ABSTRACT

STEED, LAURIE ELAINE. Nutraceutical and Rheological Properties of Purple-Fleshed Sweetpotato Purees as Affected by Continuous Flow Microwave-Assisted Aseptic Processing. (Under the direction of Dr. Van-Den Truong.)

Antioxidants play a key role in protecting the body from damage to lipid membranes, proteins, DNA, and carbohydrates by quenching reactive oxygen species. Recently, foods found to be sources of high antioxidant contents are gaining popularity with consumers. Purple-fleshed sweetpotatoes have the highest antioxidant activity compared to sweetpotatoes with other flesh colors. The deep purple color of these sweetpotatoes is due to an accumulation of mono- and di-acylated anthocyanins, which contributes to high antioxidant capacity. In Asian countries, such as Japan and Korea, purple-fleshed sweetpotatoes have found market success for use as a natural food colorant or functional food ingredient. In contrast, the United States predominantly utilizes orange-fleshed cultivars. However, the sweetpotato industry is beginning to investigate purple-fleshed sweetpotato cultivars as a way to increase consumer awareness of the health benefits of eating sweetpotatoes and consequently expand the markets.

The nutraceutical contents of flesh and whole roots of a North Carolina grown purple-fleshed sweetpotato cultivar were analyzed. Total phenolic content of the flesh and whole roots were between 401.6 and 469.9 mg CAE/100 g fw, and anthocyanin content ranged from 80.2-107.8 mg/100 g fw. The DPPH radical scavenging activities were 75.5 to 79.8 μmol TE/g fw, and the oxygen radical absorbance capacity (ORAC) values fell between 53.1-60.0 μmol TE/g fw. The results indicated that the nutraceutical properties of the purple-fleshed

sweetpotatoes were within the spectrum of fruits and vegetables known as high antioxidant food commodities.

Purple-fleshed sweetpotatoes have higher dry matter contents than orange-fleshed sweetpotatoes and form a thick, paste-like material when thermally processed. In this highly viscous form, purple-fleshed sweetpotatoes have limited processing opportunities. Adding water to the purple-fleshed sweetpotatoes to adjust the dry matter content to 18% was found to reduce the viscosity and make a flowable puree from storage roots. Purple-fleshed sweetpotato puree decreased in viscosity with an increase in temperature and exhibited non-newtonian, pseudoplastic behavior with a yield stress that fit the Herschel-Bulkley model. As a puree, purple-fleshed sweetpotatoes have more potential in the food processing industry because purees are the intermediate form for most processed products including soups, patties, dehydrated flakes, beverages, baby foods, and other value-added products.

Orange-fleshed sweetpotato purees are commercially available in frozen or canned forms. Frozen purees have high quality but require a large investment of capital and space for storage and transportation. Furthermore, thawing processes for purees are non-uniform and time consuming. Canned purees have the advantage of being shelf stable, but required retort schedules lead to overprocessing of the puree, degrades nutritional components and color, and causes off-flavors in the finished product. These shortcomings led to the exploration of other thermal processes. A continuous flow microwave system offers an alternative to

conventional thermal process methods and was successfully applied to orange-fleshed sweetpotato purees and other flowable food materials.

It is imperative that processing methods applied to purple-fleshed sweetpotatoes maintain color and antioxidant activity. For this reason continuous microwave application and aseptic processing were evaluated for feasibility in respect to purple-fleshed sweetpotato purees. This processing scheme was successfully applied and the aseptically packaged purple-fleshed sweetpotato puree was shelf-stable and had the same antioxidant activity as fresh purees. These positive results increase the potential of this food commodity to be utilized as a functional food ingredient.

Gel strength (storage modulus, G' and loss modulus, G") of the purple-fleshed sweetpotato purees increased with microwave processing. Depending on the desired food processing application this can be a positive or negative attribute. Future research should focus on the effects of processing treatments on rheological properties of the purple-fleshed sweetpotato purees associated with various applications of this functional food ingredient in the food industry.

NUTRACEUTICAL AND RHEOLOGICAL PROPERTIES OF PURPLE-FLESHED SWEETPOTATO PUREES AS AFFECTED BY CONTINUOUS FLOW MICROWAVE-ASSISTED ASEPTIC PROCESSING

by

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Chair of Advisory Committee

DEDICATION

For my Mom and Dad, who love me unconditionally and constantly support me in every adventure.

BIOGRAPHY

Laurie Elaine Steed was born in Wilmington, NC on February 12, 1983 to Lawrence and Sandra Steed. Two of her happiest days as a child were marked by the births of her younger brothers; Aaron was born on April 13, 1987 and Trevor was born on January 23, 1992. In May 2001 Laurie graduated from New Hanover High School as a member of the top ten in her graduating class. The following August she moved to Raleigh to pursue an engineering degree at NC State. At the end of her freshman year she matriculated into Chemical Engineering but one short year later, dropped out of the program. Tired of being a number and studying something that didn't interest her, Laurie began shopping for a new major. She was introduced to Food Science by Melody Milroy and Tiffany Brinley, and the rest is history. For her senior design research project, Laurie studied the anti-hyperglycemic components of white-fleshed sweetpotatoes and was introduced to Dr. Truong in the USDA. Due to her performance and leadership in the capstone class, Dr. Truong offered Laurie a graduate school position in his lab to study purple-fleshed sweetpotatoes. A year into her studies he convinced her to consider continuing on with a Ph.D. and it wasn't long before she was committed to a project further investigating microbial validation of the microwave system (and more sweetpotatoes).

After her defense, Laurie plans to take a much deserved break and prepare her first manuscript for publication. At the end of July she will attend IFT in Chicago, IL where her research poster titled: Microwave-assisted aseptic

processing and packaging of purple-fleshed sweetpotato puree for functional foods, is a top ten finalist in the fruit and vegetable products division. Her Ph.D. assistantship begins on June 1, 2007. Laurie will serve as the Vice President for the Food Science Club in the upcoming academic year and has also been named a Provost Fellow. She is constantly excited and amazed by the opportunities the future holds.

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First and foremost I would like to thank my family. They love me and I love them. It's that simple. I would never have made it this far without your love and support. Mom and Dad, your guidance and example have made me the person that I am today. You never wavered in your trust and belief in me, even though I have sometimes doubted myself. I've never felt that I had to prove myself to you, because you already knew I was capable of great things. Every accomplishment I have means more because I can share it with you. Aaron, in the last four years we've built our brother-sister bond into an amazing relationship. I enjoy our conversations about life, birds and most of all music. Every day you amaze me with the person you are becoming and I hope that I find myself in your thesis acknowledgements some day. Trevor, I still can't believe that my baby brother is growing up, nor can I wait to see how you will surprise me next. Your ridiculousness makes me laugh constantly and I wouldn't want it any other way.

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happened had I missed such a wonderful opportunity to become a lifetime member of the sweetpotato club!

Josip, your enthusiasm over the purple sweetpotato is what made bad processing days seemingly turn out okay. I'm holding play dough that refuses to become puree, or praying we don't blow up the 60 kW system and you're yelling "Look at this stuff! This is really exciting stuff!" I might not have admitted it at the time, but I agree. I look forward to our next three years together and expanding my knowledge on the microwave.

K.P., I would like to thank you for this past semester. Despite my test scores, I do feel that I learned a lot from your class and also enjoyed the opportunity to work more closely with you. I appreciate your continued support in exploring processing and publishing opportunities.

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Jaclyn, I can't imagine what this would have been like without having you by my side. I'm so excited about your future sometimes I forget about my own. You've taught me so much in our four years of friendship and I can't wait to plan weddings, shop for baby clothes, and buy houses near each other. Matt and Megan, you're two of the best perks that come from a friendship with Jaclyn.

Along the way we have developed our own strong friendships and I’m truly grateful to have a family with you guys here in Raleigh.

To my foodies; Audrey, Tri, Erika, Wayne, Jeb, Adam, Meagan, Nick and Room 330 - for a bunch of nerds we still manage to have a pretty good time! Thank you for listening to the complaining about all the mistakes I made, and helping to check my freakouts with words of advice.

To all those that provided technical support, thank you to: Sabine, for your help in acquainting me with the lab and answering my random questions; Roger T for explaining procedures and showing me how to work with sweetpotatoes; Sharon R for saving me in the world of rheology and Stresstechs; Jack C and Karl H for pilot plant processing assistance; Gary C and Prabhat for making microwave processing look easy; Suzanne for helping make my seminar successful; Sandy for making paperwork and administrative issues “disappear”.

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CHAPTER 1

LITERATURE REVIEW

1.1 – SWEETPOTATOES

1.1.1 – The Origin and Production of Sweetpotatoes

Sweetpotatoes (*Ipomoea* batatas) originated in Central America and were brought to Western Europe after the first voyage of Columbus in 1492. (Srisuwan et. al, 2006). In the 16th century they were introduced to China and due to their broad adaptability, hardiness and ability to multiply planting material rapidly from few roots, sweetpotatoes spread throughout Asia, Africa and Latin America in the 17th and 18th centuries. Today sweetpotatoes are considered 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 reaching 105 million metric tons in 2004, which accounts for approximately 85% of global production. This is due to the multiple uses of the crop as animal feed and varied processed forms for human consumption, such as noodles, starch and alcohol (FAO, 2005b).

By contrast, the United States only produced 730 thousand metric tons and utilizes sweetpotatoes in fewer ways than Asia (FAO 2005a). Per capita consumption has changed little since 2003 and was 4.5 lbs in 2005. With the exception of California, sweetpotatoes are mostly grown in southern regions. North Carolina is the largest producer of sweetpotatoes and accounts for 40% of the annual US sweetpotato production. In 2005 this equaled 270 thousand metric tons.

1.1.1.1 – Health Benefits of Sweetpotatoes

Sweetpotatoes have long been depended upon as a valuable source of energy in developing countries. Because of their non-specific growing conditions, they are also valuable in times of civil crisis and natural disasters (CIP, 1999). Furthermore, they are well-known as a nutritionally rich crop, complete with vitamins (B1, B2, C and E), minerals (calcium, magnesium, potassium and zinc), dietary fiber, and non-fibrous complex carbohydrates (Suda et al., 2003).

One of the most important dietary compounds found in sweetpotatoes is beta-carotene. This pigment is responsible for the characteristic orange color of sweetpotatoes and represents 86.4 to 89.0% of the carotenoids in yellow and orange fleshed sweetpotatoes (Woolfe, 1992). Beta-carotene is important because of its role as a vitamin A precursor which maintains and protects eye tissues, but it has also been linked to enhanced immune response and suppressed cancer development. Humans cannot synthesize carotenoids, therefore dietary sources have to provide sufficient levels (Kopsell and Kopsell, 2006). Sweetpotatoes in the United States have been found to supply anywhere from 1 to 190 µg/g dry wt. beta-carotene. This is substantial when considering that 6 µg beta-carotene is equivalent to 1 µg retinol equivalents. The recommended dietary allowance of retinol equivalents is 1000, which makes sweetpotatoes a viable and important dietary source of retinol (Kays et al, 1993).

The nutritional superiority of sweetpotatoes has led this food commodity to a recent surge in popularity. The Center for Science in the Public and the

Nutrition Action Health Letter awarded the sweetpotato first place rankings when compared to the nutritional characteristics of other vegetables (NCSPC, 2007). Many new cultivars with flesh colors including white, deep yellow, orange and purple have been developed worldwide and 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 et al., 2003).

1.1.2 – Purple-Fleshed Sweetpotatoes

Purple-fleshed sweetpotatoes have intense purple color in the skins and flesh of the storage root due to the accumulation of anthocyanins (Philpott et al., 2003; Terahara et al., 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. Prominent examples are the Japanese cultivars, Ayamurasaki, which is the second generation of Yamagawa-murasaki. Both were developed at the National Agricultural Research Center for Kyushu Okinawa Region and are used to make commercial products including natural colorants, juices, and fermented beverages available in eastern Asia (Suda et al., 2003; Yamakawa and Yoshimoto, 2002). Ayamurasaki contains four times the concentration of anthocyanins as Yamagawa-murasaki which makes it a prime candidate for use as a functional natural food colorant (Terahara et al., 2000).

1.1.2.1 - Health Benefits of Purple-Fleshed Sweetpotatoes

Anthocyanins isolated from the roots of purple sweetpotatoes show a great amount of promise in relation to their physiological function. Extracts from purple-fleshed sweetpotatoes were shown to inhibit reverse mutation in *Salmonella typhimurium* TA 98 while extracts from other flesh colors rarely exhibited inhibition (Yoshimoto et al., 1999; 2001). Mutation inhibition was conjectured to be due to the anthocyanin pigments, which were absent from the other cultivars examined. Yoshimoto et al. (1999) confirmed this by testing the antimutagenicity of two deacylated anthocyanins isolated from Ayamurasaki and found them both to have high instances of inhibition against four common mutagens. Of these two pigments, one was found to have higher antimutagenicity and this was concluded to be due to structural differences. It was confirmed in a later study that cyanidin-type anthocyanins are superior to peonidin types when antimutagenic activity is concerned (Yoshimoto et al., 2001).

A di-acylated peonidin-type anthocyanin isolated from Ayamurasaki has been evaluated for its ability to suppress glucose metabolism by α-glucosidase inhibitory action in rats. This inhibitory effect provides a way to manage non-insulin-dependent diabetes by delaying glucose absorption in the small intestine and preventing an excessive rise in blood glucose levels (BGL). Administration of the anthocyanin to the rats led to a decreased BGL by 16.5% at doses much lower than the therapeutic drug acarbose suggesting that the anthocyanins

present in purple-fleshed sweetpotato could be useful in preventing hyperglycemia when ingesting carbohydrates (Matsui et al., 2002).

Recently, Japanese markets have coupled anthocyanin extracts from purple-fleshed sweetpotato with cordyceps mushroom extracts as a memory enhancing dietary supplement. In 2003 it was found by Cho et al., that in vitro inhibition of lipid peroxidation in rat brain was accomplished greatly by the sweetpotato extract, and decreased by the addition of the mushroom extract which showed that a synergistic effect between the two extracts does not exist. A synergistic effect between the two extracts was further denounced by DPPH radical scavenging activity. Both sweetpotato and mushroom extracts had roughly the same radical scavenging activity separately, but DPPH activity was not increased by combining the two extracts. Furthermore sweetpotato extracts were shown to improve performance of ethanol-treated mice, while mushroom extract did not. Overall, the anthocyanins were proved to be the active component in memory enhancement (Cho et al., 2003).

Rats have frequently been used to show in vivo physiological function of anthocyanins. Suda et al. (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 anthocyanins reported to be absorbed in rats or humans indicating that other types of acylated anthocyanins could also be absorbed (Suda et al., 2002).

1.1.2.2 – Antioxidants in Purple Sweetpotatoes

Aside from the naturally present nutrients, purple-fleshed sweetpotatoes are also a great source of phytochemicals, which contribute little to overall nutrition but play other important roles in maintaining health (Van Der Sluis et al., 2002). Major antioxidants found in purple-fleshed sweetpotatoes include phenolic compounds and anthocyanins.

Phenolic Compounds

Phenolic compounds are naturally occurring secondary metabolites in plants that find their way into the human diet through fruits, vegetables, leaves, nuts, seed, flowers and barks (Shahidi, 1995). They derive their antioxidant activity from the functional group consisting of one or more hydroxyls attached to a benzene ring. These two components form the simplest member of this class of compounds, phenol. Other common phenolic compounds include benzoic acids, flavonoids, tannins, lignins, and anthocyanins (Ribereau-Gayon, 1972).

As antioxidants, phenolic compounds can either participate in H-atom transfer or single electron transfer. Both result in quenching free radicals and lead to the production of an aromatic free radical in the antioxidant which is stabilized through aromatic ring stabilization (Wright, 2001).

In 1947 chlorogenic acid was isolated from sweetpotato and since then the phenolic compounds present in the roots and leaves of this plant have been of much interest. Originally phenolics were investigated for negative reasons as their accumulation was found in roots attacked by black rot fungus and they were believed to play a role in darkening of canned sweetpotatoes and the formation

of off-colors (Walter and Purcell, 1979). Recently, phenolic compounds and their subsequent contribution to the antioxidant capacity of sweetpotatoes have been receiving more attention as they add functional properties to the sweetpotato and are considered positive attributes. Sweetpotato cultivars found in the United States have phenolic contents ranging from 78.6 to 181.4 mg CAE/100 g fresh weight (fw) (Truong et al., 2007).

While the focus is generally on the storage roots as an important food crop, in many countries the tops of sweetpotatoes are consumed as leafy vegetables. Sweetpotatoes tops are more resistant to disease, pests, and can be harvested several times a year, which makes the annual yield much higher than other green vegetables. In 2002, six caffeic acid derivatives were isolated from sweetpotato leaves, including chlorogenic acid and the total phenolic content was found to be range from 1.42 to 17.1 g/100 g dry weight (dw) in 1,389 cultivars collected worldwide. It was also found that the quantity of total phenolic compounds present in the leaves was greater than concentrations in the stems and storage roots (Islam et al., 2002).

Due to concentration of phenolic compounds in the leaves of sweetpotatoes, their antioxidant capacity is much higher than other vegetables. Furthermore, the phenolic compounds isolated from sweetpotato leaves have shown antimutagenicity in *Salmonella typhimuium* TA, antidiabetic effects in non-insulin-dependent diabetes mellitus, and has shown antibacterial efficacy against *Escherichia coli* O-157 (Islam, 2006). An important phenolic compound found in sweetpotato leaves, 3,4,5-tri-*O*-caffeoylquinic acid, has been linked to a

depression in the growth of stomach cancer, colon cancer, and promyelocytic leukemia cells. Also, caffeic acid was shown to have an exceptionally higher effect against leukemia than other caffeoylquinic acids (Kurata et al., 2007). While in vivo techniques, including molecular marker analysis, have not been investigated, future research will reveal how these properties translate into greater health benefits (Islam, 2006).

Anthocyanins

Anthocyanins are a group of water soluble phenolic compounds in the flavonoid series responsible for red, orange, blue and purple pigments found in many flowers and food products (Tian et al., 2005). Though they accumulate in the epidermal cells in fruits and flowers, anthocyanins are frequently present in the leaves and roots of many plants (Brouillard, 1983). Anthocyanin structure is characterized by the flavylium nucleus (Figure 1) or ring and usually contains a group of sugars and a group of acyl acids (Francis, 1989). Anthocyanin pigments are relatively unstable and often undergo degradative reactions during processing and storage (Wrolstad and Guisti, 2001).

In 1966, the major anthocyanins in the stems of sweetpotatoes were identified as dicaffeyl 3-diglucoside-5-glucosides of cyanidin and peonidin (Imbert et al., 1966). Purple-fleshed sweetpotatoes accumulate anthocyanins in the storage root and much research has been done to identify the structure of those responsible for imparting color.

