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EFFECT OF CULTIVAR, STORAGE, COOKING METHOD AND TISSUE TYPE ON THE ASCORBIC ACID, THIAMIN, RIBOFLAVIN AND VITAMIN B6 CONTENT OF SWEETPOTATO [IPOMOEA BATATAS (L.)] LAM

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

In

The School of Plant, Environmental and Soil Sciences

by
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B.S., EARTH University, 2005
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May 2014

To my mother Guillermina Ayala, my father Jacinto Barrera, and my friends Miriam Gil and José Ricardo Ortiz for believing in my dreams when I was a teenager.

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ABSTRACT

The effect of cultivar, curing, storage, tissue type, and cooking method on the ascorbic acid (AA), thiamin, riboflavin, and vitamin B6 content of sweetpotato was determined. A simplified and sensitive reverse-phase high performance liquid chromatography (HPLC) methodology was developed for the simultaneous determination of thiamin and riboflavin in sweetpotato. Curing of sweetpotatoes did not significantly change the content of AA, thiamin, and vitamin B6, but resulted in a decrease in riboflavin content. Thiamin and riboflavin contents were mostly stable after curing. However, compared to at harvest, storage for 6 months resulted in a decrease in AA content in cultivars 07-146, Covington, and Beauregard; and a gradual increase in vitamin B6 content in 07-146, Orleans, and Covington. Although 07-146 contained higher vitamin B6 content, no cultivar was superior or inferior for all the vitamins throughout 6 months of storage. Exposure of sweetpotatoes to chilling injury temperatures of 1 °C and 6 °C for 2 or 4 weeks did not result in consistent changes in AA, thiamin, and riboflavin. However, transfer of the low temperature-stored roots to 14 °C for an additional 7 days generally resulted in AA decreases and stable thiamin and riboflavin contents. Water-soluble vitamin concentration differed between tissue types. Leaf tissue contained no detectable amounts of thiamin, but contained the highest concentrations of AA, riboflavin, and vitamin B6. Cooking methods, including microwaving, boiling, and baking resulted in lower AA compared with raw tissue, but in little differences in thiamin, riboflavin, and vitamin B6. The overall results of this research suggest that while AA is detrimentally affected during commonly used sweetpotato cooking methods, and during typical storage conditions, thiamin, riboflavin, and vitamin B6 contents remain mostly stable. Additionally, they confirm previous reports indicating vegetative tissues can be a good source of AA and multiple B vitamins in human diets.

CHAPTER 1. INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a dicotyledonous plant species that belongs to the Convolvulaceae family. It is an herbaceous perennial plant, but is generally cultivated as an annual crop from vegetative tissues using storage roots or vegetative cuttings (Huaman, 1992). Sweetpotato is one of the world's most important food crops in terms of human consumption, particularly in sub-Saharan Africa, parts of Asia, and the Pacific Islands. The sweetpotato was first domesticated more than 5,000 years ago in Latin America, and it is grown in more developing countries than any other root crop (International Potato Center, 2010).

In 2011, sweetpotato ranked ninth in terms of worldwide production, after maize, rice, wheat, potatoes, soybean, cassava, tomatoes, and bananas; but in developing countries, it is the seventh most important food crop (FAOSTAT, 2013). In 2012, sweetpotato accounted for 12.9% of the world's root and tuber production with a total production of 105 million tons. China is the world's leading sweetpotato producing country with 75.4 million tons (FAOSTAT, 2013). In developing countries, sweetpotato is considered a subsistence or security crop and is often grown under marginal conditions. In developed countries, it is commercially grown as a high-value vegetable crop under intensively managed conditions (Padmaja, 2009).

In the Unites States, sweetpotato production is largely concentrated in North Carolina, Mississippi, Louisiana, and California; although limited production also occurs in other states (Smith et al., 2009). In 2012, North Carolina accounted for 46.8% of the national production, while California, Mississippi and Louisiana produced 23.3%, 13.3% and 7.4%, respectively (NASS, 2013). In 2012, the total US sweetpotato production was estimated at 1.35 million metric tons with an economic value of $500 million dollars (NASS, 2013).

The high nutritional value of sweetpotato has been recognized among various food crops. Sweetpotato contains many nutrients, including crude protein, carbohydrates, minerals, dietary fiber, and vitamins (Bovell-Benjamin, 2007; Picha, 1985) and is a very good source of antioxidants (Islam et al., 2002; Padda, 2006). A very high content of vitamin E , a potent anti-oxidant, has also been reported in sweetpotato roots (Holland et al., 1991). Orange- fleshed sweetpotatoes are considered one of the best sources of vitamin A and global efforts are being devoted to increase their consumption, especially in countries where vitamin A deficiency is a problem (Attaluri and Ilangantileke, 2007; Ndolo et al., 2007; Odebode et al., 2008). Sweetpotato leaves, are also a rich source of vitamins, minerals, and protein (Ishida et al., 2000; Villareal et al., 1979). Sweetpotato has potential to help prevent and reduce food insecurity, malnutrition, and under-nutrition in developing and developed countries because of its nutritional composition and unique agronomic features (Woolfe, 1992). Despite the beneficial nutritional properties of sweetpotato, its consumption is still low in most developed countries. For instance, sweetpotato per-capita consumption in 2009 was 2.4 kg in the Unites States and 0.1 kg in Europe; while the world average was 8.3 kg (FAOSTAT, 2013). Increasing the availability of nutritional information, and exploring the health beneficial properties of sweetpotatoes may increase consumer awareness of the positive attributes of this vegetable and may enhance consumption worldwide.

