool Out
105	14:21:28	14:22:07	14:22:35	14:23:14	14:23:56	14:24:35	14:25:55	*

[1] – "App" refers to the 3 microwave applicator sections.
* - The last three particles did not have clear trigger times for their exit from the cooling section.

APPENDIX III

PLATE COUNT DATA FOR ALL RECOVERED IMMOBILIZED SPORE BEADS

Set A		
Particle	Plate Count	Log Reduction Value[1]
Positive Control	1.46E+07	-
Positive Control	1.53E+07	-
Average	1.49E+07	
1	8.25E+06	0.25
2	1.79E+06	0.92
3	2.19E+05	1.83
4	1.75E+06	0.93
5	4.30E+06	0.54
6	6.80E+05	1.34
6B*	1.15E+06	1.11
7	7.10E+05	1.32
8	2.08E+06	0.85
9	1.77E+06	0.92
10	8.05E+05	1.26
11	2.10E+06	0.85
12	2.61E+06	0.75
13	1.61E+05	1.96
14	5.70E+05	1.41
16^	5.30E+06	0.45
17	1.19E+06	1.09
18	1.08E+06	1.14
19	2.44E+06	0.78
20	2.15E+06	0.84
21	1.55E+06	0.98
22	2.74E+06	0.73
25	2.51E+06	0.77
26	1.63E+06	0.96
27	1.05E+06	1.15
28	1.33E+05	2.05
29	4.65E+05	1.50
30	1.85E+06	0.90
31	2.18E+06	0.83

Particle	Plate Count	Log Reduction Value
32	1.23E+06	1.08
33	5.55E+05	1.43
34	6.90E+05	1.33

Set B		
Particle	Plate Count	Log Reduction Value
Positive Control	1.59E+07	-
Positive Control	1.52E+07	-
Average	1.56E+07	
1	2.01E+05	1.89
2	4.35E+05	1.55
3	1.71E+05	1.96
5	2.42E+06	0.81
6	2.04E+06	0.88
7	7.95E+06	0.29
8	7.60E+06	0.31
9	8.00E+06	0.29
10	9.95E+05	1.19
11	5.45E+06	0.45
12	8.60E+06	0.26
13	1.17E+06	1.12
15	1.83E+06	0.93
16	1.66E+06	0.97
17	4.90E+06	0.50
18	1.93E+05	1.90
19	7.40E+05	1.32
20	6.05E+06	0.41
21	2.67E+06	0.76
22	9.20E+05	1.23
23	8.75E+05	1.25
24	8.20E+06	0.28
26	6.25E+05	1.39
27	9.70E+05	1.20
28	1.84E+06	0.93
29	8.05E+06	0.28
30	2.18E+06	0.85

Particle	Plate Count	Log Reduction Value
31	5.45E+06	0.45
32	4.10E+06	0.58
33	9.50E+06	0.21
34	2.05E+05	1.88
35	2.69E+06	0.76

Set C		
Particle	Plate Count	Log Reduction Value
Positive Control	1.90E+07	-
Positive Control	1.86E+07	-
Average	1.88E+07	
1	7.90E+06	0.37
2	1.34E+06	1.14
3	6.25E+05	1.47
4	5.50E+06	0.53
5	3.25E+05	1.76
6	4.55E+06	0.61
7	1.18E+07	0.20
8	6.35E+06	0.47
9	8.40E+06	0.35
10	6.85E+06	0.43
11	4.50E+06	0.62
12	3.55E+06	0.72
13	6.40E+06	0.46
14	2.46E+06	0.88
15	6.75E+06	0.44
16	8.90E+06	0.32
17	7.60E+06	0.39
18	9.90E+06	0.27
19	9.00E+06	0.32
20	4.30E+05	1.64
21	5.15E+06	0.56
22	7.75E+06	0.38
23	5.75E+06	0.51
24	7.15E+06	0.42
25	4.85E+06	0.58

Particle	Plate Count	Log Reduction Value
26	1.05E+06	1.25
27	2.06E+06	0.96
28	2.30E+06	0.91
29	1.47E+06	1.10
30	8.55E+06	0.34
31	6.75E+06	0.44
32	7.40E+05	1.40
33	4.35E+06	0.63
34	6.35E+06	0.47
35	2.27E+06	0.91

[1] – Log Reduction Value calculated using the average of the positive controls as starting population.
[*] - Sample 6 was found to have 6 immobilized spore beads instead of three, so these were divided into groups of 3 and analyzed, resulting in 6B.
[^] – Skipped numbers indicate lost samples. In the case of sample A23, immobilized spore beads were accidentally left out of the particle. A15, A24, A35, B4, B14, and B25 were lost due to the incubator completely drying out plates so that they were uncountable.