Odake et al. (1992) isolated six major pigments from the cultivar Yamagawa-murasaki. Of these, two were identified as 3-

caffeylferulysophoroside-5-glucosides of cyanidin and peonidin. Two more pigments were isolated and identified by Goda et al. (1996) as 3-*O*-(6-*O*-trans-caffeyl-2-*O*-β-glycopyranosyl-β-glucopyranoside)-5-*O*-β -glucoside of cyanidin and peonidin. To date, eight acylated anthocyanins have been isolated from yamagawamurasaki and of those six are diacylated (Terahara et al., 1999). The general structure of these anthocyanins can be seen in Figure 2.

In 2000 the focus switched to Ayamurasaki due to its higher anthocyanin content. Terahara et al. (2000, 2004) have isolated and identified six anthocyanins which are all cyanidin or peonidin 3-*O*-sophoroside-5-*O*-glucosides acylated with *p*-hydroxybenzoic, caffeic, or ferulic acids (Suda et al., 2003). The acylation found in sweetpotato anthocyanins is an important characteristic because it adds heat and light stability to the pigment, which is important in food processing applications (Odake et al., 1992).

Since the 1970's the United States Department of Agriculture/Agricultural Research Service (USDA/ARS) has undergone a collaborative effort with the breeding programs of several universities to develop new clones of sweetpotato cultivars. These new clones have different desired traits including better resistance to disease and pests and higher concentrations of phytochemicals leading to a wide range of flesh colors (Jones and Bouwkamp, 1992).

1.1.2.3 – Quantification of Sweetpotato Antioxidants

Extraction of Antioxidant Compounds

In order to evaluate the antioxidant compounds in food commodities, they must be extracted from the food matrix. Due to the chemical nature of food phenolics, and their wide range of structures, there is no method of solvent extraction that will isolate all classes of phenolics present in a food. Generally, solvents such as methanol, ethanol, and acetone, usually with some percentage of water have been used successfully in the past. These solvents can be used at room temperature, heated or cooled depending on the application (Shahidi and Naczk, 2004).

Organic solvents efficiently extract anthocyanins by destroying cell membranes to dissolve the pigments. Traditionally acidified solutions of the same solvents listed above are used with methanol extraction being the most effective solvent. Solvents are typically acidified with weak organic acids such as formic, acetic, or citric acid, or low concentrations of strong acids such as hydrochloric or trifluoroacetic acid. Weak organic acids denature the cellular membranes to facilitate solubilization of the pigments, but addition of excess acid can result in hydrolysis of labile, acyl, and sugar residues and may break down complexes with metals and co-pigments (Shahidi and Naczk, 2004). Furthermore, higher temperatures have been found to improve efficiency of extraction, but can also increase the rate of anthocyanin degradation. Conventional methods are conducted in the 20-50 °C temperature range (Ju and Howard, 2003).

High-temperature-short-time extractions minimize degradation of anthocyanins and extract under the best conditions. One of the best ways to achieve this is through pressurized liquid extraction (PLE) also known as accelerated solvent extraction (ASE). This method of extraction provides rapid extraction of anthocyanins in a closed and inert environment under high pressures (3.3 to 20.3 MPa) and temperatures (40 to 200 °C). This is a major advantage over manual extraction procedures because pressurized solvents will remain liquids above their boiling points which will improve solubility from the matrix. This method was successfully applied to grape skin and found that high temperatures (80-100 °C) using acidified water was effective for isolating anthocyanins but acidified 60% methanol was the most efficient for extracting the highest levels of anthocyanins, while a solvent mixture extracted the highest levels of acylated anthocyanins and total phenolics (Ju and Howard, 2003).

Quantifying Total Phenolic Compounds

Ways of measuring total phenolics in samples include bonding to insoluble polymers (Rexyn and polyvinylpyrrolidone), reaction with the Folin-Ciocalteau reagent, computation from absorbance at 323 nm, coupling with diazotized p-phenylazoaniline and measurement by HPLC (Walter and Purcell, 1979). All methods have been applied to the measurement of sweetpotato phenolics. Walter and Purcell (1979) found that bonding to a Rexyn polymer provided the best procedure for measuring phenolic compounds in that it was rapid and accurate in comparison to HPLC. While HPLC gives accurate and specific results, it requires expensive equipment and more time than other assays.

Folin-Ciocalteau (FC) colorimetry originated in 1927 as a method for tyrosine analysis in proteins (Folin and Ciocalteu, 1927; Prior et al., 2005). In 1965, it was adapted to wine analysis by Singleton and Rossi and the method was improved to reduce variability and erratic results in an effort to create more reliable and predictable data (Prior et al., 2005). Due to its success in wine it shows great promise when applied to sweetpotatoes with high anthocyanin content. It is based on a chemical reduction of the FC reagent, a mixture of tungsten and molybdenum oxides, by the hydroxyl groups of the phenolic compounds. The reduction results in a blue color with broad light absorption at 725 nm. The intensity of the absorption is proportional to the concentration of phenols. The concentration of phenolics present is determined based on a standard curve created by known concentrations of a phenolic compound (either gallic acid for wines, or chlorogenic acid for sweetpotatoes) and their corresponding absorbance (Singleton et al., 1999; Wrolstad and Guisti, 2001).

While this method is simple and only requires a spectrophotometer, there is a major disadvantage. Due to the general nature of the assay it is susceptible to a number of interfering factors including sugars, aromatic amines, sulfur dioxide, ascorbic acid, iron and organic acid, all of which should be corrected for. Furthermore, if the steps to the process outlined by Singleton and Rossi (1965) are not followed consistently there is little basis for comparison. Both of these factors can lead to inflated and therefore, inaccurate values of total phenolic compounds in a food sample (Prior et al., 2005).

Quantifying Anthocyanins

The measurement of total anthocyanin pigment provides a basis for comparison and is useful in assessing the color quality of many foods. Accurate quantification of anthocyanins and their degradation is of special interest when comparing fresh and processed fruits and vegetables and can also be a useful tool in assessing the color quality of food colorants (Wrolstad and Giusti, 2001). In the past HPLC was a preferred method due to simple sample preparation, small sample quantities needed, and the ability to obtain relatively pure pigments from complex mixtures. Chandra et al. (2001) successfully applied this technique to identify and quantify individual and total anthocyanins in botanical raw materials utilized in herbal supplements. HPLC was also utilized by Nielsen et al. (2003) to quantify the anthocyanins present in commercial black currant juices. However, HPLC analysis is complicated and a simpler approach based on difference in absorption at different pH values can also be used to quantify total anthocyanins. This concept was first introduced by Sondheimer and Kertesz in 1948 who analyzed strawberry jams using pH values of 2.0 and 3.4. Francis (1989) suggested pH values of 1.0 and 4.5 when working with cranberries and these values serve as the basis for the pH-differential method (Wrolstad and Guisti, 2001).

The pH-differential method is based on the reversible transformations that occur in anthocyanins with a change in pH. At pH=1.0 the flavylium cation is found in the colored oxonium form while at a pH=4.5 the colorless hemiketal form dominates. This transformation is shown in Figure 3.

At absorbances in the visible range these two anthocyanin structures are identified by different absorbance spectra. Methods that utilize spectroscopy allow for rapid and easy quantification of monomeric anthocyanins, even in the presence of polymerized degrading pigments and interfering compounds (Wrolstad, 2001).

Polymeric Color

Indicies for anthocyanin degradation of an aqueous extract can be obtained by treating the sample with bisulfite. This will bleach the monomeric anthocyanins and leave the resistant anthocyanin-tannin and melanoidin pigments, which can be read at 420 and 530 nm. Through calculations the color density and polymeric color values can be found. These absorbance readings are usually used to track color quality and used as an index for browning.

Research completed on red wines, and beverages made from fermented black carrot showed an increase in polymeric color over storage time with an accompanying decrease in total anthocyanin content. In the context of wine this was not a disadvantage as the polymeric material was resistant to the sulfur dioxide and changes in pH taking place in the wine (Somers and Evans, 1974). But, in the black carrot beverage this was used as an index for the browning that was occurring over time, and while being stored at different temperatures (Turker et al., 2004).

1.1.2.4 – Antioxidant Mechanism and Assay Methods

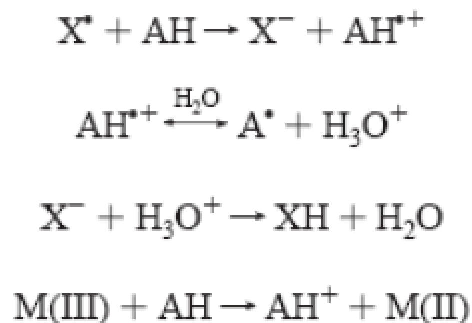
The transfer of electrons from one atom to another is called oxidation and is an essential component in aerobic metabolism. However, the electron flow can become uncoupled and transfer unpaired single electrons through the electron transport system which ends with oxygen as the final acceptor. This leads to the creation of oxygen centered free radicals, also called reactive oxygen species (ROS). Depending on the type of ROS, these molecules can be extremely reactive and rapidly attack nearby cells to cause unavoidable damage. Due to the fact that they can attack lipids in cell membranes, proteins in tissues and enzymes, carbohydrates and DNA which in turn induces oxidations, membrane damage, protein modification and DNA damage, ROS play a key role in causing several degenerative diseases (Pietta, 2000).

Antioxidants prevent cell damage by intercepting and reacting with free radicals faster than the substrate. This is accomplished by one of two pathways: H-atom transfer and electron transfer. In H-atom transfer, the "chain breaking antioxidant" interrupts the reaction to quench free radicals by donating a hydrogen atom. This reaction is:

$$X^{\bullet} + AH \rightarrow XH + A^{\bullet}$$

The efficacy of the antioxidant is dependent upon the Bond Dissociation Enthalpy (BDE) between the oxygen and the hydrogen atoms of the antioxidant. The weaker this bond, the faster the antioxidant can react with the free radical and donate a hydrogen atom.

The second mechanism of antioxidant activity is electron transfer, illustrated below.



Here a radical cation is formed, followed by a rapid and reversible deprotonation in solution. While the net result is the same as the H-atom transfer mechanism, radical cations can attack substrates if given enough time (Wright et al., 2001).

Determining Antioxidant Activity

There are several methods to evaluate the antioxidant activity of the pigments present in food systems. All are based on the addition of a free radical and the subsequent measurement of the antioxidant's ability to prevent the free radical from causing damage. Common free radicals used include 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), which is commonly used in the oxygen radical absorbance capacity (ORAC) and total radical-trapping antioxidant parameter (TRAP) assays (Prior et al., 2005; Cao et al., 1993).

Oxygen Radical Absorbance Capacity (ORAC) Assay

The oxygen radical absorbance capacity (ORAC) assay measures antioxidant inhibition of peroxyl radicals induced by 2,2'-azobis(2-

amidinopropane) dihydrochloride (AAPH) at 37 °C (Ou et al., 2001). This reflects the antioxidant's ability to break the chain of free radical formation by H-atom transfer and is measured by a loss in fluorescence intensity of the fluorescent probe. The decreased rate and amount of product formed over time create a fluorescein curve (AUC), and when compared to a curve created by a blank sample, the protective effect of an antioxidant is determined (Prior et al., 2005; Ou et al., 2001). Trolox (a water-soluble vitamin E analogue and known antioxidant) is generally used as a standard, and results are expressed as trolox equivalents (Cao et al., 1993).

B-phycoerythrin (B-PE) was the original fluorescent probe used, but proved to have shortcomings in antioxidant assays due to its inconsistency, nonspecific protein binding with polyphenols, and the fact that it wasn't photostable and bleached after exposure to excitation light for a certain time. Today the ORAC assay has been improved by the use of fluorescein (3',6'-dihydroxyspiro[isobenzofuran-1[3*H*],9'[9*H*]-xanthen]-3-one) as the probe, which was proven superior to B-PE by Ou et al. (2001). Fluorescein will react with the peroxyl free radicals and decay, which causes a loss in fluorescence intensity. In the presence of antioxidants this decay is delayed so fluorescein will maintain fluorescence intensity for a longer period of time (Ou et al., 2001).

ORAC has successfully been used to determine the antioxidant capacity of common anthocyanins. Cyanidin-3-glucoside was found to have the highest antioxidant capacity which was 3.5 times that of trolox. Peonidin anthocyanins were found to be about 1.5 times more effective than trolox (Wang et al., 1997).

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical utilized in a method that measures antioxidant efficiency by monitoring a decrease in the absorbance of DPPH at a characteristic wavelength. DPPH forms a deep purple solution that readily absorbs at 515 nm, but when it is reduced by an antioxidant it becomes light yellow in color and loses this absorbance (Brand-Williams et al., 1995). Since the test is so simple, and only requires a UV-spectrophotometer, the DPPH method is widespread. However, DPPH is a nitrogen radical that bears no similarity to the highly reactive and transient peroxyl radiacals involved in biological systems and actual relevance to an in vivo efficacy is unknown. Bondet et al. (1997) found that most phenolic compounds react slowly with DPPH and therefore antioxidant activity should be evaluated over time (Awika et al., 2003). Furthermore, DPPH is decolorized by reducing agents as well as hydrogen atom transfer, which can lead to inaccurate interpretation of results (Prior et al., 2005). Color interference of DPPH with samples that contain anthocyanins can also lead to underestimation of antioxidant activity (Awika et al., 2003). DPPH has been performed on purple sweetpotato cultivars from Japan and found that radical scavenging activity ranged from 8.6-49.0 µmol Trolox equivalent/g fresh weight (Oki et al., 2003).

1.2 – SWEETPOTATO PROCESSING

Despite the demonstrated health benefits of sweetpotatoes, worldwide production and consumption has been in a continued state of decline for the past 42 years (Kays, 2005). By contrast, white potato steadily rises in production each year due to an increased array of processed products (Kays, 1985). Sweetpotato continues to be bypassed as a preferred food source for the world's population due to poor utilization of the storage roots (Kays, 2005). Technology that converts raw sweetpotatoes into appealing processed products is one of the main strategies utilized to help boost market demand for sweetpotatoes (Truong, 1992).

Processed products include canned sweetpotatoes, purees, dehydrated flakes, chips, patties, breads, beverages and specialty products including candies and baby food (Kays, 1985). Most of these products are derived from purees, which have many production advantages (Fasina et al., 2003b). A high quality puree can be made from any size or shape of roots. Since approximately 40% of the crop is left in the field due to inadequate sizes; the production of puree alleviates this lack of utilization. Originally purees were prepared by simply cooking peeled roots and pureeing. This created highly variable products due to differences in handling, storage, and other parameters. To obtain a better product 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).

1.2.1 – Puree production

The process of making puree starts with thorough washing with cold pressurized water in a drum washer. The peels are then removed by hot lye (5-6 minute exposure to 10-20% lye solution at 104°C) or high pressure steam and re-washed. The roots are then moved to a sorting station where unacceptable roots are removed and surface blemishes and the ends of the roots are cut off. Pureeing is achieved by pushing the roots through a 0.8 mm screen using the blades of a pulper, or hammermill.

Steam injection follows and serves to activate the natural amylolytic enzymes by rapidly heating to 74-85 °C. This heat treatment gelatinizes the starch and then the enzymes partially degrade it to maltose and dextrins. Maltose production is completed after 10 minutes, but additionally compositional changes in starches and dextrins can last for an hour and have a direct effect on the texture of the final puree. Adjusting the time allowed for partial hydrolysis is important in maintaining a consistent product. The puree then goes into a flash heat exchanger to raise product temperature from 88-100 °C which inactivates the enzymes and finishes cooking the puree. Sweetpotato puree packaged aseptically, in cans, or frozen (Kays, 1985, Collins and Walter, 1992).

1.2.2 – Current Commercial Forms of Puree

There are two main forms of commercially available sweetpotato puree, canned and frozen. Canned purees require high temperatures in retorting in order to guarantee adequate heating of the cold spot. Sweetpotato purees can be processed for over 80 minutes in a 303x307 size can and up to 165 minutes for industrial size cans. 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.

Due to the poor quality product created by canning, frozen packaging has become increasingly popular. Overall it has much lower degradation of the nutritional and aesthetic properties of the puree, but 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 et al., 2005; Kays, 1985).

1.2.3 – Issues Associated with Sweetpotato Puree Processing

1.2.3.1 – Thermal Properties of Sweetpotato Puree

Sweetpotato purees are difficult materials to heat based on their thermal properties. These include specific heat, thermal conductivity and thermal diffusivity and will affect the heating rate of the purees.

Specific heat (C_p) is the quantity of heat that is gained or lost by a unit mass of product to accomplish a unit change in temperature without a change in state. This property is a function of the various components including moisture content, temperature, and pressure and it is generally increased with increasing moisture content (Singh and Heldman, 2001). Fasina et al. (2003a) reported that the specific heat of sweetpotato puree made from Beauregard roots increased linearly over a 5-80 °C temperature range, but the change was less than 0.1 kJ/kg K (3.70-3.78 kJ/kg K). This follows the trends of moist foods and shows that pureeing the roots does not affect their ability to store thermal energy.

Thermal conductivity is important to consider when determining heat transfer. It is the amount of heat that will be conducted per unit time through a unit thickness of the material if a unit temperature gradient exists across the thickness. High moisture foods will have thermal conductivity values close to that of water (Singh and Heldman, 2001). The thermal conductivity of sweetpotato puree was found to generally increase with temperature but at lower temperatures this change was very small. It was reported that thermal conductivity was 0.53 W/m K at 20°C, which compared well with a previously reported value of 0.54 W//m K at 24°C (Fasina et al., 2003a; Stewert et al., 2000).

Thermal diffusivity is a ratio involving thermal conductivity, density and specific heat. It's the rate that heat diffuses by conduction through a food product (Singh and Heldman, 2001). For sweetpotato puree, thermal diffusivity follows the same trend as thermal conductivity and is generally 2-4 times that of

sweetpotato roots due to the reduction in density of puree during heating (Fasina et al., 2003a).

1.2.3.2 – Rheological Properties of Sweetpotato Purees

Sweetpotato purees are naturally viscous and thicker than other processed purees from products such as carrots or white potato. This can lead to several processing problems due to pumping requirements and behavior during heating and cooling in the system. Fasina et al. (2003b) found that purees exhibit gel behavior illustrated by a larger storage modulus (G') than loss modulus (G") through oscillatory rheology. This characteristic defines solid-like behavior of a food material. This gel network was further strengthened by the addition of alginate and calcium salts to form a firmer puree (Fasina et al., 2003b).

1.3 – MICROWAVE APPLICATION TO FOOD PROCESSING

Conventional methods of thermal processing for low acid foods that achieve commercial sterility and shelf-stability often cause a degradation of color, flavor, texture, and nutrients (Wang et al., 2003). One 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). It has also been investigated for drying and

blanching fruits, vegetables and herbs, and as a means to cook meats and breads (Brewer, 2005).

1.3.1 – Microwave Application to Sweetpotato Purees

A process for rapid sterilization and aseptic packaging of sweetpotato puree using a continuous flow microwave system operating at 915 MHz was successfully developed (Coronel et al., 2005). Microwave heating offers a way to overcome the problems presented by canning and freezing methods. 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). Continuous flow microwave heating is an emerging technology in food processing due to the fast and efficient heating it offers.