Besides being considered a rich source of vitamin A, sweetpotato is also considered a good source of vitamin C (ascorbic acid) and certain B vitamins (Padmaja, 2009). A fair amount of research has been conducted on factors affecting beta-carotene (pro-vitamin A) in sweetpotato by different workers (Ezell and Wilcox, 1952; K'Osambo et al., 1999; Bengtsson et al., 2008; Van Jaarsveld et al., 2006; Kidmose et al., 2007). However, research conducted on water-soluble

vitamins (including vitamin C and B vitamins) has been more limited, particularly for B vitamins.

Water soluble vitamins are essential for normal cell function, growth, and development (Bellows and Moore, 2012). The B vitamins are an important part of coenzymes that catalyze multiple metabolic reactions in plants and animals. They help the body obtain energy from food, are important for normal appetite, good vision, healthy skin, adequate functioning of the nervous system, and red blood cell formation (Bellows and Moore, 2012). Vitamin C is widely known as an antioxidant, it is important for collagen formation, and has been associated with the reduction of certain degenerative diseases such as cataracts, cancer, and cardiovascular diseases (Bendich and Langseth, 1995; Salonen et al., 1997). Each of these vitamins play an essential role in human nutrition and their deficiency can cause health disorders (Kawasaki and Egi, 2000; Nielsen, 2000; Padayatty et al., 2003; Ubbink, 2000).

Differences in ascorbic acid and B vitamin content among sweetpotato cultivars have been previously reported (Aina et al., 2009; Bradbury and Singh, 1986a; Bradbury and Singh, 1986b; Reddy and Sistrunk, 1980). Long term storage, a common practice to enhance year-round availability of sweetpotatoes, was reported to affect sweetpotato ascorbic acid content (Ezell et al., 1948; Hollinger, 1944; Reddy and Sistrunk, 1980). However, little is known on the effect of curing on water-soluble vitamin content, a common postharvest process usually conducted at 32 °C and 90-95% relative humidity for 5-7 days to facilitate healing of the wounds incurred during harvest and extend the postharvest life of the roots.

Several studies have indicated a detrimental effect of various sweetpotato cooking methods on ascorbic acid (Babalola et al., 2010; Chukwu et al., 2012; Lanier and Sistrunk, 1979). However, limited studies have been conducted on the effect of cooking method on

sweetpotato B vitamins. Little is known about the distribution of water soluble vitamins, particularly B vitamins, in sweetpotato root and leaf tissues. A major reason for the limited availability of studies on the effect of various postharvest and processing factors on B vitamin content of sweetpotato is the lack of adequate analytical methodologies for routine analysis of these nutrients.

The objectives of this work were to determine the effect of various postharvest factors, cooking methods, and intra-plant tissue distribution on vitamin C (ascorbic acid), thiamin, riboflavin, and vitamin B6 content in currently important commercial sweetpotato cultivars. An additional goal was to develop a simple, rapid, and reliable methodology to quantify these vitamins in sweetpotato.

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CHAPTER 2. SIMULTANEOUS ANALYSIS OF THIAMIN AND RIBOFLAVIN IN SWEETPOTATO BY HPLC

INTRODUCTION

Thiamin (vitamin B1) and riboflavin (vitamin B2) are water soluble B-vitamins required in the human diet for normal metabolic activity. These vitamins serve as coenzymes in numerous metabolic reactions that produce energy (Nielsen, 2000; Takashi, 2000) and their insufficiency can lead to vitamin deficiency disorders (Zhuang, 2003). Various fruits and vegetables, including sweetpotato, are considered moderate to good sources of these compounds, which contributes to their quality and nutritional value.

Information on fruit and vegetable nutritional value and composition requires appropriate analytical methodologies for B-vitamin quantification. However, the separation and quantification of low tissue concentration B-vitamins, like thiamin and riboflavin, in complex matrices such as fruits and vegetables can have significant analytical challenges. Classical techniques for the analysis of thiamin and riboflavin from biological samples include microbiological and chemical analyses, which have been accepted as standard for many years (Eitenmiller and Landen JR, 2000). However, these procedures are often time consuming, usually specific for the determination of single vitamins, variable in precision, and may not have the sensitivity required for low analyte level quantification in fruit and vegetable tissue (Blake, 2007; Eitenmiller and Landen JR, 2000). Recent advances in HPLC have placed it as the methodology of choice for the measurement of most of the water-soluble vitamins. Reverse phase HPLC combines the properties of speed, precision, and accuracy in the separation of vitamins in food and biological samples (Nielsen, 2000; Takashi, 2000), (Blake, 2007; del Carmen Mondragón-Portocarrero et al., 2011).

Although numerous HPLC methodologies have been published on the analysis of thiamin and riboflavin in different food products (Augustin, 1984; del Carmen Mondragón-Portocarrero et al., 2011; Esteve et al., 2001; Fellman et al., 1982; Fernando and Murphy, 1990; Finglas and Faulks, 1984; Jakobsen, 2008; Sánchez-Machado et al., 2004; Sims and Shoemaker, 1993), few have achieved simultaneous extraction and analysis of multiple vitamins in low analyte containing tissues such as fruits and vegetables. Obtaining adequate simultaneous separation and resolution of thiamin and riboflavin analyses by HPLC in complex sample matrices can be a significant challenge due to the presence of interfering compounds, poor resolution, inconsistency, and low sensitivity. Most of the HPLC methods used for analysis of thiamin and riboflavin in food are based on the same principle with modifications only in the extraction, cleanup, and chromatographic conditions (Sánchez-Machado et al., 2004). The sample extraction procedure generally consists of autoclaving in acid solution, followed by enzymatic hydrolysis to release bound forms of the vitamins. Since thiamin molar absorptivity is rather low, it is generally oxidized to thiochrome under alkaline conditions (Blake, 2007). Thiochrome is a highly fluorescent compound that increases thiamin detection in samples with low analyte concentration. HPLC separation of thiamin and riboflavin has been conducted with various solvents, including mixtures of ammonium acetate and methanol (del Carmen Mondragón-Portocarrero et al., 2011; Sánchez-Machado et al., 2004; Sims and Shoemaker, 1993), methanol and water (Esteve et al., 2001), and other solutions (Augustin, 1984). Detection of thiamin and riboflavin has been conducted with fluorescence detection to increase system selectivity and sensitivity.

The objective of this research was to develop and optimize conditions for the analysis of thiamin and riboflavin with HPLC in fruits and vegetables. This work resulted in a methodology

with new conditions for the simultaneous chromatographic separation of thiamin and riboflavin. Additionally, the extraction methodology of these vitamins from fruit and vegetable tissues was optimized.

MATERIALS AND METHODS

Reagents

Thiamin hydrochloride, riboflavin, taka-diastase from *Aspergillus oryzae*, sodium acetate, potassium ferricianyde (III), potassium phosphate monobasic, and sodium hexane sulfonate were obtained from Sigma Aldrich (St Louis, MO). All reagents were HPLC grade unless otherwise stated. Hydrochloric acid and phosphoric acid were supplied by Fisher Scientific (Pittsburgh, PA). Acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All standards were prepared daily for the respective analyses.

Fruit and vegetable material

Fresh fruits and vegetables including tomato (*Solanum lycopersicum*), green pepper (*Capsicum annum*), potato (*Solanum tuberosum)*, sweetpotato (*Ipomoea batatas*), broccoli (*Brassica oleracea*), mango (*Mangifera indica*), carrot (*Daucus carota* subs. *sativus*), grape (*Vitis vinifera*), banana (*Musa sp.*), navel orange (*Citrus sinensis*), and apple (*Malus domestica*) were purchased from a local supermarket and immediately processed in the laboratory.

Vitamin extraction procedure

The thiamin and riboflavin extraction methodology was adapted from Finglas and Faulks (1984) and Esteve et al., (2001). During the extraction procedure samples were handled in amber vials to prevent photo-degradation of the analytes. Five g of finely grated unpeeled tissue (tomato, green pepper, grape and apple), peeled tissue (potato, sweetpotato, banana, carrot, mango), finely chopped broccoli heads, or 5 ml of juice (orange) were transferred into a 250 ml

Erlenmeyer flask and 50 ml of 0.1M HCl were added. The mixture was autoclaved for 30 min at 121° C. It was allowed to cool to room temperature and the pH was adjusted to 4.5±0.1 with 2M sodium acetate. In each individual sample, 100 mg of taka-diastase was added, followed by gentle stirring for 10 s. All samples were then put in an incubator (Innova™ 4000 Incubator shaker, New Brunswick Scientific Co, Inc., Enfield, CT) at 37° C, with agitation speed of 60 revolutions per minute (rpm) for 12 hr. The volume was then brought to 100 ml with distilled water and filtered through Whatman #4 paper (GE Healthcare Co., Buckinghamshire, UK). Then, 300 µl of potassium ferricyanide was added to 5.0 ml of sample extract, followed by 15 s of vigorous stirring. The sample extract was placed in the dark for 10 min in order to reduce thiamin to thiochrome. The pH of the sample was then adjusted to 7.0 with a solution of 17% (w/v) ortho-phosphoric acid, followed by filtration through a 0.45 µm nylon membrane syringe filter (Phenex, Phenomenex Inc., Torrance, CA) and injection into the HPLC system for simultaneous determination of thiamin and riboflavin. All analyzes were conducted on 4 units (replicates) of each fruit or vegetable (n=4). Thiamin and riboflavin standards solution were treated with the same enzymatic extraction and derivatization procedure.

HPLC analysis

The HPLC equipment (Waters Corp., Milford MA) included a model W600 pump, a 717 Plus autosampler, and a model 474 scanning fluorescence detector. The software used for programming and data collection was Waters Empower 3. Multiple reverse-phase C-18 analytical columns were tested for their B vitamin separation performance. The column which provided the best resolution and sensitivity for analysis of thiamin and riboflavin was a Synergy 4-um Hydro-RP, C-18 (4.6 x 150 mm) from Phenomenex Inc. The mobile phase used for B vitamin separation consisted of a gradient of two solvents: (1) 20 mM potassium phosphate in

0.1% hexane sulfonic acid, and (2) acetonitrile. The total chromatographic run time was 25 min and the flow rate 1.5 ml/min. Column temperature was maintained at 32 °C during analyses. The optimal gradient was 97:3 (solvent 1 and 2, respectively) from 0-3 min, followed by a uniform transition to 70:30 from 3-18 min, and a reverse uniform transition back to 97:3 from 18-22 min. The sample injection volume into the HPLC system was 50 µl. The fluorescence detector was programmed for a two-event run, with a 360:430 excitation:emission wavelength from 0 to 15.3 min, followed by 420:525 during the remainder of the run.

Validation of the method

Thiamin and riboflavin standard solution concentrations of 0.05, 0.25, 0.50 and 1.0 µg/ml were injected to assess the response linearity and fitted to the appropriate regression line against optical density.

Recovery efficiency was analyzed by spiking sweetpotato samples at three different concentrations for each vitamin. The concentrations tested were 0.25, 0.5 and 0.75 µg/ml. Identities of the B vitamin peaks were established by comparison with retention times of pure standards.