Heat is generated in the puree 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 the puree and undercooking of the center which is seen with conventional heating methods, like canning (Coronel et al., 2005). Furthermore, the heat generated by microwaves can significantly reduce the time required for pasteurization and sterilization to provide a better quality product (Sumnu and Sahin, 2005). Thus, microwave heating is a viable technology that can be employed to convert raw storage roots

into a high quality shelf stable puree industry can utilize as a functional food ingredient.

1.3.2 – 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 (Reigier and Schubert, 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. This outcome is generally determined by the dielectric properties of the material. Heating occurs when materials convert the electromagnetic energy into thermal energy and occurs through ionic polarization or dipole rotation (Singh and Heldman, 2001).

1.3.2.1 – Ionic Polarization

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 play be the major mechanism of heating the material (Tang, 2005).

1.3.2.2 – 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).

Sweetpotatoes contain 52-85% moisture and it has been reported that foods with moisture contents greater than 35% will have a substantial amount of free water dominating overall dielectric behavior (Sumnu and Sahin, 2005). Dipoles rapidly oscillate at a rate based on the microwave frequency, which can be millions to billions of times per seconds. 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). With the high percentage of polar

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

Dipole rotation is dependent upon frequency. The frequency must be low enough so that the dipoles have time to respond to the alternating electromagnetic field, but not so low that the dipole rotation simply follows the electric field. Temperature increases also effect dipole rotation. Higher temperatures increase thermal agitation so that fewer dipoles can re-orient with the changing electromagnetic field, which causes a loss of rotation in a food material (Tang, 2005).

1.3.3 – 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 a food's heating behavior in microwave systems. Dielectric properties include the dielectric constant (ε‘) and the dielectric loss factor (ε“). The dielectric constant is the ability of the food to store energy when in an electric field while the loss factor is the ability of a material to dissipate microwave energy into heat (Tang, 2005 and Sumnu and Sahin, 2005).

Dielectric properties are dependent upon food composition, temperature and frequency (Sumnu and Sahin, 2005). Because different foods have unique compositions it is impossible to develop a generic equation that will predict dielectric properties based on composition, rather equations need to be product

specific. Measurements for each product should be found for broad temperature and frequency ranges (Guan et al., 2004).

Dielectric properties for sweet potato puree fall within the range for food materials with >60% moisture (Datta et al., 1995). Dielectric constant decreases with an increase in both temperature and frequency. The loss factor initially decreased at low temperatures until reaching a minimum and then increased with frequency. This response is caused by the ionic conductivity of bound water relaxation. At temperatures higher than 35°C the loss factor decreased with an increase in frequency showing that at high temperature levels ionic conductivity is predominantly responsible for loss factor. Also, dielectric properties of sweetpotato puree were higher at 915 MHz due to more dipole rotation at lower frequencies. The loss factor increased with temperature due a reduction in viscosity of the puree which led to increased mobility of the ions and higher conductivity (Fasina et al., 2003).

1.3.4 – Aseptic processing

Aseptic processing involves the separate sterilization of a product and container, followed by packaging in a sterile environment under aseptic conditions (Singh and Heldman, 2001). Dole first introduced aseptic processing in the 1930's as the Aseptic Canning system. Advantages of aseptic processing include retention of nutritional and sensory properties of the food and the lack of need for preservatives. Aseptic processing also creates products with long shelf life (>2 years) at room temperature storage. Commercial aseptic processing

takes place at high temperatures with short holding times. Shorter holding times are allowed because the high temperatures utilized in aseptic processing lead to a faster reduction in microbial population. Typical process temperatures range from 120-150 °C with accompanying hold times from 5-90 s (Reuter, 1987; Colonel, 2005). Components necessary for continuous aseptic processing include: pump, flow controller, heating section, holding tube, cooling section, and packaging system (Singh and Heldman, 2001). The entire system is sterilized before use by hot water, saturated steam, or sterile air.

Aseptic packages are sterilized before they are formed. One of the recent developments with aseptic processing is the development of aseptic pouch fillers. Inpaco, located in Pennsylvania and Robert A. Bosch from Germany, are two companies that introduced fillers that have received FDA approval for aseptic packaging of low acid foods. These fillers operate by sterilizing the packaging material with hydrogen peroxide followed by sterile hot air for drying. They operate at approximately the same rate and can fill 24-32 #10 (3 liters) pouches a minute. The machines can also fill 1-5 liter pouches. This is expected to have a large economic impact as the cost of #10 pouches is 45-50% less expensive than #10 cans. The flexible pouches offer greater safety, lower warehousing and shipping costs, and less space required for storing (Szemplenski, 1995).

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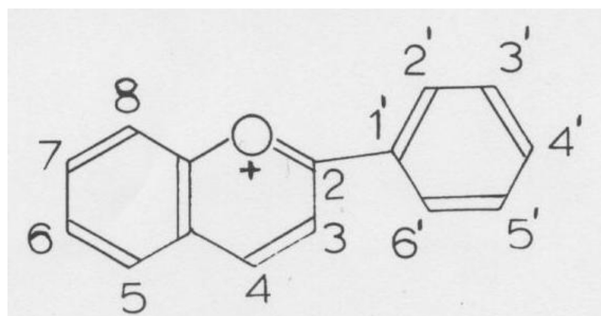
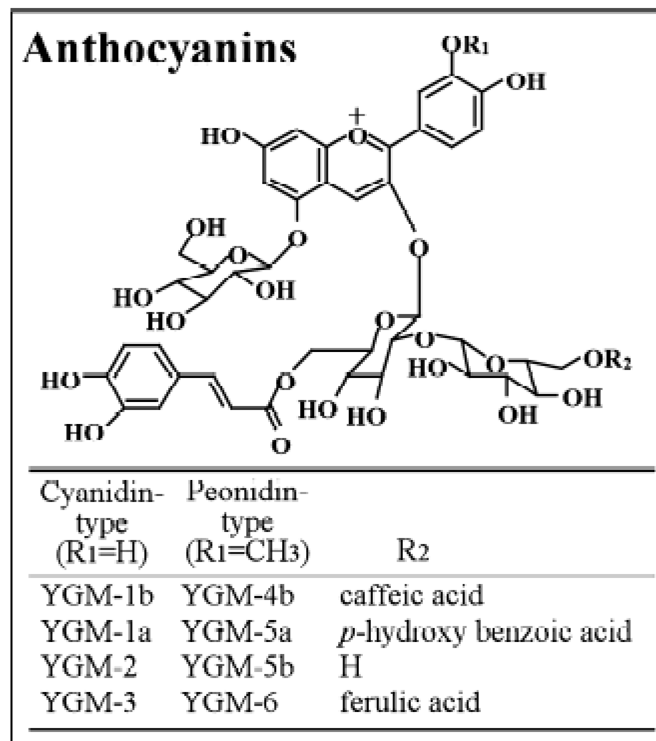


Figure 1: The flavylium nucleus (Francis, 1989)



Cyanidin-type (R_1=H)	Peonidin-type (R_1=CH_3)	R_2
YGM-1b	YGM-4b	caffeic acid
YGM-1a	YGM-5a	*p*-hydroxy benzoic acid
YGM-2	YGM-5b	H
YGM-3	YGM-6	ferulic acid

Figure 2: General structure of 8 isolated anthocyanins from Yamagawa-murasaki.

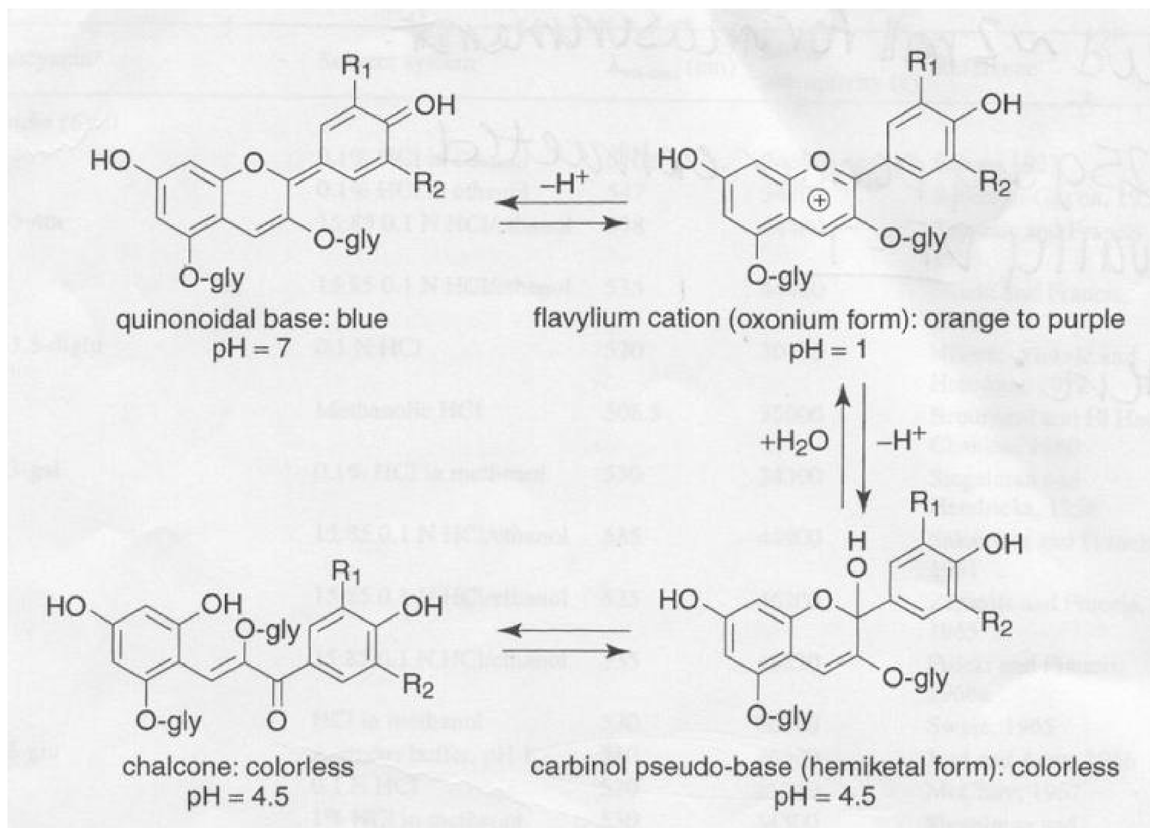


Figure 3: Predominant structural forms of anthocyanins present at different pH levels

CHAPTER 2

NEUTRACEUTICAL AND RHEOLOGICAL PROPERTIES OF PURPLE-FLESHED SWEETPOTATO PUREES

2.1 – ABSTRACT

With high levels of polyphenolic compounds, purple-fleshed sweetpotatoes have been utilized as a healthy food choice for consumers, and a source for natural food colorants in Asian countries. In the United States, there are growing interests in the sweetpotato industry to explore these market opportunities for purple-fleshed sweetpotato genotypes. A locally grown purple-fleshed sweetpotato cultivar was analyzed for nutraceutical properties. Total phenolics of the flesh and whole roots were between 401.6 and 469.9 mg chlorogenic acid equivalent/100 g fw, and anthocyanin content ranged from 80.2-107.8 mg/100 g fw. The DPPH radical scavenging activities were 75.5 to 79.8 µmol trolox equivalent/g fw, and the oxygen radical absorbance capacity (ORAC) values fell between 53.1-60.0 µmol trolox equivalent/g fw. Steam cooking had no effect on these nutraceutical values for both flesh and whole roots except in the case of total monomeric anthocyanins. Unlike orange-fleshed sweetpotatoes, the steamed roots of purple-fleshed sweetpotatoes formed a thick paste which required a process modification to produce flowable purees. Rheological testing indicated that adjusting the dry matter of purple-fleshed sweetpotatoes to 18% produced purees with viscosities similar to orange-fleshed sweetpotato purees. Nutraceutical values of the purple-fleshed sweetpotato purees were lower than values for flesh and whole roots, but they were still similar to fruits and vegetables with high polyphenolic content and antioxidant capacity.

2.2 – INTRODUCTION

Purple-fleshed sweetpotatoes have intense purple color in the skins and flesh of the storage roots due to the accumulation of anthocyanins (Philpott et al., 2003; Terahara et al., 2004). Sweetpotato anthocyanins are mono- or di-acylated forms of cyanidin and peonidin. Recent research on the nutraceutical properties of purple-fleshed sweetpotatoes indicated that the extracted anthocyanins exhibited strong radical scavenging activity, anti-mutagenic activity, and significantly reduced high blood pressure and carbon tetrachloride-induced liver injury in rats (Suda et al., 1997; Yoshimoto et al., 1999; Oki et al., 2002). Other physiological functions of anthocyanins include anti-inflammatory, antimicrobial, and ultraviolet protection effects (Suda et al., 2003).

During the past few years new sweetpotato cultivars with deep purple flesh colors have been developed in Japan to meet a growing demand in the health food markets. Prominent examples are the cultivars Yamagawa-murasaki and Ayamurasaki. Both cultivars were developed at the National Agricultural Research Center for Kyushu Okinawa Region and are used in processed commercial products including natural food colorants, juices, bread, noodles, jams, confectionary, and fermented beverages available in eastern Asia (Suda et al., 2003, Yamakawa and Yoshimoto, 2002).

While many new cultivars with flesh colors including white, deep yellow, orange and purple have been developed worldwide and are finding market success, the United States predominantly consumes orange-fleshed

sweetpotatoes (Suda et al., 2003; Collins and Walter, 1992). Utilizing new and different cultivars that have the same nutritious benefits as orange sweetpotatoes, but also contain additional functional pigments including flavones, beta-carotene, phenolic acids and anthocyanins, is one way to expand the market opportunities for the sweetpotato industry, especially in North Carolina, which produces 40% of the United States sweetpotato production (Suda et al., 2003; NCSPC, 2007).

Despite the well published health benefits of sweetpotatoes, worldwide consumption has been in a state of decline for the last 42 years (Kays, 2005). Some of the strategies to combat this declining trend are to expand the selection of processed products available to consumers including dehydrated flakes, casseroles, pudding, pies, cakes, patties, breads, soups, beverages and baby foods (Truong, 1992; Kays, 1985). Most processed products begin by transforming sweetpotatoes into a puree. Orange-fleshed sweetpotatoes have successfully been processed into purees that are viscous, but flowable, and can be used in various processing operations (Truong et al., 1995; Coronel et al., 2005). However, purple-fleshed sweetpotatoes have a higher dry matter content than orange-fleshed sweetpotatoes, and potentially different starch profile, which presents challenges for the commercial production of flowable puree. In order for a purple-fleshed sweetpotato puree to be used as a functional food ingredient, it must flow so that it can be mixed and pumped through processing equipment.

The goal of this research was to examine the nutraceutical characteristics of purple-fleshed sweetpotatoes that have recently been grown in limited

commercial production in the United States. Furthermore, the rheological properties of the purple-fleshed sweetpotato purees were evaluated aiming to develop a process for producing flowable purees which can be utilized as functional ingredients in the food industry.

2.3 – MATERIALS AND METHODS

2.3.1 – Chemicals

Chlorogenic acid, Folin-Ciocalteau reagent, and Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma-Aldrich (Milwaukee, WI) while AAPH [2,2'-azobis(2-amidinopropane) dihydrochloride] was from Wako Chemicals USA (Richmond, VA). All solvents and chemicals were of analytical grade.

2.3.2 – Sample Preparation of Raw and Steamed Roots

Purple-fleshed sweetpotatoes (Stokes Purple cultivar) were procured from Saura Pride Sweetpotatoes (Walnut Cove, NC). This New cultivar was coded NC 414 in the germplasm collections of the Sweetpotato Breeding Program, NC State University. Representative samples of the roots (n=10) from two batches were taken for analysis. These roots were hand washed and allowed to air dry overnight. Each root was cut longitudinally into fourths and divided into four groups. Two groups were used to create a raw sample set and two were used for a steamed sample set. To prepare the raw samples, one group was peeled

and quickly cut into slices of about 0.75 cm thickness while the other group was sliced whole. From the chopped roots about 250 g representative samples were taken and double bagged into quart size Ziploc® bags (Racine, Wisconsin). This formed 3 raw samples: peels, flesh, and whole roots. All samples were put into a -80 °C freezer within 5 minutes after slicing to minimize pigment degradation. Once frozen, they were removed from the freezer and weighed out into plastic containers. The weights were recorded and the containers were covered with cheesecloth, placed back in plastic bags, and kept frozen at -80 °C for freeze-drying. This was also done within 5 minutes to prevent thawing of the samples.

The two remaining groups were peeled, sliced in the same way, and each sample was steamed for 30 minutes in an 8 qt pot outfitted with a steamer basket (Home Essentials, Kmart, Troy, MI). After steaming, the sweetpotatoes were held in containers with lids until cool to prevent moisture loss. Steamed samples were then homogenized in a Robotcoupe mixer (Model RSI 2YI Ridgeland, MS). Representative samples were taken in the same way as the raw samples, but placed in plastic containers before freezing. Their weights were recorded, the containers were covered with cheesecloth, and frozen at -80 °C to create the final three sample sets: steamed peels, steamed flesh, and steamed whole roots.

Samples were removed from -80 °C storage and placed into a VirTis Genesis 25XL freeze dryer (Gardiner, NY) that operated at -35 to -40 °C. They were allowed to dry for one week and then removed and placed in Ziploc® bags until they warmed up to room temperature. The samples were weighed and grinded into powder using a Mr. Coffee® precision coffee grinder (Sunbeam

Boca Raton, FL). The powders were placed in sample vials and kept in -80 °C storage until analysis.

2.3.3 – Puree Production

Puree samples with various dry solid contents were adjusted by adding water to the cooked slices which adjusted the dry matter contents to a range of 16.8 to 21.2%. Steamed slices and water were weighed, put into a food processor (Black and Decker Power Pro II Model FP1500K, Shelton, CT) and grinded for 2 min to obtain a homogeneous puree. These puree samples were analyzed by rheological testing.

Puree production was scaled up (Figure 1) to convert 500 kg of purple-fleshed sweetpotatoes into puree in the Fruit and Vegetable Pilot Plant, Department of Food science, NCSU. The roots were tumble washed, sliced to 0.65 cm thickness (Louis Allis Co. Slicer, Milwaukee, WI), and steam cooked for 20 min in a thermoscrew steam cooker (Rietz Manufacturing Co., Santa Rosa, CA). A ribbon mixer (Keebler Engineering Co., Chicago, IL) was used to add water to the slices which adjusted dry matter content to 18%. From the ribbon mixer sweetpotato puree was then pureed using a hammer mill (Model D, Fitzpatrick Co., Chicago, IL) fitted with a 0.15 cm screen. Puree was placed in 5 gallon buckets with lids and frozen at -20 °C until later use.

Samples were taken at each stage of the pilot-scale puree production process (raw slices, steamed slice, puree), placed into containers, and freeze-dried as described previously.

2.3.4 – Nutraceutical Analysis

2.3.4.1 – Preparation of the Extracts

Extraction of polyphenolic compounds from dried sweetpotato powders was performed using an accelerated solvent extractor (Dionex ASE 200, Sunnyvale, CA) equipped with a solvent controller. Three cellulose filters were placed in the bottom of a 22 ml stainless steel extraction cell and covered with 2 g of sea sand (Fisher Scientific, Pittsburgh, PA). Sweetpotato powder (0.25 g) was mixed with 26 g of sand, loaded into the cell, and then sealed tightly. Extraction parameters for all extracts were set as followed: pressure, 1500 psi; temperature, 100 °C; extraction time, 3x5 minute cycles; flushing volume, 60%; and nitrogen purge time, 60 seconds. All powders were extracted with a solvent containing 7% acetic acid in 80% methanol that was purged with nitrogen gas prior to use to prevent phenolic oxidation during the extraction. Extracts were collected in UV-proof glass vials, adjusted to 50 ml volume with solvent, dispersed into 10 ml serum tubes and kept at -80 °C until nutraceutical analysis.

2.3.4.2 – Total Phenolics

Total phenolic compounds were quantified using a modified Folin-Ciolctau method (Singleton et al., 1999). Chlorogenic acid was used as the standard. Samples and standards were diluted in 4 ml of water and 0.5 ml of the FC reagent was added and allowed to react for 3 minutes. Then, 0.5 ml 1 N sodium carbonate was added and allowed to react for one hour. Samples were read for

absorbance at 725 nm using a Varian Spectrophotmeter (Cary WinUV Model 300, Palo Alto, CA). A blank was used to calibrate the machine that contained 0.25 ml water instead of sample, along with the same amount of water for dilution, FC reagent, and sodium carbonate. Total phenolic values were reported in milligrams chlorogenic acid equivalents per 100 grams fresh weight (mg CAE/100 g fw).

2.3.4.3 – Total Monomeric Anthocyanins

Total monomeric anthocyanin content was determined using the pH-differential method (Wrolstad and Guisti, 2001). Two dilutions were performed on each sample. The first used potassium chloride (0.025 M) at pH 1 and the second was with sodium acetate (0.4 M) at pH 4.5. Samples were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 minutes before absorbance at 530 and 700 nm were recorded using a spectrophotometer calibrated with distilled water as the blank.

The difference in absorbance between pH's and wavelengths was calculated:

$$A = (A_{530nm} - A_{700nm})_{pH1.0} - (A_{530nm} - A_{700nm})_{pH4.5}$$

This was used to calculate monomeric anthocyanin pigment concentration using:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

where MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivitiy, and 1 is for a standard 1cm pathlength. The molecular weight (MW = 449.2) and molar absorptivity (ε = 26,900) correspond to the most predominant

anthocyanin in the sample and values for cyanidin-3-glucoside were used. Total monomeric anthocyanins were reported as mg anthocyanins per 100 g fresh weight (mg anthocyanins/100 g fw).

2.3.4.4 – 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant activity determined by the DPPH assay is based on the methodology of Brand-Williams et al. (1995). Trolox was used as a standard and concentrations ranging from 0-0.5 μM were used to create a standard curve. Samples were diluted 10-fold and then 100 μl was added to 1.9 mL of DPPH solution and allowed to react for 3 hours. Absorbances of standards and samples were read at 515 nm with a spectrophotometer. Results were expressed in μM trolox equivalents per gram fresh weight (μM TE/g fw).

2.3.4.5 – Oxygen Radical Absorbance Capacity (ORAC)

The ORAC procedure established by Prior et al. (2003) was followed. Fluorescence intensity measurements were performed using a Safire monochromator based microplate reader equipped with Magellan V4-W reader software (Tecan USA, Research Triangle Park, NC). Samples were loaded into 96-well transparent Costar polystyrene flat bottom plates (Corning, Acton, MA). The concentrations of reagents prepared were the same as described by Prior et al. (2003) except that the samples were diluted 100-fold. Wells were filled with 70 μl of phosphate buffer, 60 μl of fluorescein solution, and 60 μl of standard or sample. For blank wells, phosphate buffer was used in place of the sample. The

plate was incubated at 37 °C for 15 minutes before 60 µl of AAPH was rapidly added to each well. Plates were shaken orbitally for 5 seconds at the start and between 1 minute reading intervals. Measurements were performed with 80 cycles using excitation and emission filter wavelengths of 485 and 520 nm, respectively.

ORAC values were calculated using a regression equation Y = mx + b for a linear regression on the range of 6.25-100 µM trolox standards. Y is the concentration and x is the net area under the fluorescence decay curve. The area under the curve was calculated as follows:

$$AUC = (0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + \ldots + f_i/f_4) \times CT$$

where f_4 is the initial fluorescence reading at cycle 4, f_i is the fluorescence reading at cycle i, and CT is the cycle time in minutes. The net area under the curve was obtained by subtracting the area under the curve for the blank values from the curves of samples and standards. ORAC values were expressed in µM trolox equivalents per gram of fresh weight (µM TE/g fw).

2.3.4.6 – Color Measurements

Hunter L*a*b* values were measured with a Hunter colorimeter (D25/DP9000 Tristimulus Colorimeter, Hunter Associate Laboratories Inc., Reston, VA). The cooked sweetpotato samples were filled into a 35 mm petri dish, covered, and pressed against the surface to remove air bubbles. The colorimeter was calibrated against a standard white tile (L* = 92.75, a* = -0.76, b* = -0.07) and sample measurements were taken at 3 different locations, with

duplicates performed for each sample. Averages of these readings are reported. Hue angle (h°) was calculated using arc tan(b*/a*), chroma (C*) was calculated using $[a^{*2} + b^{*2}]^{1/2}$.

2.3.4.7 – Moisture Analysis

Moisture analysis was measured two ways for each sample. The first was based on the initial and final weights of the freeze dried samples. Moisture content was also established based on the AOAC oven drying method, (AOAC, 2006) 24 hours at 100°C. Moisture content was converted to dry matter for both methods and for the purpose of calculations dry matter established by freeze-drying was used.

2.3.4.8 – pH Measurements

The pH of sweetpotato samples was measured by performing two dilutions on the sample. First, dry matter content was adjusted to 18.1% by adding distilled water to the flesh and whole samples to create the same dilution used in scaled-up puree production. Then all samples were diluted 1:1 with distilled water. Samples were homogenized using a Tissumizer (Tekmar, Cincinnati, OH) and pH was measured using an Accumet AR50 pH meter (Fisher Scientific, Pittsburgh, PA).

2.3.5 – Rheological Testing

Rheological properties of the lab scale puree samples were evaluated using a stress-controlled ATS Stresstech Rheometer (Rheosystems, Bordentown, NJ) outfitted with serrated cup and bob geometry. Samples were covered with a thin layer of mineral oil to prevent moisture loss, and samples were pre-sheared at 20 s^{-1} for 30 s before testing began. Shear rate sweeps were performed at 5, 25, 70, and 90 °C with shear rate ramped from 1 to 300 s^{-1}. At each temperature change samples were allowed to equilibrate for 60 s. The orange-fleshed sweetpotato puree from Beauregard cultivar (George Foods, Pembroke, NC) was measured under the same conditions. Apparent viscosity measurements at 25 °C were compared between the orange puree which was used as a standard and the purple puree samples with different water concentrations.

The puree samples from the scale up pilot plant experiments were also evaluated and results were used to model the behavior of the puree using the Herschel-bulkley model:

$$\sigma = \sigma_0 + K\gamma^n$$

where σ is the shear rate, σ_0 is the yield stress, γ is shear rate, K is the consistency coefficient, and n is the flow behavior index (Steffe, 1996).

2.3.6 – Statistical Analysis

Group differences were evaluated using analysis of variance (ANOVA) F-tests using the SAS Statistical Analysis System v8.1 (SAS Institute Inc., Cary,

NC) with $p<0.05$ considered to be a statistically significant difference. Means were separated by the Student-Newman-Keuls (SNK) procedure. This was chosen due to the unequal sample sizes of the lab samples, and this procedure accounts for that by using the harmonic mean. The SNK procedure is less conservative than other methods of means separation, which means that it is more likely to declare a difference between values. Color data was evaluated using Tukey's studentized range because sample sizes were equal. Regression analysis was carried out for correlation determination with a significant R^2 having $p<0.05$.

2.4 – RESULTS AND DISCUSSION

2.4.1 – Total Phenolics

The total phenolic content of the samples ranged from 313.6 mg CAE/100 g fw for puree to 1,483.7 mg CAE/100 g fw for raw peels (Table 1). Phenolic contents of the peels were significantly different from the flesh and whole roots for both raw and steamed samples. The peel samples had at least three times the phenolic content of flesh and whole roots. Also, the steamed and raw peels were significantly different from one another but this trend was not seen for raw and steamed flesh and whole roots. These results are in accordance with a previous report by Truong et al. (2007) for the raw and steamed tissues of orange-fleshed sweetpotatoes. Unpeeled carrots have also reported higher total phenolic values than their peeled counterparts (Talcott et al., 2000).

Total phenolic content values for the peels were comparable with purple corn and red-fleshed sweetpotato which have reported the contents of 1,756 mg CAE/100 g fw and 945 mg CAE/100 g fw, respectively (Cevallos-Casals and Cisneros-Zevallos, 2003). As expected, the purple-fleshed sweetpotatoes examined in this study had much higher phenolic contents than those of orange-fleshed sweetpotatoes. Walter and Purcell (1979) examined a range of orange clones and found the highest content to be 75 mg CAE/100 g fw which is approximately 25% of the values for the purple-fleshed sweetpotato puree in this study (Table 1). Truong et al. (2007) reported higher total phenolic contents for Beauregard tissues ranging from 78.6 to 181.4 mg CAE/100 g fw. However, purple-fleshed sweetpotato samples have phenolic contents much lower than those found in the leaves of commercial sweetpotato cultivars grown in the United States (Truong et al., 2007). In a study evaluating 1,389 sweetpotato genotypes in Japan, the foliar phenolic content ranged from 6.4 to 221.0 mg CAE/100 g dw (Islam et al., 2006).

Berries are one of the highly antioxidant foods that contribute substantial amounts of phenolic compounds to the diet. Wild chokeberries contain 2,556 mg CAE/100g fw which is far greater than the contents of lingonberries, cranberries and blueberries (Zheng and Wang, 2003). Chun et al. (2005) analyzed the total phenolic content in gallic acid equivalents (GAE) for a wide range of fruits and found that plums have 368.7 mg GAE/100g fw while strawberries and cherries have 225.0 and 83.6 mg GAE/100g fw, respectively. Blueberries can also have

high phenolic contents and range from 399.3 ± 149.1 to 556.1 ± 216.9 mg GAE/100g fw (Sellappan et al., 2002).

For the pilot plant experiments on the purple-fleshed sweetpotato purees, the phenolic contents of the raw slices were significantly lower than that of the steamed slices. Similar findings have been reported for the orange-fleshed sweetpotatoes, though the differences were not significant (Truong et al., 2007). This effect was not observed in any other sample set of the laboratory scale experiments, and these results can be attributed to the difference in sampling methods. For all the lab scale experiments, the raw samples were frozen at -80 °C within 5 minutes of slicing which likely minimized pigment degradation. However, in the pilot plant experiments representative samples of slices were taken throughout the processing scheme and were kept in Ziploc® bags at room temperature and exposed to air and oxygen for a much longer time, about 3-4 hours. Jang et al. (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. Ascorbic acid has been shown to inhibit the decrease in caffeic acid derivatives of sweetpotatoes, and this inhibition supports the involvement of PPO in degradation of phenolic compounds (Takenaka et al., 2006). Therefore, thermal and/or chemical treatment to inactivate PPO should be applied to maintain high retention of the phenolic and other nutraceutical components during processing.

The puree samples were significantly lower in phenolic content than all other samples presented in Table 1. Adjustment of dry matter content to 18% by

adding water to the steamed slices diluted the nutraceutical components. However, we found that this step was necessary in producing flowable purple-fleshed sweetpotato purees for industrial applications. Klopotek et al. (2005) processed strawberries to many different products and found a significant decrease in total phenolic content from 257.1 mg GAE/100 g fw in strawberries to 73.6 mg GAE/100 g fw for strawberry puree. Decreases in chlorogenic acid (5 to 15%) have also been shown for the production of pumpkin purees from the raw fruits (Dragovic-Uzelac et al., 2005).

2.4.2 – Total Monomeric Anthocyanins

Anthocyanins are the most important group of phenolic compounds present in purple-fleshed sweetpotatoes. They contribute to the characteristic color and have been linked to anti-hyperglycemic, anti-cancer, and anti-mutagenic health benefits (Suda et al., 2003; Yoshimoto et al., 1999; Matsui et al., 2002). Since they are a sub-category of phenolic compounds, the anthocyanin content of the samples analyzed were expected to follow the same trends as seen in the total phenolic assay. Total monomeric anthocyanins range from 57.54 mg/100 g fw for puree to 174.74 mg/100 g fw for raw peels. The anthocyanin content in the peels was about 1.3 to 1.7 times higher than the flesh and whole roots (Table 1). However, the peel only contributes about 10 to 15% of the total weight of the sweetpotato roots (Truong et al., 2007). There was no significant difference ($p<0.05$) in anthocyanin values between the flesh and whole root. Steaming resulted in significant decreases ($p<0.05$) in the anthocyanin

content of all the lab experiment samples. A higher total monomeric anthocyanin value of steamed slices compared to raw slices for the samples from the pilot plant experiments were due to the long exposure of the sample to ambient conditions as described above.

A decrease in total monomeric anthocyanin values of steamed samples with reference to raw sweetpotato samples (Table 1) is in contrast to the previous reports. Teow (2005) reported higher total phenolic and total monomeric anthocyanin contents for the steamed samples as compared to the chopped samples of the raw roots from most of the purple-fleshed sweetpotato clones evaluated. In a study on anthocyanin content in purple-fleshed sweetpotatoes in Guam, Yang et al. (2006) also reported that the steamed samples had higher anthocyanin contents than the raw slices exposed to drying at 60 °C. The results in our study are attributable to the high activity of PPO that was discussed above. The same reasoning can also be used to explain why the pilot plant slices have significantly lower anthocyanins than the steamed slices. The lab scale sampling procedure quickly transferred sliced roots to -80 °C storage, which is believed to have stopped enzymatic degradation of the pigments catalyzed by PPO.

The difference in anthocyanin content between samples is reflected in the Hunter L*a*b* values and summarized in Table 2. Differences for each attribute amongst the samples are significant ($p<0.05$). L* is a lightness index and ranges from 0-100 with 0 representing black and 100 representing white. Puree was the darkest sample while the whole steamed roots were darker than steamed flesh. Therefore, the utilization of the whole roots would not increase total phenolic and

anthocyanin content, but result in darker color of the purple-fleshed sweetpotato products. Truong et al. (2007) reported similar effects in orange-fleshed sweetpotatoes. The intensity of red color is represented by a* value while blue color is represented by a negative b* value. Masuda et al. (2002) reported Hunter color values for Ayamurasaki and Kyushu-132 as L*: 44.0 and 45.5, a*: 21.6, 21.5, and b*: -6.7, and -7.7, respectively. Color results for the samples evaluated in our study are similar except in the case of the L* values. Generally, the cultivar used in this study was almost twice as dark as the Japanese cultivars. The puree samples were the most intense for all color attributes, which leads to an increased chroma (C*) value and hue angle (h°) that signifies more of a blue-ish purple. Originally, the higher saturation and different hue were thought to be caused by a higher pH, which is known to cause darker purple and blue colors (Suda et al., 2003). However pH measurements for all samples were all about 6.0. Instead, the darker color of the puree may be due to metal ions present in the tap water used to dilute steamed slices in the puree process. These ions could potentially form complexes with the anthocyanins in purple-fleshed sweetpotatoes and lead to differences in color. Cornflower anthocyanin structure has a metal complex formed between anthocyanins and a ferric ion. This complex causes the characteristic blue color of the cornflower, but its absence in roses makes the same anthocyanin appear red (Takeda, 2006).

Purple color in sweetpotatoes is due to anthocyanins that are mono- or di-acylated forms of peonidin and cyanindin (Terahara et al., 2000; 2004). Yoshinga et al. (1999) found that purple-fleshed sweetpotato clones could be

classified into two groups based on their b*/a* ratio. Clones that have ratios less than -1.4 are blue dominant which tend to have more cyanidin than peonidin while clones with ratios greater than -1.1 are red dominant and have more peonidin. Based on this finding, the samples evaluated in this study were more peonidin rich since the b*/a* ratios ranged from -0.59 to -0.51.

Anthocyanins are present in a wide range of fruits, including mostly berries, and some vegetables. Wu et al. (2006) reported that chokeberries and elderberries have the highest anthocyanin content of all food commodities examined with 1,480 and 1,375 mg anthocyanins/100 g fw respectively. Black currant, blueberries and red cabbage are more common food commodities that are known to have higher anthocyanin contents, ranging from 322 ± 40.8 to 476 ± 115 mg anthocyanins/100 g fw. Red fleshed potatoes have up to 25.5 mg anthocyanins/100 g fw, while red-fleshed sweetpotatoes are much higher with 182 ± 2 mg anthocyanins/100 g fw (Rodriguez-Saona et al., 1998; Cevallos-Casals and Cisneros-Zevallos, 2003).

The anthocyanin contents for purple-fleshed sweetpotatoes in the study were slightly lower than those for red-fleshed sweetpotato, but comparable with sweet cherries, grapes, plum, raspberries, eggplant, and red radishes, showing that they fall in the middle of the spectrum of high anthocyanin fruits and vegetables (Cevallos-Casals and Cisneros-Zevallos, 2003). The purple-fleshed sweetpotato puree was on the lower end of the spectrum having anthocyanin contents comparable to black bean, red onion, and strawberries that range from 41.7 to 48.5 mg/100 g fw (Wu et al., 2006).

2.4.3 – Antioxidant Activity

Based on the DPPH assay the sample with the highest antioxidant activity is raw peels with 87.36 μmol TE/g fw and the lowest is represented by puree with 46.98 μmol TE/g fw. Raw peels have almost twice the antioxidant activity of puree. There was no significant difference in DPPH values among the raw and steamed samples for both flesh and whole roots (Table 1). This range of DPPH values was higher than the DPPH radical scavenging activity ranging from 8.6 to 49.0 μmol TE/g fw for a group of sixteen purple-fleshed cultivars reported by Oki et al. (2003). Kano et al. (2005) reported that the DPPH radical scavenging activity of a purple-fleshed sweetpotato cultivar, Ayamurasaki, was higher than those of red cabbage, grape skin, elderberry or purple corn. This is an important finding as purple-fleshed sweetpotatoes do not have quite as high of an anthocyanin content as found for elderberry and purple corn. Orange-fleshed sweetpotatoes (cultivar Beauregard) have lower DPPH values ranging from about 2.0 μmol TE/g fw for flesh to 7.1 μmol TE/g fw for peels, and as great as 38.2 μmol TE/g fw for leaves (Truong et al., 2007).