RESULTS AND DISCUSSION

Enzyme impurities and extraction methodology optimization

Taka-diastase is commonly used in thiamin and riboflavin extraction procedures to free protein-bound and phosphorylated forms of these vitamins in foods (Blake, 2007). During the optimization of the enzyme specific activity to use in my extraction methodology, trace amounts of thiamin and riboflavin were detected in taka-diastase (Figures 2.1 and 2.2). Thiamin and riboflavin content detected increased linearly with higher amounts of taka-diastase used during the analyses as shown by Pearson correlation coefficients (r).

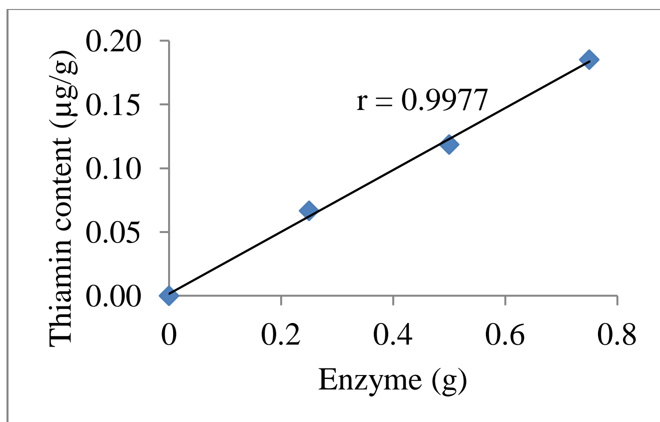

Figure 2.1 Thiamin content detected in commercial taka-diastase.

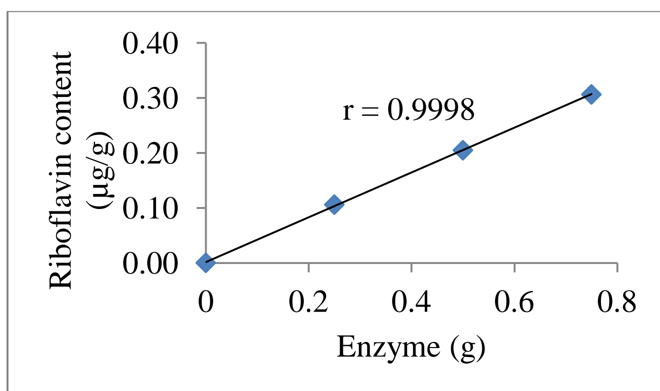

Figure 2.2 Riboflavin content detected in commercial taka-diastase.

To prevent over-estimation of the analytes in the samples, the standards solutions were treated with the same amount of enzyme and then subjected to the same thiamin derivatization procedure. This methodology provided good analyte recovery and precision (Table 2.1). Differences in efficiency and impurity content in taka-diastase originating from different sources can exist (Ndaw et al., 2000). However, no information on thiamin and riboflavin trace content was available from the product supplier (Sigma-Aldrich, St. Louis MO). To the best of my knowledge, the only reference to riboflavin trace content in taka-diastase is indicated in AOAC method 981.15 for the analysis of riboflavin in foods and vitamin preparations. Various earlier publications have not reported thiamin and riboflavin trace content in taka-diastase enzymatic

extraction. Whether this problem occurs only in isolated lots of the enzyme is not clear. However, any taka-diastase contamination may lead to overestimation of thiamin and riboflavin in food samples.

Table 2.1 Parameters of the developed methodology for the analysis of thiamin and riboflavin in sweetpotato.

Parameter	Thiamin	Riboflavin
Y-intercept	-3199	2193
Slope	164281	35360
r	0.9985	0.9979
Calibrated range (ng/ml)	1.13-227	1.13-227
LOD (ng/ml)[z]	1.07	0.31
Precision (n=5)		
Mean content of sweetpotato samples (µg/g)	0.37[y]	0.16[y]
Relative standard deviation (RSD)	3.0	0.93

[z]LOD=Limit of detection of at least 3 times the background signal noise, based on guidelines for data acquisition and data quality evaluation in environmental chemistry (American Chemical Society (ACS) Subcommittee on Environmental Analytical Chemistry, 1980).
r= Pearson correlation coefficient.
[y]Analyses conducted on sweetpotato cultivar Beauregard.

The use of clara-diastase was initially considered as a possible alternative to avoid the impurity issue. Clara-diastase provided adequate extraction of thiamin and riboflavin in seaweeds (Sánchez-Machado et al., 2004), and in green leafy vegetables (del Carmen Mondragón-Portocarrero et al., 2011). However, clara-diastase extraction efficiency for riboflavin was lower than taka-diastase in our tests (Table 2.2). For this reason, it was decided to use taka-diastase.

Gradient optimization

The best resolution and separation of thiamin and riboflavin was obtained using a ramping gradient to 70:30 (solvent 1:2) at 18 minutes after injection. Thiamin eluted at 14.25 min, while riboflavin eluted at 15.55 min (Figures 2.3 and 2.4). Shorter run times and higher or lower solvent ratios resulted in poor peak resolution or interferences. Fluorescence detector

wavelength switching during the run resulted in optimal thiamin and riboflavin signal resolution in a single run.

Table 2.2 Comparison of thiamin and riboflavin extraction efficiency with different amounts of clara-diastase and taka-diastase in sweetpotato tissue samples.

Enzyme/amount	Thiamin content (μg/g)	Riboflavin content (μg/g)
Original sample (no enzyme added)[z]	0.27±0.06	0.031±0.004
Taka-diastase		
50 mg	0.41±0.019	0.168±0.033
250 mg	0.37±0.019	0.275±0.026
500 mg	0.41±0.011	0.277±0.010
Clara-diastase		
50 mg	0.41±0.031	0.042±0.012
250 mg	0.36±0.014	0.048±0.018
500 mg	0.39±0.053	0.062±0.001

Values for each treatment represent the mean of 3 replicates ± the standard deviation.
[z]Sweetpotato raw tissue sample. Before enzyme addition, 5.0 g of sweetpotato raw tissue were placed in a solution of 0.1 M HCl, autoclaved at 120 °C for 30 min, cooled to room temperature, and the pH adjusted to 4.5 with 2M sodium acetate.