While DPPH is an easy and cost efficient method to evaluate antioxidant activity, it is a nitrogen free radical that is dissimilar from the reactive and transient radicals involved in biological systems. DPPH can also be reduced by radical reaction or reduction and is therefore not a competitive reaction. ORAC is another antioxidant assay that provides a controllable source of free radicals to model reactions of antioxidants with lipids in food and physiological systems. When using fluorescein, it has been determined that antioxidants reduce free

radicals by hydrogen atom transfer (Prior et al., 2005). ORAC values were the highest for raw peels at 78.2 µmol TE/g fw. The puree sample had the lowest ORAC value at 26.4 µmol TE/g fw (Table 1). Based on these values puree is capable of a third of the amount of radical scavenging capacity as peels, and about half as much as flesh and whole root samples. Statistical analysis shows that antioxidant activities of the raw and steamed samples of flesh and whole roots were not statistically different ($p>0.05$).

The ORAC values for the purple-fleshed sweetpotato samples were below what has been reported for cranberries and lowbush blueberries with ORAC values of 92.6 µmol TE/g fw and 92.1 µmol TE/g fw, respectively. However, the range of ORAC values in Table 1 for purple-fleshed sweetpotatoes compared well with a range of 35.4-61.8 µmol TE/g fw for high antioxidant food commodities such as blackberries, cultivated blueberries, sweet cherries, raspberries and strawberries (Wu et al., 2004). Despite the ORAC value for the purple-fleshed sweetpotato puree being lower than the undiluted samples, its value of 26.4 µmol TE/g fw was still higher than the ORAC values reported for several Florida-grown tropical fruits. Of the 14 fruits evaluated, the highest ORAC value was 16.7 µmol TE/g puree for red guava followed by lower values for ripe papaya and mango with 5.3 and 2.2 µmol TE/g fw, respectively (Mahattanatawee et al., 2006).

The ORAC values typically correlate well with total phenolic content values (Prior et al., 2005). Purple-fleshed clones have reported an R^2 value as high as 0.937 for this relationship (Teow et al., 2007). For this study the R^2 value

was only 0.59, but this was statistically significant ($p<0.05$). The difference in correlations could be due to a greater number of samples evaluated in other studies. The correlation between ORAC values and total monomeric anthocyanins was higher with $R^2 = 0.71$ which was also significant. Furthermore, there is a significant correlation ($R^2 = 0.84$) between the ORAC and DPPH values. This has been reported before with DPPH and ORAC values of sorghum products with an $R^2 = 0.97$ (Awika et al., 2003).

2.4.4 – Rheological Characterization of Purple-fleshed Sweetpotato Purees

Viscosities of purple-fleshed sweetpotato purees, with different levels of added water, were compared to the viscosity of the commercial puree manufactured from the orange-fleshed sweetpotatoes (Figure 1). In an initial pilot plant scale experiment, the cooked slices of the purple-fleshed sweetpotatoes formed a sticky material that clogged a hammer mill when attempted to grind into puree. Unlike the orange-fleshed sweetpotatoes, the comminuated material from purple-fleshed sweetpotatoes was sticky and paste-like, and could not flow through the largest mesh fitted to the hammer mill. The problem was most likely due to the high dry matter content found in purple-fleshed sweetpotatoes. As indicated in Table 1, purple-fleshed sweetpotatoes had 30 to 35% dry matter, while orange-fleshed sweetpotatoes were reported with about 19 to 21% dry matter (Truong et al., 2007). Also, purple-fleshed sweetpotatoes could have high starch levels with different pasting, swelling and thermal properties. The processing hurdle in pureeing the purple-fleshed

sweetpotatoes could be overcome by either water addition, amylase hydrolysis of starch components, or a combination of the two treatments. For cost effective reasons, water addition was taken as an initial approach in processing of purple-fleshed sweetpotato purees.

The orange-fleshed sweetpotato purees are relatively thick and have been reported to have an apparent viscosity of 5.1 Pa s at 25 °C and a shear rate of 50 s^{-1}. However, this puree is flowable and has been used in processing applications including continuous flow microwave and aseptic processing (Coronel et al., 2005). Since purple-fleshed sweetpotatoes were also planned to be processed in this manner, a flowable puree was desired.

The lab scale purees with adjusted dry matter content to 16.8 and 21.2% water were evaluated first and compared to the orange-fleshed sweetpotato puree. Purple purees adjusted to 16.8% dry matter showed a viscosity over a shear rate range that very closely mimicked the orange puree. Since orange puree was slightly less viscous, when the puree process was scaled up to the pilot plant level, dry matter was adjusted to 18.1% was utilized and its viscosity is shown in Figure 1.

Pilot plant puree adjusted to 18.1% dry matter actually turned out to be less viscous than orange sweetpotato puree. This could be due to inaccuracies in measuring water weights at the pilot plant level. The available scale (Fairbanks Scales, Model 90-504, Kansas City, MO) that could handle wet processing conditions and the weights needed rounded off to one decimal place and this place was rounded to either a 0 or 5. Still, a less viscous puree is easier

to work with and the final water concentration was kept below 40%. This was the maximum concentration evaluated because at concentrations higher than this there was a large degree of syneresis, which was undesirable from the processing standpoint.

Figure 2 shows the viscosity of a purple-fleshed sweetpotato puree adjusted to 18.1% dry matter at 4 different temperatures; 5, 25, 70, and 90°C. Viscosity decreased as temperature increased, and this same behavior has been noted for orange sweetpotato purees by Kyereme et al. (1999). Furthermore, several researchers have found that orange sweetpotato puree exhibits non-Newtonian, pseudoplastic behavior with a yield stress that fits the Herschel-Bulkley model (Rao et al., 1975; Kyereme et al., 1999; Grabowski et al., 2007). The flow behavior of a purple-fleshed sweetpotato puree with 18% dry matter was modeled using the Herschel-Bulkley model and reported a consistency coefficient (K) of 14.84 Pa s^n and a flow behavior index (n) of 0.50.

The consistency coefficient found for purple-fleshed sweetpotato purees falls within the range of 2.8 and 21.5 Pa s^n reported by Kyereme et al. (1999) for orange sweetpotato purees, but the flow behavior index is greater than the reported range of 0.20-0.33. These values correspond to orange sweetpotato purees. Rao et al. (1975) examined seven different orange sweetpotato cultivars and reported similar consistency coefficient values of 1.8-24.8 Pa s^n. But, flow behavior index values were closer to the value found for purple sweetpotato puree and ranged from 0.33 to 0.56.

2.5 – CONCLUSIONS

Purple-fleshed sweetpotatoes have nutraceutical components that are competitive with other food commodities known to be good sources of antioxidants. They are high in phenolic and anthocyanin content, which correspond to high antioxidant activities. In order to make a flowable puree that will have applications in the food industry, water must be added to the steamed purple-fleshed sweetpotatoes. This process causes a significant decrease in nutraceutical content but it does not take the developed purees out of the range of high antioxidant products. Future work needs to focus on processing and packaging this product into a stable form that can potentially be used as a functional food ingredient in various food systems.

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Table 1: Nutraceutical values for purple-fleshed sweetpotato samples

Sample	Treatment	Dry Matter[1]	TP[2]	TMA[3]	DPPH[4]	ORAC[5]
Lab Scale						
Peel	Raw	28.8	1483.7±18.6^{a*}	174.7±3.3^{a}	87.4±2.0^{a}	78.2±1.0^{a}
Flesh	Raw	37.7	408.1±13.9^{de}	101.5±2.5^{b}	75.5±1.6^{bc}	58.5±1.3^{b}
Whole	Raw	36.9	469.9±13.8^{d}	107.8±1.8^{b}	79.8±1.9^{b}	58.7±1.2^{b}
Peel	Steamed	24.0	1298.1±43.3^{b}	109.8±5.2^{b}	77.0±1.0^{bc}	71.5±2.2^{a}
Flesh	Steamed	36.4	401.6±24.1^{de}	80.2±5.5^{c}	77.1±1.6^{bc}	53.1±1.7^{bc}
Whole	Steamed	37.0	465.9±13.4^{d}	84.6±2.7^{c}	75.5±1.8^{bc}	60.0±1.1^{b}
Pilot Plant Scale						
Slices	Raw	29.6	353.4±14.5^{ef}	68.7±7.1^{d}	66.6±1.7^{d}	49.0±3.0^{bc}
Slices	Steamed	28.2	570.8±8.9^{c}	83.0±2.7^{c}	70.8±1.6^{dc}	46.2±2.5^{c}
Puree[6]		18.1	313.6±4.6^{f}	57.5±1.5^{e}	47.0±2.6^{e}	26.4±1.3^{d}

1 - Dry matter determined by AOAC oven drying method (1995)
2 - TP: Total phenolics values expressed in mg CAE/100g fw
3 - Total Monomeric Anthocyanin: TMA values expressed as mg anthocyanins/100g fw
4 - DPPH values expressed as mM TE/g fw
5 - ORAC values expressed as mM TE/g fw
6 - Puree made from steamed slices adjusted to 18% dry matter content
* subscripts signify significance based on ANOVA F-tests with Student-Newman-Keuls means separation

Table 2: Color data for selected purple-fleshed sweetpotato samples

Sample	L*	a*	b*	Hue angle	Chroma
Flesh[1]	26.0±0.2[a*]	17.7±0.2[c]	-10.4±0.1[b]	-30.6±0.4[b]	20.5±0.2[b]
Whole[1]	24.2±0.1[b]	18.5±0.3[b]	-9.9±0.1[a]	-28.3±0.5[a]	21.0±0.2[b]
Puree	20.3±0.1[c]	26.2±0.1[a]	-13.3±0.1[c]	-26.9±0.2[a]	29.4±0.1[a]

1 – Flesh and whole represent values for steamed samples
* - Values reported are averages ± standard error of the means. Means with different letters differed significantly ($p<0.05$) and are separated by Tukey's studentized range procedure

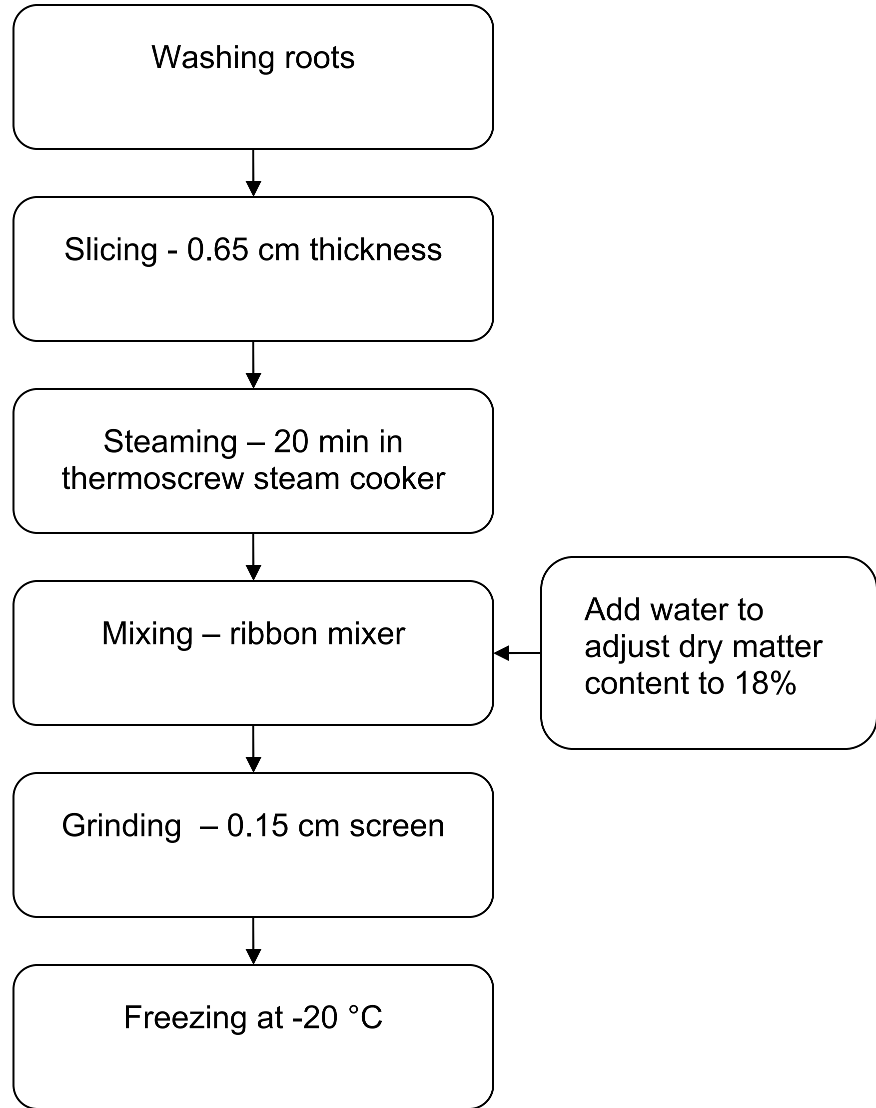
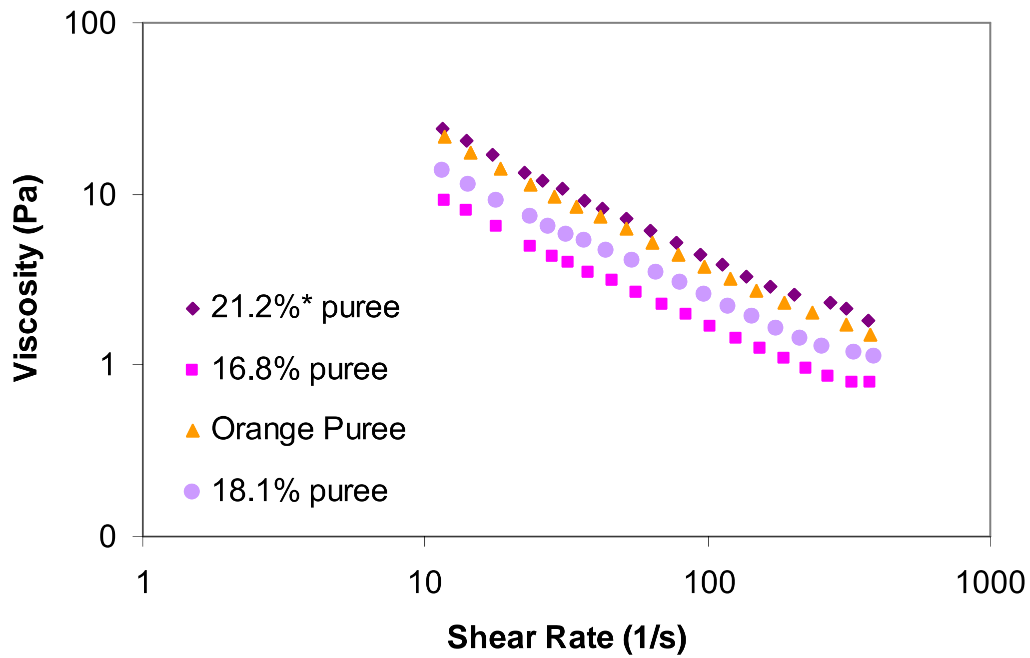


Figure 1: Pilot plant puree process



* - Percentages represent dry matter content

Figure 2: Viscosity of purple-fleshed sweetpotato purees made with different added water levels and orange sweetpotato puree at 25°C

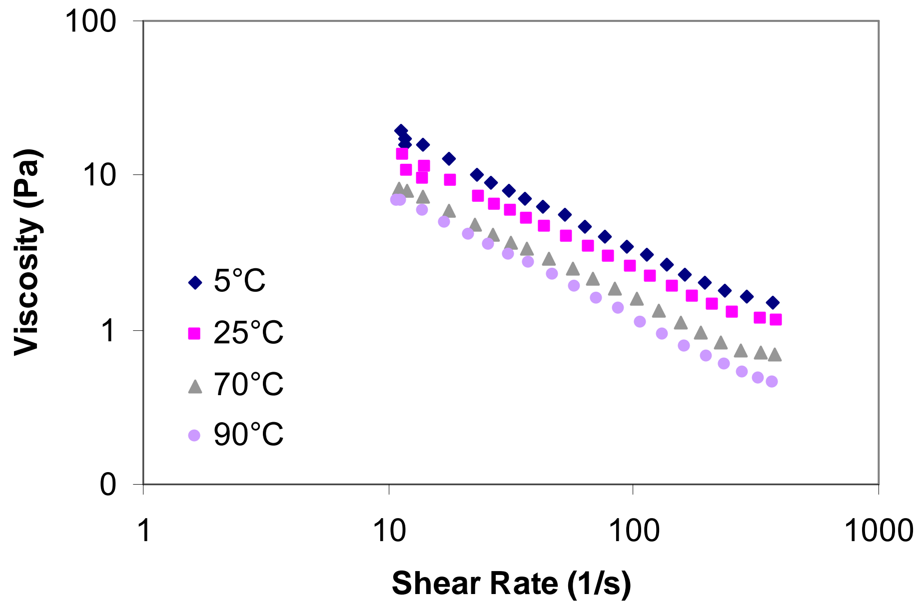


Figure 3: Viscosity of purple-fleshed sweetpotato puree (18.1% dry matter content) at different temperatures

CHAPTER 3

CONTINUOUS FLOW MICROWAVE-ASSISTED PROCESSING AND ASEPTIC PACKAGING OF PURPLE-FLESHED SWEETPOTATO PUREES

3.1 – ABSTRACT

Pumpable purees from purple-flesh sweetpotatoes were subjected to microwave heating using a 60 kW continuous flow system, followed by aseptic packaging in flexible pouches to obtain a shelf-stable product. Test runs were conducted in a 5 kW microwave system to measure dielectric properties and examine the puree temperature profiles. Results demonstrated uniformity in heating of the puree at sterilization temperatures and above, and the dielectric constant and loss factor were within the range of published values for orange-fleshed sweetpotato purees. The pilot-scale test runs in a 60 kW microwave unit produced shelf-stable puree packages for >90 days at room temperature. Nutraceutical components of the purple-fleshed sweetpotato purees were evaluated and showed that while total phenolics increased and total monomeric anthocyanins slightly decreased with microwave application, antioxidant activity determined by DPPH radical scavenging activity and oxygen radical absorbance capacity (ORAC) assays, did not significantly change due to microwave processing. Color values showed a loss of saturation and hue due to processing but the L* a* b* values were comparable with the reported values for purple-fleshed sweetpotatoes. Purple-fleshed sweetpotato purees increased in gel strength with microwave application and this could be a negative or positive attribute. Overall, high quality retention can be obtained by microwave processing and aseptic packaging of purple-fleshed sweetpotato purees for functional food ingredients.

3.2 – INTRODUCTION

A process for making purple-fleshed sweetpotato purees by adjusting the dry matter content of the steamed slices to 18% before grinding was developed (Chapter 2). With high polyphenolic content and antioxidant activities the purple-fleshed sweetpotato purees have great potential as ingredients for functional foods and nutraceutical products.