The distinguishing features of the developed methodology compared with previous methods are the unique chromatographic conditions for the simultaneous analysis of thiamin and riboflavin. Although this method requires a longer chromatograpahic run time (25 min) for simultaneous separation of thiamin and riboflavin compared to 22, 14, and 11 mins in the the methodologies developed by Augustin (1984), Sims and Shoemaker (1993), Sanchez-Machado et al., (2004) respectivelly, it provides optimal peak separation, coupled with stable baseline resolution and stability, areas in which multiple previous methods have shown deficiencies (Sims and Shoemaker, 1993). The new method also achieved optimal separation of thiamin and riboflavin in multiple fruits and vegetable samples without requiring the use of additional cleanup steps. Sep-pak cartridges have been used during sample cleanup in recent methodologies to achieve HPLC separation of these vitamins (del Carmen Mondragón-Portocarrero et al., 2011;

Sims and Shoemaker, 1993; Sanchez-Machado et al., 2004). Due to these features, the new method has the potential to be used in a wide variety of food matrixes as an alternative methodology for the analysis of thiamin and riboflavin.

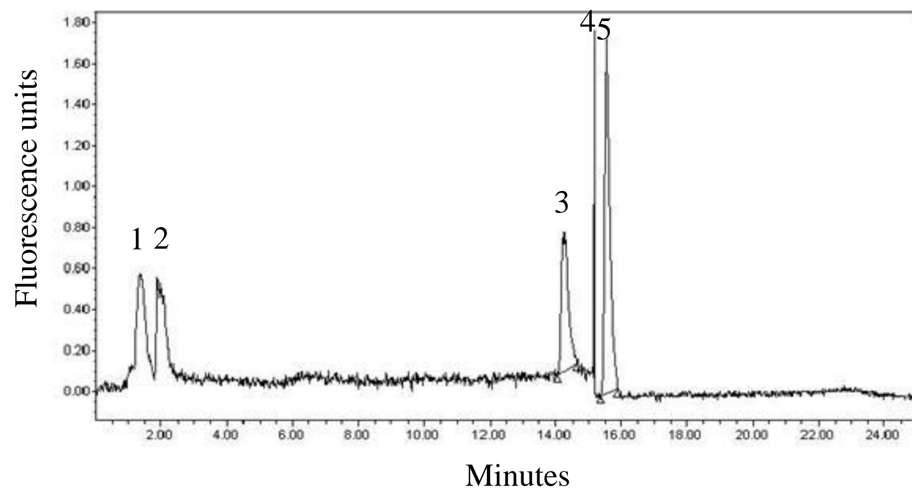

Figure 2.3 Representative chromatogram of a 50 µl standard solution of thiamin and riboflavin at 0.5 µg/ml concentration. (1) unknown, (2) unknown, (3) thiamin, (4) signal from fluorescence detector excitation: emission wavelength change, (5) riboflavin.

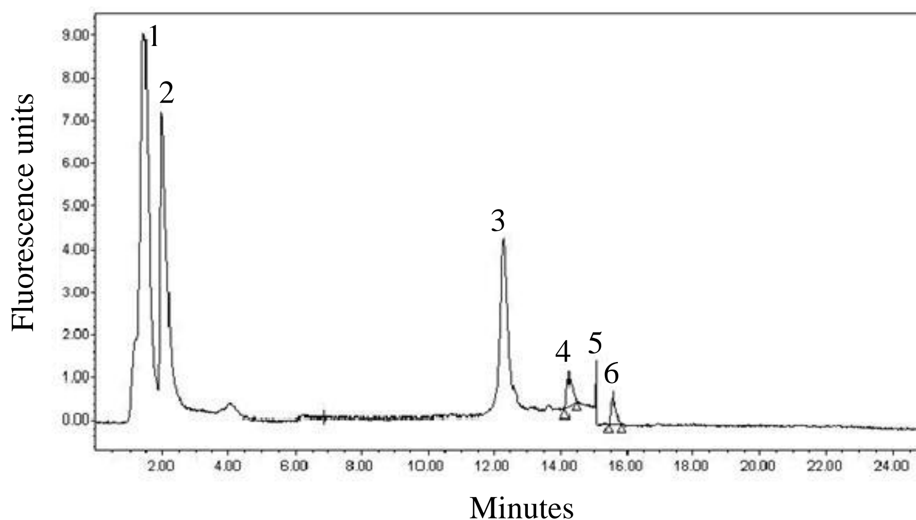

Figure 2.4 Representative HPLC chromatogram of thiamin and riboflavin from a 50 µl sweetpotato root extract. (1) unknown, (2) unknown, (3) unknown, (4) thiamin, (5) signal from fluorescence detector excitation: emission wavelength change, (6) riboflavin.

Method validation

A linear system response to the standards concentration was observed (Pearson r >0.99) with both thiamin and riboflavin. The calibration range (1.13-227 ng/ml) for thiamin and riboflavin standards covered the regular natural occurrence range of these vitamins in fruits and vegetables. The recovery for thiamin ranged from 97-100% and 97-101% for riboflavin (Tables 2.3 and 2.4). These values compare favorably with recoveries of 95.5% for thiamin and 90.1% for riboflavin in the methodology by Sanchez-Machado et al., (2004) in seaweeds, and 91.6% for thiamin and 96.7% for riboflavin obtained by Esteve et al., (2001) in mushrooms. Additionally, the methodology precision, measured as relative standard deviation (%RSD) was adequate based on the precision parameters of other analytical methods (Kawasaki, 2000; Nielsen, 2000).