In the United States, the canned and frozen purees have been produced at commercial levels from orange-fleshed sweetpotatoes for use in processed products such as breads, beverages, dehydrated flakes, patties, soups and baby foods (Kays, 1985; Fasina et al., 2003). These processing technologies can be readily applied for the purple-fleshed sweetpotato purees. However, the frozen puree requires substantial investment in frozen storage and transportation, as well as lengthy and poorly controlled defrosting steps before use. On the other hand, canning requires long retort times due to the thick viscosity of sweetpotato purees and often causes over-processing that result in poor color retention, high nutrient loss, and the formation of off-flavors. These commercially available processing methods have shortcomings that leading to the popularity of alternate forms of processing. Recently, a continuous flow microwave-assisted aseptic process was developed for orange-fleshed sweetpotato purees and other flowable foods (Coronel et al., 2005). This process offers the advantage of producing a shelf-stable product, and avoids long retort processing schedules to maintain high puree quality. This research explored the feasibility of applying

continuous flow microwave heating technology and aseptic processing to the purple-fleshed sweetpotato puree in order to produce a shelf-stable product. The study also included an evaluation of the in-line dielectric properties and heating performance of the material during microwave application, and nutraceutical analysis of the puree as affected by microwave processing.

3.3 – MATERIALS AND METHODS

3.3.1 – Chemicals

Chlorogenic acid, Folin-Ciocalteau reagent, and Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma-Aldrich (Milwaukee, WI) while AAPH [2,2'-azobis(2-amidinopropane) dihydrochloride] was from Wako Chemicals USA (Richmond, VA). All solvents and chemicals were of analytical grade.

3.3.2 – Puree Production

Purple-fleshed sweetpotatoes (Stokes Purple cultivar) were procured from Saura Pride Sweetpotatoes (Walnut Cove, NC). This new cultivar was coded NC 414 in the germplasm collections of the Sweetpotato Breeding Progam, NC State University. The harvested roots (about 500 kg) were cured at 30 °C, 85-90% relative humidity for seven days and stored at 13 °C, 85-90% relative humidity, until they were shipped to the Fruit and Vegetable Pilot Plant, Department of Food science, NCSU. The roots were tumble washed, sliced to 0.65 cm

thickness (Louis Allis Co. Slicer, Milwaukee, WI), and steam cooked for 20 min in a thermoscrew steam cooker (Rietz Manufacturing Co., Santa Rosa, CA). A ribbon mixer (Keebler Engineering Co., Chicago, IL) was used to add water to the slices to adjust dry matter content to 18.1%, and this mixture was then pureed using a hammer mill (Model D, Fitzpatrick Co., Chicago, IL) fitted with a 0.15 cm screen. Puree was placed in 5 gallon buckets, sealed with lids, and frozen at -20 °C until later use.

3.3.3 – 5 kW Test Runs

A 5 kW system was used to evaluate how purple-fleshed sweetpotato purees reacted to microwave heating. Based on the results of test runs, it could be determined if purple-fleshed sweetpotatoes were suitable for scale up to an industrial system. Figure 1 shows a continuous flow microwave heating unit (Industrial Microwave Systems, Morrisville, NC) consisting of a 5 kW microwave generator operating at 915 MHz, a waveguide of rectangular cross-section, and a specially designed focused applicator that was used to process purple-fleshed sweetpotato puree. Puree was pumped using a positive displacement pump (Model MD012, Seepex GmbH+ Co, Bottrop, Germany) with a variable speed motor (Tri-Clover Rotary Pump, Model PRE3-1M, Ladish Co., Kenosha, WI) at a rate of 0.9 L/min through a tube of 1.5” nominal diameter (0.038 m ID) made of polytetrafluoroethylene (PTFE or Teflon®) placed at the center of the applicator. Temperatures at the inlet and outlet of the applicators were monitored using a thermocouple arrangement designed by Coronel et al. (2003) and a datalogging

system (Model DAS-16, Keithley Metrabyte Inc., Taunton, MA). Purple-fleshed sweetpotato puree was continually circulated until a centerline exit temperature of 135 °C was reached. A dielectric probe (HP 85070E) was inserted at the outlet of the applicator in 1 of the 3 ports of the smart gasket (Coronel et al., 2003) to measure dielectric constant and loss factor (Kumar et al., 2007). Duplicate runs were conducted.

3.3.4 – 60 kW Scale-up Runs

Scale up to the 60 kW system involved heating the puree to 65 °C with a tubular heat exchanger using steam as the heating medium. The puree was then loaded into the hopper of a 60 kW continuous flow microwave-heating unit (Industrial Microwave Systems, Morrisville, NC) operating at 915 MHz (Figure 2). Microwaves were generated and delivered to the puree by a waveguide of rectangular cross-section which was split into two sections and led to two specially designed applicators. A PTFE tube of the same specifications as used in the 5kW system was placed at the center of each applicator and had a corresponding exposure region of 0.2m.

A positive displacement pump (Model A7000, Marlen Research Corp, Overland Park, KS) pumped the puree through the system. Temperatures were measured at the inlet and outlet of the system and each applicator by a thermocouple arrangement described by Coronel et al. (2003). The temperature at the outlet of the system was achieved by controlling the power to the microwave system and each applicator varied from 25-30 kW.

The product was heated to 135-145 °C, held for 30 s, cooled using a tubular heat exchanger, and then aseptically packaged in aluminum-polyethylene elaminated bags (Scholle Corp, Chicago, IL) using a bag-in-box unit (Model PT A.F., Astepo, Parma, Italy). Duplicate runs of purple puree were carried out in the 60kW system. Aseptically packaged puree was stored at room temperature, about 22 °C, until analyzed.

3.3.5 – Microbiological Testing

An aseptic pouch from each 60 kW run was incubated at 37 °C and observed for changes in the appearance of the bags for 14 days. After this observation period, another aseptic pouch from each run was sent to Silliker Labs Inc. (Cedar Rapids, IA) for microbiological testing. Aerobic plate counts, mesophilic anaerobic spores, thermophilic aerobic spores, and thermophilic anaerobic spores were enumerated to verify the aseptic process.

3.3.6 – Sampling

Samples were taken at each stage of the microwave process: fresh puree production, puree processed in the 5 kW system, puree heated by tubular heat exchanger and finally purees that ran through the 60 kW microwave system. Samples were taken in duplicate, 250 g was weighed into plastic containers, covered with cheesecloth and frozen at -80 °C. Samples were removed from frozen storage and placed into a VirTis Genesis 25XL freeze dryer (Gardiner, NY) that operated at -35 to -40 °C. They were allowed to dry for one week and

then removed and placed in Ziploc® bags until they warmed up to room temperature. The samples were weighed and grinded into powder using a Mr. Coffee® precision coffee grinder (Sunbeam Boca Raton, FL). The powders were placed in sample vials and kept in -80 °C storage until analysis.

3.3.7 – Nutraceutical Analysis

3.3.7.1 – Preparation of the Extracts

Extraction of polyphenolic compounds from dried sweetpotato powders was performed using an accelerated solvent extractor (Dionex ASE 200, Sunnyvale, CA) equipped with a solvent controller. Three cellulose filters were placed in the bottom of a 22 ml stainless steel extraction cell and covered with 2 g of sea sand (Fisher Scientific, Pittsburgh, PA). Sweetpotato powder (0.25 g) was mixed with 26 g of sand, loaded into the cell, and then sealed tightly. Extraction parameters for all extracts were set as followed: pressure, 1500 psi; temperature, 100 °C; extraction time, 3x5 minute cycles; flushing volume, 60%; and nitrogen purge time, 60 seconds. All powders were extracted with a solvent containing 7% acetic acid in 80% methanol that was purged with nitrogen gas prior to use to prevent phenolic oxidation during the extraction. Extracts were collected in UV-proof glass vials, adjusted to 50 ml volume with solvent, dispersed into 10 ml serum tubes and kept at -80 °C until nutraceutical analysis.

3.3.7.2 – Total Phenolics

Total phenolic compounds were quantified using a modified Folin-Ciolctau method (Singleton et al., 1999). Chlorogenic acid was used as the standard. Samples and standards were diluted in 4 ml of water and 0.5 ml of the FC reagent was added and allowed to react for 3 minutes. Then, 0.5 ml 1 N sodium carbonate was added and allowed to react for one hour. Samples were read for absorbance at 725 nm using a Varian Spectrophotmeter (Cary WinUV Model 300, Palo Alto, CA). A blank was used to calibrate the machine that contained 0.25 ml water instead of sample, along with the same amount of water for dilution, FC reagent, and sodium carbonate. Total phenolic values were reported in milligrams chlorogenic acid equivalents per 100 grams fresh weight (mg CAE/100 g fw).

3.3.7.3 – Total Monomeric Anthocyanins

Total monomeric anthocyanin content was determined using the pH-differential method (Wrolstad and Guisti, 2001). Two dilutions were performed on each sample. The first used potassium chloride (0.025 M) at pH 1 and the second was with sodium acetate (0.4 M) at pH 4.5. Samples were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 minutes before absorbance at 530 and 700 nm were recorded using a spectrophotometer calibrated with distilled water as the blank.

The difference in absorbance between pH's and wavelengths was calculated:

$$A = (A_{530nm} - A_{700nm})_{pH1.0} - (A_{530nm} - A_{700nm})_{pH4.5}$$

This was used to calculate monomeric anthocyanin pigment concentration using:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

where MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivitiy, and 1 is for a standard 1cm pathlength. The molecular weight (MW = 449.2) and molar absorptivity (ε = 26,900) correspond to the most predominant anthocyanin in the sample and values for cyanidin-3-glucoside were used. Total monomeric anthocyanins were reported as mg anthocyanins per 100 g fresh weight (mg anthocyanins/100 g fw).

3.3.7.4 – 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant activity determined by the DPPH assay is based on the methodology of Brand-Williams et al. (1995). Trolox was used as a standard and concentrations ranging from 0-0.5 µM were used to create a standard curve. Samples were diluted 10-fold and then 100 µl was added to 1.9 mL of DPPH solution and allowed to react for 3 hours. Absorbances of standards and samples were read at 515 nm with a spectrophotometer. Results were expressed in µM trolox equivalents per gram fresh weight (µM TE/g fw).

3.3.7.5 – Oxygen Radical Absorbance Capacity (ORAC)

The ORAC procedure established by Prior et al. (2003) was followed. Fluorescence intensity measurements were performed using a Safire monochromator based microplate reader equipped with Magellan V4-W reader

software (Tecan USA, Research Triangle Park, NC). Samples were loaded into 96-well transparent Costar polystyrene flat bottom plates (Corning, Acton, MA). The concentrations of reagents prepared were the same as described by Prior et al. (2003) except that the samples were diluted 100-fold. Wells were filled with 70 µl of phosphate buffer, 60 µl of fluorescein solution, and 60 µl of standard or sample. For blank wells, phosphate buffer was used in place of the sample. The plate was incubated at 37 °C for 15 minutes before 60 µl of AAPH was rapidly added to each well. Plates were shaken orbitally for 5 seconds at the start and between 1 minute reading intervals. Measurements were performed with 80 cycles using excitation and emission filter wavelengths of 485 and 520 nm, respectively.

ORAC values were calculated using a regression equation Y = mx + b for a linear regression on the range of 6.25-100 µM trolox standards. Y is the concentration and x is the net area under the fluorescence decay curve. The area under the curve was calculated as follows:

$$AUC = (0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + \dots + f_i/f_4) \times CT$$

where f_4 is the initial fluorescence reading at cycle 4, f_i is the fluorescence reading at cycle i, and CT is the cycle time in minutes. The net area under the curve was obtained by subtracting the area under the curve for the blank values from the curves of samples and standards. ORAC values were expressed in µM trolox equivalents per gram of fresh weight (µM TE/g fw).

3.3.7.6 – Color Measurements

Hunter L*a*b* values were measured with a Hunter colorimeter (D25/DP9000 Tristimulus Colorimeter, Hunter Associate Laboratories Inc., Reston, VA). The cooked sweetpotato samples were filled into a 35 mm petri dish, covered, and pressed against the surface to remove air bubbles. The colorimeter was calibrated against a standard white tile (L* = 92.75, a* = -0.76, b* = -0.07) and sample measurements were taken at 3 different locations, with duplicates performed for each sample. Averages of these readings are reported. Hue angle (h°) was calculated using arc tan(b*/a*), chroma (C*) as $[a^{*2} + b^{*2}]^{1/2}$, and ΔE as $((\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)^{1/2}$.

3.3.7.7 – Moisture Analysis

Moisture analysis was measured two ways for each sample. The first was based on the initial and final weights of the freeze dried samples. Moisture content was also established based on the AOAC oven drying method, (AOAC, 2006) 24 hours at 100°C. Moisture content was converted to dry matter for both methods and for the purpose of calculations dry matter established by freeze-drying was used.

3.3.8 – Rheological Testing

The gel properties of fresh and microwaved purees were evaluated by small amplitude oscillatory rheology (SAOR) using a stress-controlled ATS Stresstech Rheometer (Rheosystems, Bordentown, NJ) outfitted with parallel

plate (20 mm diameter) geometry. Frequency sweeps were conducted for a range of 0.1 to 20 Hz with stress held constant at 20 Pa, which was within the linear viscoelastic region. Fresh puree samples were evaluated under a gap height of 1.5 mm. For microwaved purees, cylindrical samples (diameter = 25 mm) were taken at room temperature using a metal cylindrical punch. This cylinder was then trimmed to 3.5 mm thickness and the cylindrical samples were glued (Loctite 401 Instant Adhesive, Henkel Corp, Louisville, KY) to the upper and lower plate of the rheometer with a gap of 3 mm. The diameter of the cylinder was trimmed to match the parallel plate and edges were covered with lubricant (Super Lube®, Bohemia, NY) to prevent the sample from drying out. Measurements were taken at 25 °C and the G modulus was examined for gel characteristics.

3.3.9 – Statistical Analysis

Group differences were evaluated using analysis of variance (ANOVA) F-tests using the SAS Statistical Analysis System v8.1 (SAS Institute Inc., Cary, NC) with $p<0.05$ considered to be a statistically significant difference. Means were separated by the Student-Newman-Keuls (SNK) procedure. This was chosen due to the unequal sample sizes of the lab samples, and this procedure accounts for that by using the harmonic mean. The SNK procedure is less conservative than other methods of means separation, which means that it is more likely to declare a difference between values.

3.4 – RESULTS AND DISCUSSION

3.4.1 – 5 kW test runs

Purple-fleshed sweetpotato purees were processed in a 5 kW microwave unit to determine the suitability of the materials for microwave processing based on temperature profiles and dielectric properties. Inlet temperatures showed uniformity at the center, intermediate and walls of the heating tube (Figure 3). Larger temperature differences were noted for the outlet streams, especially between the center/intermediate space and the walls (Figure 4). As microwave heating increases the temperature of the puree to sterilization (121 °C) the temperature differences among these streams become closer (Figure 4). Smaller temperature differences in outlet streams is indicative of increased uniformity in heating of the puree at sterilization temperatures and above.

The dielectric properties of purple-fleshed sweetpotato purees were measured under continuous conditions with a probe placed at the exit of the applicator in the 5 kW system. Dielectric constant (ε') and dielectric loss factor (ε") with respect to temperature are shown in Figure 5. The values for ε' decrease with an increase in temperature which is in accordance with the observations of Datta et al. (1997) for food products with greater than 60% moisture. Also, it is established that dielectric constant decreases with increasing temperature due to a decrease in dielectric relaxation time. Relaxation time is associated with the time for the dipoles to revert to random orientation when the electric field is removed and decreases as temperature

increases (Simnu and Sahin, 2005). Dielectric constant decreased from 70.4 at 20 °C to 47.7 at 135 °C. These values were slightly higher than those reported by Brinley et al. (2007) for purees made from the Okinawa and NC 415 purple-fleshed cultivars which were measured under static conditions. Since Kumar et al. (2007) has shown similar dielectric properties measured under static and continuous conditions for vegetable purees, the discrepancy can be attributable to the differences in moisture content among the purees. Brinley et al. (2007) did not dilute the purees to make them flowable and the moisture content in the Okinawa and NC 415 purees were 30 and 32%, respectively, as compared to 18.1% for the puree prepared in this study. Dielectric constant values for purple-fleshed sweetpotato purees were very close to those reported for orange-fleshed sweetpotatoes (Brinley et al., 2007; Coronel et al., 2005). This was favorable since the viscosity of purple-fleshed sweetpotato puree was adjusted to mimic that of an orange-fleshed sweetpotato puree. Viscosity and moisture content are among the main factors that affect dielectric properties in microwave processing. Brinley et al. (2007) reported that moisture content had a significant effect on dielectric constant of sweetpotato purees derived from various cultivars. An increase in free water in the puree will lead to an increase of the number of polar molecules that will re-orient with changes in electric field to cause an elevated dielectric constant (Sumnu and Sahin, 2005).

Dielectric loss factor was shown to increase with an increase in temperature which was also in accordance with the previous reports (Datta et al., 1997). Ionic conductivity and dipole rotation both contribute to the increase in

dielectric loss factor (Wang et al., 2003). Herve et al., (1998) showed that the reduction in viscosity of cottage cheese at higher temperatures led to an increased mobility of ions and electrical conductivity to ultimately cause an increase in heating. Dielectric loss factor values increased from 14.1 at 20 °C to 39.4 at 135 °C for the purple-fleshed sweetpotatoes (Figure 5). These values are similar to those of purple-fleshed and orange-fleshed sweetpotato purees (Brinley et al., 2007; Coronel et al., 2005).

The results of the 5 kW test runs indicated that purple-fleshed sweetpotato puree was interacting with microwave heating in a similar manner to orange-fleshed sweetpotato purees. Therefore, processing and aseptic packaging of the purple-fleshed sweetpotato puree in a 60 kW microwave system would be highly feasible as demonstrated in the following section.

3.4.2 – 60 kW microwave runs

The temperature of purple-fleshed sweetpotato puree was measured with thermocouples positioned at the inlet of the system, the inlet of each applicator and the inlet and exit of the holding tube (Figure 2). Figures 6 and 7 show time-temperature profiles recorded during microwave processing of purple-fleshed sweetpotato puree during the first run. The puree entered the microwave heating section at about 50 °C and exited the second applicator at a target temperature of 135 to 150 °C (Figure 6). Purple-fleshed sweetpotato passed through the heating section at a flow rate of about 4.0 L/min and spent an estimated 25 s in the 2.4 m holding tube (ID = 22.9 mm). Outlet temperatures for the cooling

section indicated that puree was pumped out of the system at 10 to 20 °C (Figure 7). This temperature was too low and led to a thickening of the puree resulting in large increase in the back pressure of the microwave system. For a batch of sweetpotato roots which were not properly cured after harvesting, the high starch content in the puree resulted in gel formation that clogged the system and increased the back pressure to dangerous levels. With this experience the cooling temperature was maintained at 40 °C in the duplicate test run and the system ran without any problems (Figure 8). On this second run there was a greater temperature difference between the center and intermediate space of the holding tube (Figure 8).