Table 2.3 Recovery results for sweetpotato tissue spiked with different amounts of thiamin (μg/ml).

Amount added	Original thiamin	Expected quantity	Observed quantity^z	Recovery %
0.25	0.51	0.76	0.74±0.03	97.4
0.50	0.51	1.01	0.99±0.04	98.0
0.75	0.51	1.26	1.26±0.03	100.0

^{z}All values are the average 3 individual samples ± standard deviation and expressed in fresh weight basis.

Table 2.4 Recovery results for sweetpotato tissue spiked with different amounts of riboflavin (μg/ml).

Amount added	Sample	Expected quantity	Observed quantity^z	Recovery %
0.25	0.19	0.44	0.44±0.007	100.0
0.50	0.19	0.69	0.67±0.018	97.1
0.75	0.19	0.94	0.95±0.015	101.1

^{z}All values are the average 3 individual samples ± standard deviation and expressed in fresh weight basis.

The sensitivity of this method, expressed as limit of detection (LOD), was 1.07 ng/ml for thiamin and 0.31 ng/ml for riboflavin. These values provide high sensitivity for low analyte

concentration samples, and are similar to the LOD values reported by Augustin (1984) in other HPLC methodology for the determination of thiamin and riboflavin in various foods.

Thiamin and riboflavin content in fruits and vegetables

Adequate peak separation and resolution of thiamin and riboflavin was observed during the analysis of different fruits and vegetables. The thiamin content in the various fruit and vegetable products was found within the range of reference values reported in the USDA National Nutrient Database for Standard Reference (2012), except in sweetpotato and green pepper (Table 2.5). Riboflavin content found was similar to previous results for mango, potato, broccoli and green pepper; but was below the reference range in the other fruits and vegetables. The discrepancies could be due to various factors, including different cultivars and analytical procedures. Thiamin was determined with a fluorometric method (AOAC, 942.23), while riboflavin was determined with either a fluorometric method (AOAC, 970.65) or microbiological method (AOAC, 940.33) for the USDA nutrient database analyses (USDA-ARS, 2012).

Table 2.5 Comparison between thiamin and riboflavin content obtained in various fruits and vegetables by HPLC and reference values available from the USDA National Nutrient Database for Standard Reference (2012).

	Thiamin (µg/g fresh weight)		Riboflavin (µg/g fresh weight)	
Fruit/vegetable	HPLC	USDA reference values	HPLC	USDA reference values
Sweetpotato	0.44-0.47	0.67-0.98	0.17-0.20	0.54-0.76
Mango	0.067-0.27	0.14-0.41	0.18-0.23	0.20-0.72
Orange	0.40-0.78	0.27-1.16	0.05-0.08	0.34-0.64
Tomato	0.15-0.34	0.20-0.60	0.09-0.10	0.12-0.28
Grape	0.40-0.54	0.59-0.82	0.07-0.10	0.49-1.08
Apple	0.10-0.26	0.07-0.30	0.07-0.09	0.15-0.49
Potato	0.62-0.82	0.60-0.95	0.15-0.20	0.16-0.53
Carrot	0.18-0.25	0.20-0.85	0.25-0.33	0.40-0.87
Banana	0.11-0.18	0.10-0.51	0.15-0.19	0.50-1.15
Broccoli	0.50-0.81	0.64-0.90	1.17-1.42	0.86-1.41
Green pepper	0.25-0.28	0.37-0.67	0.19-0.26	0.23-0.33

Lower thiamin and riboflavin content has been found using HPLC determinations compared to fluorometric AOAC methods in some foods (Finglas and Faulks, 1984; Nielsen, 2000; Skurray, 1981). Whether variability in thiamin and riboflavin observed between the different studies is enhanced by unintended additions of trace amounts of riboflavin to food samples during enzymatic digestion with taka-diastase remains unclear.

CONCLUSIONS

A new HPLC method was developed for simultaneous analysis of thiamin and riboflavin in fruits and vegetables. It is distinguished from previous methods for its unique conditions for chromatographic separation of thiamin and riboflavin. The new method considered trace content of thiamin and riboflavin in commercial enzyme used for the extraction of these vitamins in foods, thereby increasing accuracy in quantification results. Additionally, it showed adequate recovery values (95-100%), high sensitivity, and precision, and was successfully employed in the quantification of thiamin and riboflavin in multiple fruits and vegetables. The new method has the potential to be used in a wide variety of food matrixes as an alternative methodology for the analysis of thiamin and riboflavin.

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CHAPTER 3. EFFECT OF CULTIVAR, CURING AND STORAGE ON THE CONTENT OF ASCORBIC ACID, THIAMIN, RIBOFLAVIN AND VITAMIN B6 IN SWEETPOTATO