The F_0 values were calculated based on the time-temperature history of the holding tubes. For run 1, F_0 value at the center for the fastest liquid particle was 3.25 min, while for run 2 this value was 5.25 min. This is logical because the center temperature of the holding tube for run 2 was consistently a few degrees higher than the center temperature of the holding tube in run 1. Higher processing temperatures will increase F_0 values. Furthermore these F_0 values fall within the range reported by Brinley et al. (2007) of 0.65, 2.80 and 10.10 min for target temperatures of 126, 132 and 138 °C, respectively.

3.4.3 – Microbiology

Aseptic pouches incubated at 37 °C were monitored for two weeks for changes in appearance. This environment was used to perfect conditions for thermophilic spoilage which would indicate inadequate processing for

commercial sterility. Thermophilic spoilage would lead to gas production and cause a ballooning of the bag. Since there was no visible change noted after two weeks of incubation, it was likely that the thermal treatment was adequate. The results from Sillikar Laboratories summarized in Table 1 confirmed the sterility of the packaged purees. All tests were negative or reported values below the detection limits except for one aerobic plate count. Since aerobic plate counts will rarely enumerate organisms of concern in thermal processing, colonies that formed could be due to less important spoilage organisms that somehow survived the microwave process or are to do some form of post-process contamination.

3.4.4 – Nutraceutical Properties

3.4.4.1 – Total Phenolics

Total phenolic content ranged from 313.6 to 353.8 mg CAE/100 g fw for purple-fleshed sweetpotato puree and 60 kW microwave samples, respectively. Phenolic content for the 60 kW microwaved samples was significantly higher than all other samples evaluated. This increase places 60 kW microwaved puree in the range found for raw and steamed flesh and whole purple-fleshed sweetpotato results (Presented in Chapter 2). These values are much higher than the total phenolic content range of 78.6 to 181.4 mg CAE/100 g fw for orange-fleshed sweetpotatoes (Truong et al., 2007). The phenolic content in the microwaved purple-fleshed sweetpotato puree was within the range of other high

antioxidant food commodities. However, the phenolic contents of red-fleshed sweetpotato and purple carrot are much higher and are reported as 945, and 1756 mg CAE/100g fw, respectively (Cevallos-Casals and Cisneros-Zevallos, 2003). Wild chokeberries contain 2,556 mg CAE/100 g fw which is far greater than the contents of lingonberries, cranberries and blueberries (Zheng and Wang, 2003). Chun et al. (2005) analyzed the total phenolic content in gallic acid equivalents (GAE) for a wide range of fruits and found that plums have 368.7 mg GAE/100 g fw while strawberries and cherries have 225.0 and 55.8 mg GAE/100 g fw, respectively. Blueberries can also have high phenolic contents and range from 399.3 ± 149.1 to 556.1 ± 216.9 mg GAE/100g fw (Sellappan et al., 2002).

Generally, thermal processing to sterilization levels shows a decrease in phenolic content. Strawberry phenolic content decreased by almost 50% from 61 to 35.6 mg GAE/100 g fw, while canned wild and cultivated blueberry products also exhibited significant decreases in phenolic compounds (Klopotek et al., 2005; Schmidt et al., 2005). However, hot air dried tomatoes had a significant increase of 13 and 29% in total phenolics as referred to the fresh samples from two different cultivars (Chang et al., 2006). Oregano treated by industrial microwaves was also shown to increase in total phenolic content from 112.1 to 135.3 mg GAE/g fw (Bertelli et al., 2004). Pepper and basil increased in phenolic content as well, though not significantly. Microwave application has also been shown to stimulate the production of phenolic compounds by 700% when applied to germinated sprouts of fava beans (Randhir and Shetty, 2004). These reports

are in agreement with a 12% increase in total phenolic content of the 60 kW microwave samples as compared to the fresh puree (Table 2).

Chang et al. (2006) postulated that an increase in phenolic contents could be due to the liberation of phenolic compounds from the matrix during food processes. Phenolic compounds are secondary metabolites that usually accumulate in the vacuoles and are therefore located in outer tissues. The breakdown of cellular membranes resulting from food processing could lead to an accelerated release of bound phenolic compounds (Chang et al., 2006).

3.4.4.2 – Total monomeric anthocyanins

Total monomeric anthocyanins were found to decrease from 57.5 for fresh puree to 46.1 mg/100 g fw for a 60 kW microwave sample (Table 2). This 20% difference in anthocyanin components was found to be significant ($p<0.05$) but both 5 and 60 kW microwave treatments showed the same level of destruction. Ancos et al. (1999) reported that strawberry purees maintained anthocyanin content when treated by microwaves at powers ranging from 285-850 W for 15, 30, 45 and 60 s. However, microwaves operating at 2450 MHz were utilized for this experiment, which are known to deliver less power and have a smaller penetration depth than those of the industrial microwave systems that operate at 915 MHz (Singh and Heldman, 2001). The fact that purple-fleshed sweetpotato purees were treated with a higher powered microwave system could account for a slight decrease in anthocyanin content.

The difference in anthocyanin content between samples was reflected in the Hunter L*a*b* values and summarized in Table 3. L* is a lightness index and ranges from 0-100 with 0 representing black and 100 representing white. The pre-heated and 60 kW microwave samples were significantly ($p<0.05$) darker than the untreated puree and 5 kW samples. The 5 kW puree sample was the lightest, and this was in accordance with a lightening of strawberry purees that undergo microwave treatment by Ancos et al. (1999). However, Lee et al. (2002) showed that pasteurized blueberry juice darkened from L* = 36.4 to 23.3 when an initial blanching step was employed. The intensity of red color is represented by a* value while blue color is represented by a negative b* value. Both red and blue intensity decreased for purple-fleshed sweetpotato samples processed by microwave heating.

Masuda et al. (2002) reported Hunter color values for Ayamurasaki and Kyushu-132 as L*: 44.0 and 45.5, a*: 21.6, 21.5, and b*: -6.7, and -7.7, respectively. Despite microwave processing, purple-fleshed sweetpotato purees made from cultivar NC 414 compared well with these ranges, except in the case of L*. Generally, the cultivar used in this study was almost twice as dark as the Japanese cultivars. Puree and pre-heated samples had higher hue angles signifying a more blue-ish purple color than microwave treated samples. Puree also had a significantly higher ($p<0.05$) chroma value showing an increase in saturation of purple color (Table 3). Overall, microwave processing caused a decrease in hue angle and saturation but these color attributes were not different from the reported values for other cultivars of purple-fleshed sweetpotatoes. This

is further supported by the overall color change, represented by ΔE (Table 3) which is not significant for any of the microwave samples.

Purple color in sweetpotatoes is due to anthocyanins that are mono- or di-acylated forms of peonidin and cyanindin (Terahara et al., 2004). Yoshinga et al. (1999) found that purple-fleshed sweetpotato clones could be classified into two groups based on their b*/a* ratio. Clones that have ratios less than -1.4 are blue dominant which tend to have more cyanidin than peonidin while clones with ratios greater than -1.1 are red dominant and have more peonidin. Based on this finding, the samples evaluated in this study were more peonidin rich since the b*/a* ratios ranged from -0.59 to -0.51.

Anthocyanins are present in a wide range of fruits, including mostly berries, and some vegetables. Wu et al. (2006) reported that chokeberries and elderberries have the highest anthocyanin content of all food commodities examined with 1480 and 1375 mg anthocyanins/100g fw respectively. Black currant, blueberries and red cabbage are more common food commodities that are known to have higher anthocyanin contents, ranging from 322 ± 40.8 to 476 ± 115 mg anthocyanins/100 g fw. Red fleshed potatoes have up to 25.5 mg anthocyanins/100 g fw, while red-fleshed sweetpotatoes are much higher with 182 ± 2 mg anthocyanins/100 g fw (Rodriguez-Saona et al., 1998; Cevallos-Casals and Cisneros-Zevallos, 2003). Purple-fleshed sweetpotato puree (fresh and microwaved samples) were on the lower end of the spectrum having anthocyanin contents comparable to black bean, red onion, and strawberries that range from 41.7 to 48.5 mg/100 g fw. While these are lower values, they are still

greater than anthocyanin contents found in apples, and red grapes which are 12.3 and 26.7 mg/100 g fw, respectively (Wu et al., 2006).

3.4.4.3 – Antioxidant Activity

Based on the DPPH assay, antioxidant activity ranged from 47.0 to 50.2 μmol TE/g fw for puree and 5 kW samples, respectively (Table 2). No sample showed significantly ($p<0.05$) lower or higher antioxidant activity. These DPPH values are about half of what was reported for raw peels of purple-fleshed sweetpotatoes in earlier studies. While the process of transforming whole purple-fleshed sweetpotato roots into puree causes a decrease in antioxidant activity, it is important to note that once puree is formed and microwave processed, there is no further decrease. This means that despite increases in total phenolic content and decreases in total monomeric anthocyanins; microwave application maintains antioxidant activity as determined by the DPPH assay.

This range of DPPH values for microwave samples was on the higher end of DPPH radical scavenging values from 8.6 to 49.0 μmol TE/g fw for a group of sixteen purple-fleshed cultivars reported by Oki et al. (2003). Kano et al. (2005) reported that the DPPH radical scavenging activity of a purple-fleshed sweetpotato cultivar, Ayamurasaki, was higher than those of red cabbage, grape skin, elderberry or purple corn. This is an important finding as purple-fleshed sweetpotatoes do not have quite as high of an anthocyanin content as found for elderberry and purple corn. Orange-fleshed sweetpotatoes (cultivar Beauregard)

have lower DPPH values ranging from about 2.0 μmol TE/g fw for flesh to 7.1 μmol TE/g fw for peels, and as great as 38.2 μmol TE/g fw for leaves (Truong et al., 2007).

The ORAC values ranged from 25.9 to 26.8 μmol TE/g fw for pre-heated and 60 kW samples, respectively (Table 2). The ORAC values followed the same trend as the DPPH values in that no sample presented significantly different ($p<0.05$) radical scavenging ability. Based on ORAC values previously reported, puree and microwave samples are capable of a third of the amount of radical scavenging capacity as peels, and about half as much as flesh and whole root samples (Presented in Chapter 2).

The ORAC values for the purple-sweet potato samples are below what have been reported for cranberries and lowbush blueberries with ORAC values of 92.56 μmol TE/g fw and 92.09 μmol TE/g fw, respectively. However, the range of ORAC values in Table 2 for purple-fleshed sweetpotato puree and microwave samples compared well with fuji and gala apples found to have 25.7 and 28.0 μmol TE/g fw, respectively. Despite the ORAC value for these samples being on the lower range of the spectrum samples, puree and microwave samples were still higher than the ORAC values reported for several Florida-grown tropical fruits. Of the 14 fruits evaluated, the highest ORAC value was 16.7 μmol TE/g puree for red guava followed by lower values for ripe papaya and mango with 5.3 and 2.2 μmol TE/g fw, respectively (Mahattanatawee et al., 2006).

3.4.5 – Rheological Testing

When the samples tested in the 5 kW microwave cooled it was noticed that gel structure of the puree seemed stronger. Samples processed in the 60 kW system exhibited the same behavior and therefore underwent small amplitude oscillatory rheology (SAOR) to obtain the G* modulus. This has two components, G' the storage modulus or elastic component and G" the loss modulus, or viscous component. Viscoelastic materials are further defined by the relationship between G' and G". Gel behavior is described the parallel slopes of G' and G" with G' greater than G" throughout the frequency range. This illustrates a dominance of the solid behavior for the viscoelastic material (Steffe, 1996). The G' and G" values as a function of frequency of the fresh puree and puree that has been processed in a 60 kW system are shown in Figure 6.

The G' values for puree and 60 kW microwave processed puree are greater than their G" counterparts, and all run parallel to one another. However, the G' and G" values for 60 kW microwave processed puree are nearly a magnitude greater than the values for fresh purees. Values for G' and G" of purple-fleshed sweetpotato puree were similar but slightly higher than values reported by Fasina et al. (2003) for orange-fleshed sweetpotato puree. Orange-fleshed sweetpotato puree did not exhibit gel strengthening due to microwave processing, so this phenomenon is most likely linked to a difference in carbohydrate components present in the two cultivars.

Depending on the processing application, an increase in gel strength can be a negative or positive attribute. Future work needs to explore other means of

reducing the viscosity of purple-fleshed sweetpotato purees rather than the addition of water. Alpha-amylase has successfully been used to reduce the viscosity of orange-fleshed sweetpotato purees for use in spray-drying. Grabowski et al. (2006) found that alpha-amylase at concentrations of 7.5 and 3.75 ml per kg puree and reaction times up to 30 minutes reduced the viscosity sufficiently for spray-drying. The viscosity of purple-fleshed sweetpotato purees will not be to be reduced that low, but it is possible that the use of enzymes will cleave starch molecules so that they are unable to form such strong networks after processing.

3.5 – CONCLUSIONS

Purple-fleshed sweetpotato puree was successfully processed and aseptically packaged. Total phenolic content showed increases while total monomeric anthocyanins decreased due to processing. Color data showed that microwave processing led to a loss of saturation in blue-ish purple color, but values were still comparable to reported data for other purple-fleshed sweetpotato cultivars. DPPH and ORAC radical scavenging assays showed that despite these changes, purple-fleshed sweetpotato purees maintained their antioxidant activity through microwave processing. The gel strength was significantly increased by microwave processing in a 60 kW system and this problem will need to be evaluated and rectified in future research to ensure that purple-fleshed sweetpotato puree finds success as a functional food ingredient.

This is the first report of purple-fleshed sweetpotato puree being treated by a continuous 60 kW system followed by aseptic packaging.

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Table 1: Microbiological results from Silliker Laboratories

Analyte	Results	Method Reference
Aerobic Plate Count	30/g	AOAC 966.23
Aerobic Plate Count	<10/g	AOAC 966.24
Aerobic Plate Count	<10/g	AOAC 966.25
Mesophilic Anaerobic Spores - MPN 3	<3/g	CMMEF, 4th ed.
Thermophilic Aerobic Spores	<5/10g	CMMEF, 4th ed.
Thermophilic Anaer. Spores (Pos/6)	0/6 tubes positive	CMMEF, 4th ed.

Table 2: Nutraceutical measurements for purple-fleshed sweetpotato puree and microwaved counterparts.

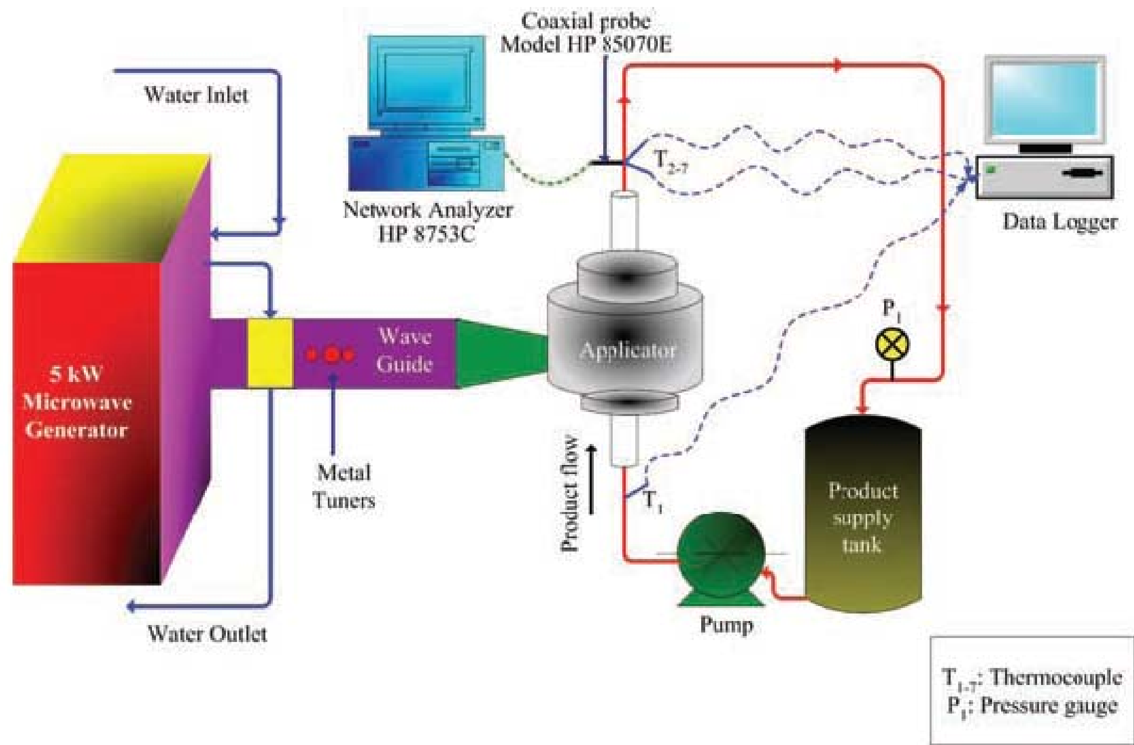
Sample	Dry Matter[1]	TP[2]	TMA[3]	DPPH[4]	ORAC[5]
Puree[6]	18.1	313.6±4.6[b*]	57.5±1.5[a]	47.0±2.6[a]	26.4±1.3[a]
5 kW Micro	17.67	329.1±5.5[b]	46.4±1.3[c]	50.2±2.0[a]	26.7±1.1[a]
Pre-heated[7]	18.28	333.9±4.4[b]	53.9±1.3[b]	49.2±2.2[a]	25.9±0.8[a]
60 kW Micro	18.17	353.8±7.4[a]	46.1±0.7[c]	49.2±2.0[a]	26.8±0.6[a]

1 - Dry matter determined by AOAC oven drying method (1995)
2 - TP: Total phenolics values expressed in mg CAE/100g fw
3 - Total Monomeric Anthocyanin: TMA values expressed as mg anthocyanins/100g fw
4 - DPPH values expressed as mM TE/g fw
5 - ORAC values expressed as mM TE/g fw
6 - Puree made from steamed slices adjusted to 18% dry matter content
7 - Pre-heated puree that was heated to 65°C before 60kW microwave
* subscripts signify significance based on ANOVA F-tests with Student-Newman-Keuls means separation