INTRODUCTION

Sweetpotato is considered a good source of ascorbic acid (AA) and a moderate source of several B vitamins in the human diet. Ascorbic acid is well known for its antioxidant properties and for its physiological functions as a coenzyme in multiple enzymatic processes in the human body (Nyyssonen and Salonen, 2000). Thiamine, riboflavin and vitamin B6 play important roles as coenzymes in multiple reactions that produce energy and also serve as building blocks for the biosynthesis of other important biological molecules (Kawasaki and Egi, 2000; Nielsen, 2000; Ubbink, 2000). The insufficiency of these vitamins in the diet can cause specific diseases (Kawasaki and Egi, 2000; Nielsen, 2000; Nyyssonen and Salonen, 2000). Historically, AA has been the most studied water-soluble vitamin (WSV) in sweetpotato. Previous reports have indicated significant differences in AA content among cultivars (Aina et al., 2009; Ezell et al., 1948; Hollinger, 1944; Lanier and Sistrunk, 1979; Reddy and Sistrunk, 1980). Long term-storage and curing were also found to affect the AA content of sweetpotato cultivars (Ezell et al., 1948; Hollinger, 1944). The AA content was lower in sweetpotato cultivar Centennial after 30 days of storage and then remained stable through two months in storage (Watada, 1987). Also, AA was lower after seven months of storage, although no indication of storage effect on different cultivars was provided (Reddy and Sistrunk, 1980). Studies on the effect of curing and storage on the AA content in current US sweetpotato commercial cultivars have not been carried out.

On the other hand, limited studies have addressed the effect of cultivar, curing, and storage on B vitamin composition of sweetpotato. Significant differences in riboflavin (vitamin B2) niacin (vitamin B3) and pantothenic acid (vitamin B5) content were observed among

cultivars (Lanier and Sistrunk, 1979; Reddy and Sistrunk, 1980). However, in another study including three cultivars from the South Pacific, no particular cultivar contained consistently higher levels of thiamin (vitamin B1), riboflavin and niacin, compared with others (Bradbury and Singh, 1986). No studies have addressed the effect of curing and storage on B vitamin composition in sweetpotato. The objective of this study was to determine the effect of curing and storage on AA, thiamin, riboflavin and vitamin B6 composition in currently important commercial sweetpotato cultivars in the United States.

MATERIALS AND METHODS

Reagents

Thiamin hydrochloride, riboflavin, L-ascorbic acid, vitamin B6 vitamers (pyridoxine hydrochloride, pyridoxamine dehydrochloride, and pyridoxal hydrochloride), taka-diastase from *Aspergillus oryzae*, acid phosphatase from potato, Beta-glucosidase from almonds, meta-phosphoric acid (MPA), sodium acetate, potassium ferricyanide (III), potassium phosphate monobasic, 1-octane sulfonic acid, trethylamine, sodium phosphate monobasic, sodium hexane sulfonate, tris(2carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Sigma-Aldrich Co. (St Louis, MO). All reagents were HPLC-grade unless otherwise stated. Hydrochloric acid and orthophosphoric acid were supplied by Fisher Scientific (Pittsburgh, PA). Acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All standards were prepared daily for the respective analyses.

Harvesting and storage of roots

Sweetpotato roots from four different cultivars (Beauregard, Covington, Orleans and 07-146) were harvested on August 22, 2012 from subplots of the same field at the Burden Research Center, Baton Rouge LA. The four cultivars had received the same cultural management during

the growing season. The roots were randomly divided into two lots. One lot was cured at 31° C and 90% relative humidity (RH) for 7 days, and then placed in storage at 14 °C and 85% RH. The second lot was immediately sampled for WSV determinations. Five individual root replications per cultivar were collected at harvest, after curing, at three months, and six months of storage for WSV analysis.

Vitamin extraction and analysis procedures

All samples were handled in amber vials during extraction procedures to prevent photo-degradation of the WSV analytes.

Ascorbic acid. The AA extraction methodology was adapted from Chebrolu et al. (2012). Three g of finely grated tissue from the central pith region of the root were placed in an amber vial, and 6 ml of 3% (w/v) meta-phosphoric acid were added. The tissue was homogenized at 3,000 rpm for 30 s with a 1.0 cm diameter VirtiShear homogenizer (The Virtis Co., Gardiner, NY), and then the homogenate was transferred to a 15-ml polypropylene test tube. The sample was centrifuged at 12,857 *x g* for 10 minutes. About 3 ml of the supernatant was carefully filtered through a Phenex 25 mm, 0.45 µm nylon membrane syringe filter, (Phenomenex Inc., Torrance, CA). Exactly 0.5ml of the filtered sample was transferred to an amber 8 x 40mm (1ml) HPLC vial and then mixed with 0.5ml of 5mmol/L of tris(2carboxyethyl)phosphine hydrochloride (TCEP). The sample was manually agitated for 15 s and then allowed to remain at room temperature (21 °C) for 30 min for complete reduction of dehydroascorbic acid to AA.

The sample was then analyzed for total AA content by injecting 5µl of the sample in an HPLC system (Waters Corp., Milford MA) consisting of a model W600 pump, a 717 Plus autosampler, and a 2487 UV detector. The separation was achieved with a reverse phase C18

GraceSmart column (150mmx4.6mm, 3µm particle size) from Grace Davison Discovery Sciences Corp (Deerfield, IL). An isocratic mobile phase was used consisting of 25 mM monobasic sodium phosphate, with the pH lowered to 2.5 with 17% (w/v) orthophosphoric acid. The flow rate of the mobile phase was 1ml/min and the run time was 10 mins. The AA signal was detected at 254 nm. The software used for HPLC programming and data collection was Waters Empower 3.