Table 3: Color data for microwave samples

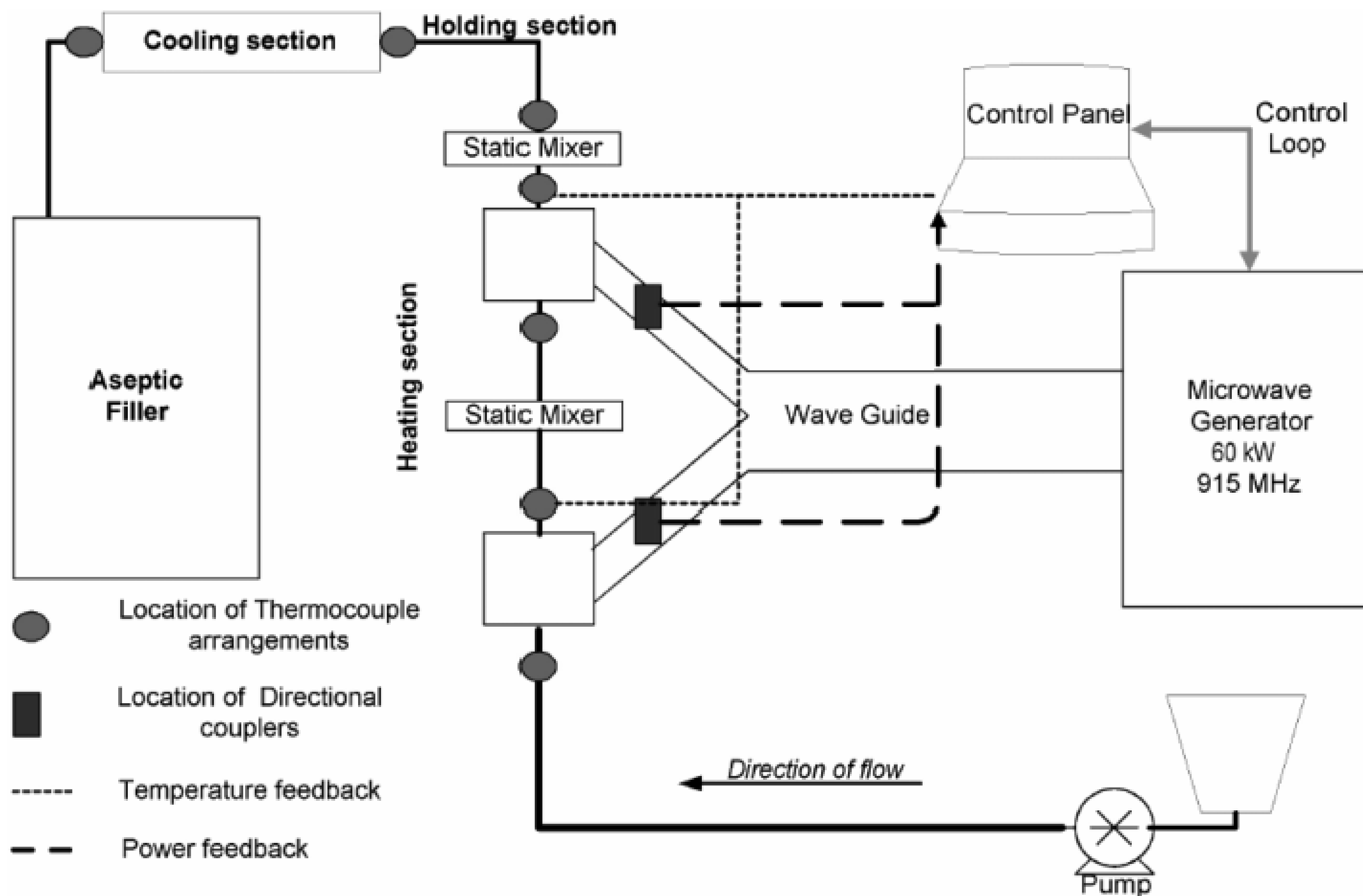
Sample	L*	a*	b*	Hue	Chroma	ΔE
Puree[1]	20.3±0.1[b]	26.2±0.1[a]	-13.3±0.1[a]	-26.9±0.2[b]	29.4±0.1[a]	--
5 kW	20.6±0.1[a]	23.2±0.1[b]	-9.3±0.0[d]	-21.8±0.1[a]	25.0±0.1[b]	5.1±0.1[a]
Heated[2]	19.3±0.0[c]	21.6±1.3[b]	-10.6±0.3[b]	-27.4±0.7[b]	24.1±1.3[b]	5.7±1.2[a]
60 kW	19.2±0.1[c]	22.1±1.3[b]	-8.0±0.3[c]	-18.5±2.4[a]	23.5±1.3[b]	7.7±1.0[a]

1 – Puree made from steamed slices adjusted to 18% dry matter content
2 – Heated puree underwent tubular exchage heating to 60°C before 60 kW microwave processing
3 – ΔE calculated using the average values of L*, a*, b* for Puree



Adapted from Kumar et al., 2007

Figure 1: Schematic Diagram of the 5 kW System



Adapted from Coronel et al., 2005

Figure 2: Schematic diagram of the 60 kW System

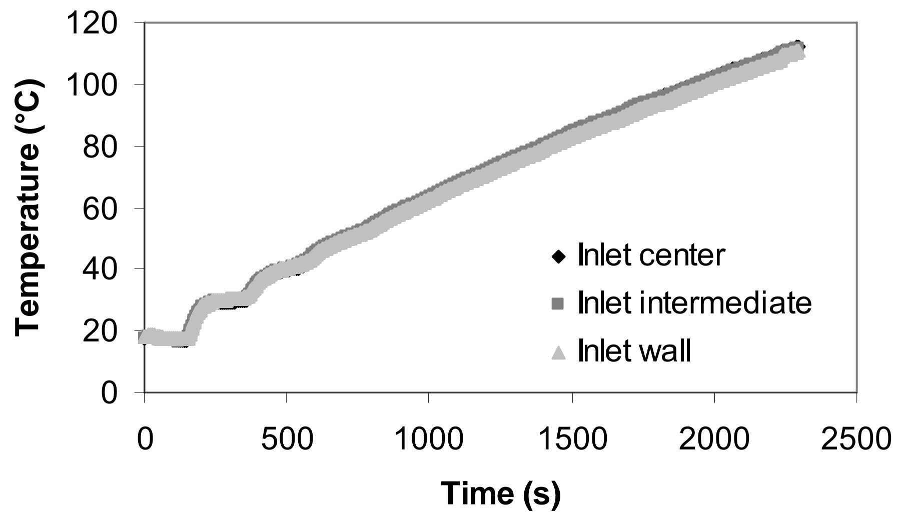


Figure 3: Time-temperature data for inlet streams during 5 kW processing

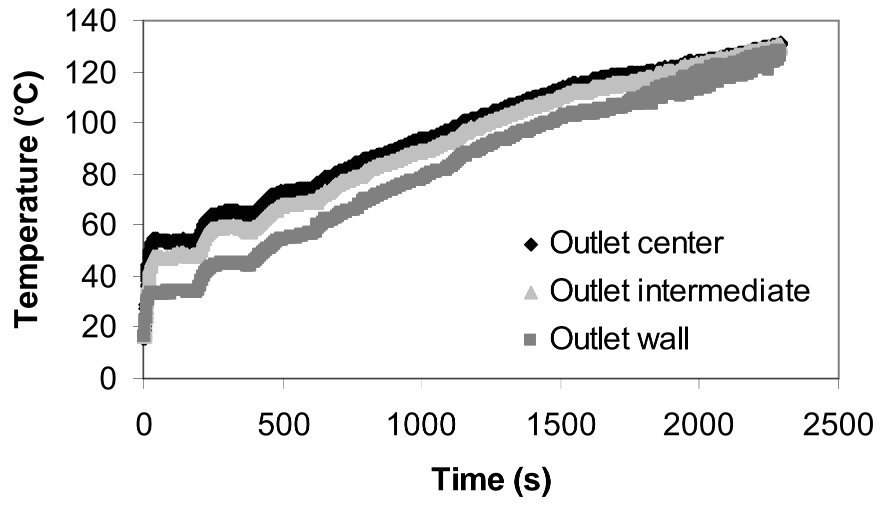


Figure 4: Time-temperature data for outlet streams during 5 kW processing

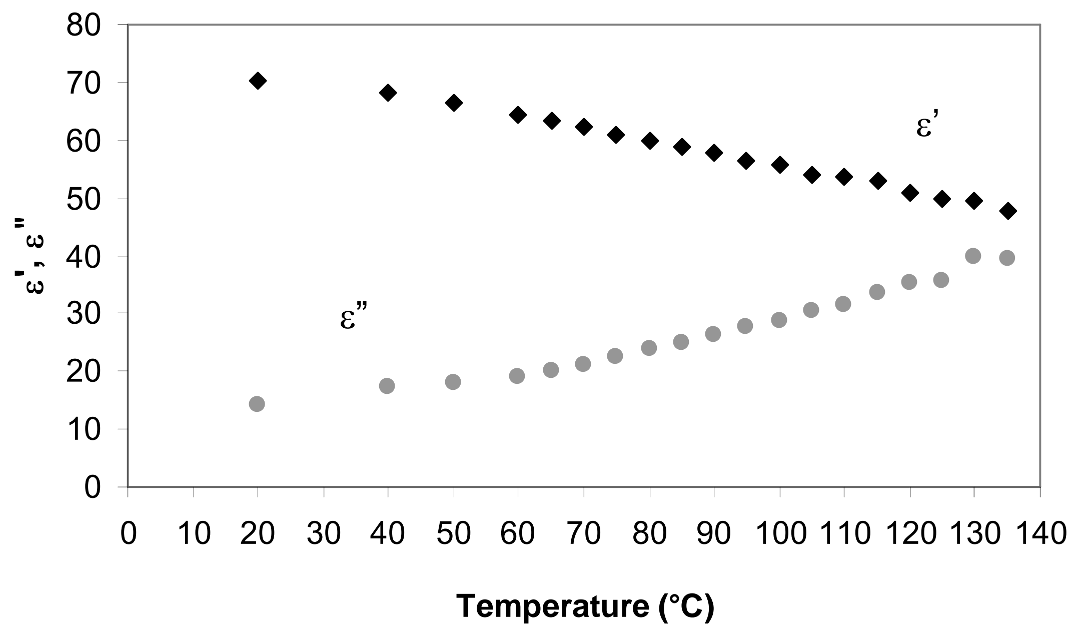


Figure 5: 5 kW In-line dielectric measurements for purple-fleshed sweetpotato puree

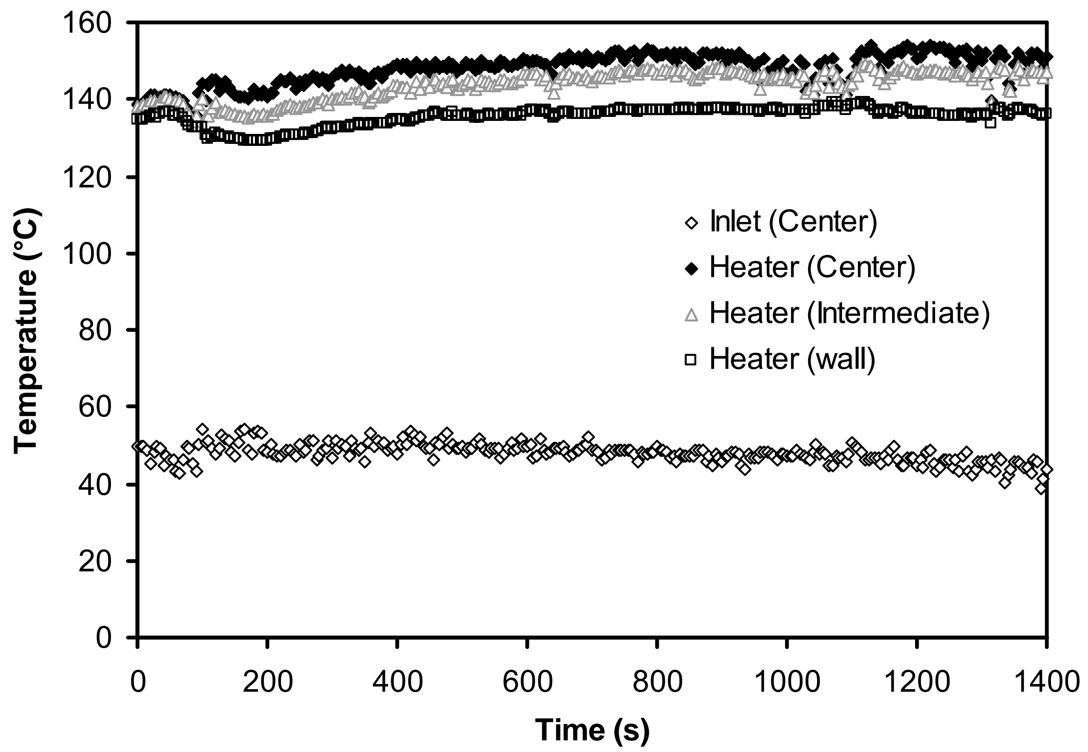


Figure 6: Typical time-temperature history at the inlet of the microwave heating section and outlet of second microwave applicator (Run 1)

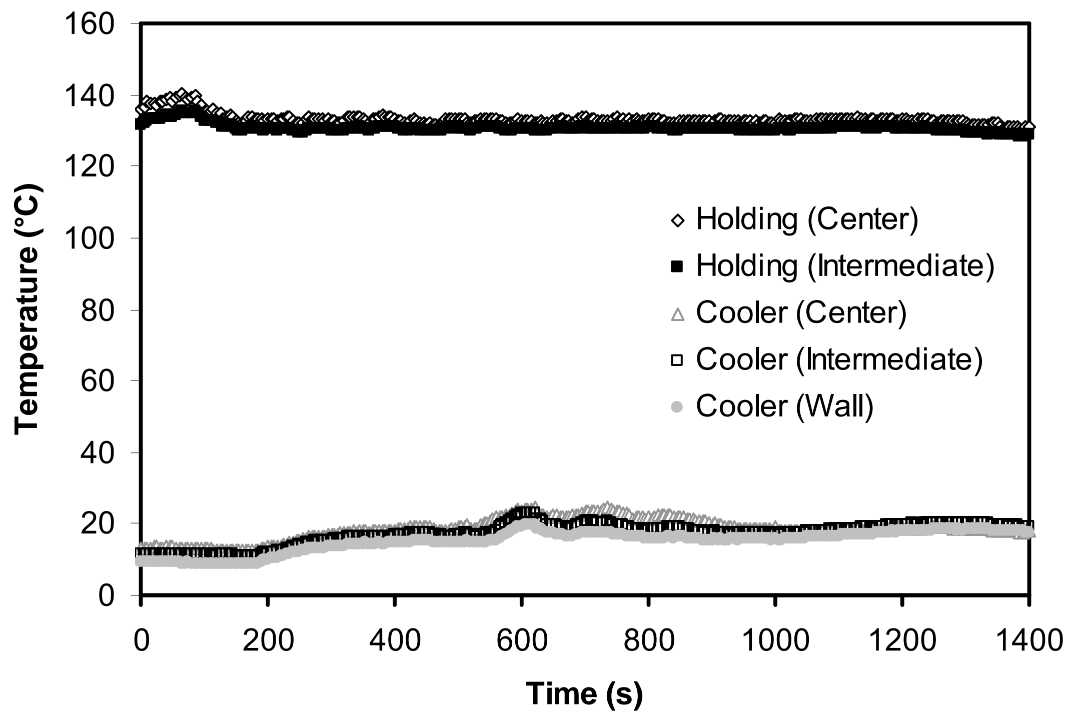


Figure 7: Typical time-temperature history at the exit of the holding and cooling sections (Run 1)

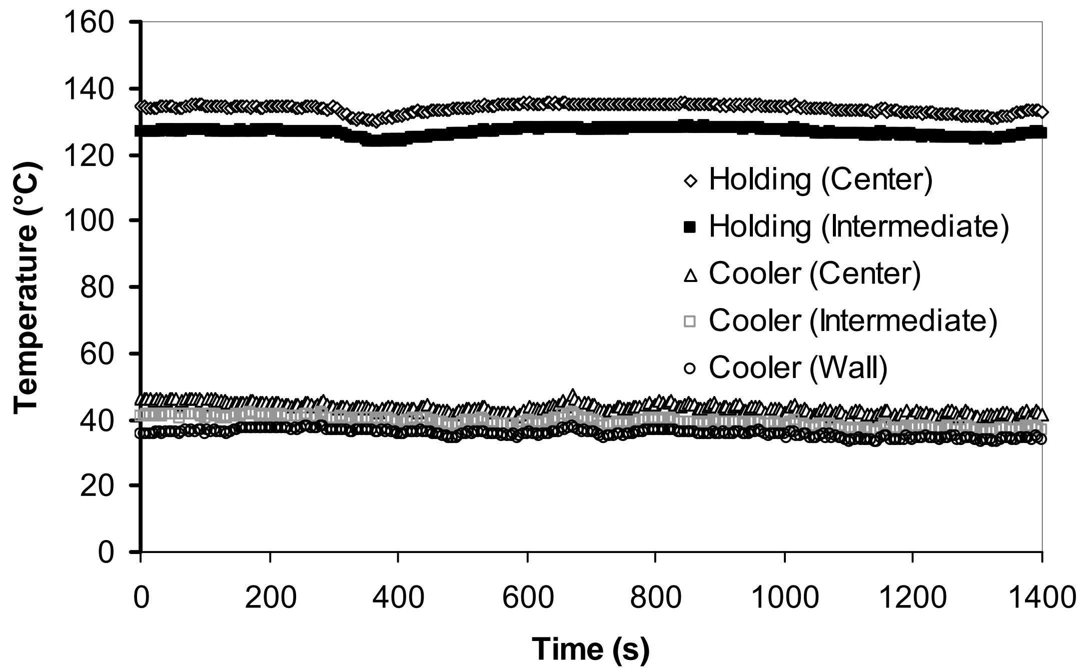


Figure 8: Typical time-temperature history at the exit of the holding and cooling sections (Run 2)

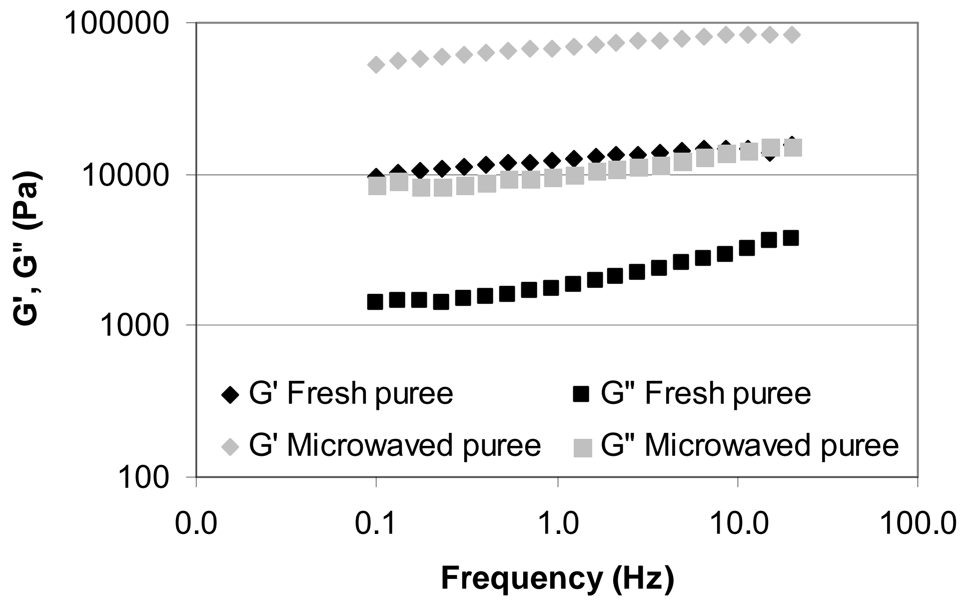


Figure 9: Comparison of G' and G" for fresh and 60 kW microwave processed puree