Thiamin and riboflavin. Thiamin and riboflavin were extracted based on the methodology of Barrera and Picha (2013, unpublished). Five g of finely grated tissue from the central pith region of the root were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl was added. The mixture was autoclaved for 30 min at 121 °C. It was allowed to cool and the pH was adjusted to 4.5±0.1 with 2M sodium acetate. In each individual sample, 100 mg of taka-diastase was added, followed by gentle manual stirring for 10 s. All samples were then put in an incubator (Innova™ 4000 Incubator shaker, New Brunswick Scientific Co, Inc., Enfield, CT) at 37° C, with agitation speed of 60 revolutions per minute (rpm) for 12 hr. The volume was then brought to 100 ml with distilled water and filtered through Whatman #4 paper (GE Healthcare Co., Buckinghamshire, UK). Exactly 300 µl of 0.03M potassium ferricyanide were added to 5.0 ml of sample extract, followed by 15 s of vigorous manual stirring. The sample extract was placed in the dark for 10 min in order to reduce thiamin to thiochrome. To prevent degradation of analytical column performance the pH of the sample was then adjusted to 7.0 with a 17% (w/v) orthophosphoric acid dilution, followed by filtration through a 0.45 µm nylon membrane syringe filter (Phenex, Phenomenex Inc., Torrance, CA) and injected in the HPLC system for simultaneous determination of thiamin and riboflavin.

The analysis of thiamin and riboflavin was conducted by HPLC with a reverse phase Synergy Hydro-RP, C18 column (150mm x 4.6mm, 4µm particle size), (Phenomenex Inc). Column temperature was kept at 32 °C during analyses. The mobile phase consisted of a gradient of two solvents: (1) 20 mM potassium phosphate in 0.1% hexane sulfonic acid, and (2) acetonitrile. The optimal gradient was 97:3 (solvent 1 and 2, respectively) from 0-3 min, followed by a uniform transition to 70:30 from 3-18 min, and a reverse uniform transition back to 97:3 from 18-22 min. The flow rate was 1.5 ml/min and the total chromatographic run time was 25 min. The sample injection volume was 50 µl. A scanning fluorescence (Waters Corp., Model 474) detector was used for analyte quantification and programmed for a two-event run, with a 360:430 excitation:emission wavelength from 0 to 15.3 min, followed by 420:525 during the remainder of the run.

Vitamin B6. The extraction and analysis methodology for vitamin B6 was adapted from Kall (2003). Five g of finely grated tissue from the pith of the central region of the root were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl were added. The mixture was autoclaved for 15 min at 121° C. It was then allowed to cool and the pH was adjusted to 4.5 with 2M sodium acetate. The volume was then brought to 100 ml with distilled water, and then vigorously agitated for 15 s. Forty ml were transferred to a 50 ml polypropylene test tube. The sample was then centrifuged at 12,857 *x g* for 10 minutes. An aliquot of 15 ml of supernatant was transferred to a 25-ml volumetric flask, to which was added 1 ml of 25 Units/ml acid phosphatase solution plus 3 ml of 45 Units/ml of the β-glucosidase solution. Samples were incubated at 37° C, with gentle stirring, for 18 hr. Following incubation, the samples were cooled to room temperature for 15 minutes and 5 ml of 1M HCl was added. The flask was made up to

final volume of 25 ml with 0.1M HCl. An aliquot of this solution was filtered through a 0.45 µm nylon membrane syringe filter and transferred into an HPLC amber vial.

The HPLC analysis of vitamin B6 was conducted with a reverse phase C-18 HyperClone BDS column (150mm x 4.6mm, 3µm particle size) from Phenomenex Inc. An isocratic mobile phase consisting of a mixture of 93% buffer and 7% acetonitrile was used. The buffer was a solution of 2.2 mM 1-octane sulfonic acid in 81 mM potassium dihydrogen phosphate and 4.0 mM triethylamine, adjusted to pH 2.75 with 85% (w/w) orthophosphoric acid. The flow rate was 1.0 ml/min and the total chromatographic run time was 14 min. The sample injection volume was 50 µl. In order to improve the detector sensitivity, the mobile phase pH was adjusted to 7.5 with a post column infusion of 0.5M phosphate buffer (pH=7.5) at 0.3ml/min, by using a Beckman 110B solvent delivery module. Vitamin B6 vitamers pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM) were detected with a Waters Model 474 fluorescence detector programmed for excitation at 333 nm and emission at 375 nm. Total vitamin B6 was calculated as PN, HCl (pyridoxine hydrochloride) with the following equation:

PN, HCl= PN+(1.01×PL)+(0.85×PM).

The values in the previous equation are due to different molar weight of PL, HCl; PM, 2HCl (mono-hydride); and PN, HCl.

Statistical analyses

A completely randomized design with 5 replications per treatment was used. Each root was considered one replication. The data was analyzed with SAS PROC GLM procedure (SAS Institute, Cary, NC). Treatment means were separated using Tukey's HSD test. Due to lack of data normality, results for AA were analyzed with the Kruskal-Wallis non-parametric test. All WSV analysis results were expressed as mg/100 g of fresh weigh (FW).

RESULTS AND DISCUSSION

Ascorbic acid

No cultivar differences in AA content were found at harvest and after six months of storage. Beauregard showed higher AA content than 07-146 after curing and after 3 months of storage. Covington also contained higher AA concentration after curing than 07-146 (Table 3.1). Differences in AA content have been previously reported in sweetpotato cultivars (Aina et al., 2009; Lanier and Sistrunk, 1979; Reddy and Sistrunk, 1980). The results of this study are similar to those of Ezell and Wilcox (1948), who reported variable AA content differences after 2, 4 and 6 months of storage in cultivars Nancy Hall, Southern Queen, Yellow Jersey, and Porto Rico.

Through 6 months of storage, a significant decrease in AA was observed in Beauregard, Covington and 07-146. Ascorbic acid decreased by 36% in Beauregard, 53% in Covington, and 41% in 07-146. Meanwhile, Orleans's AA content remained mostly similar to the level measured at harvest. The declines are consistent with AA content decreases of 25% observed in Centennial, Goldrush, Georgia Jet, and Jasper during 7 months of storage (Reddy and Sistrunk, 1980) and with declines ranging between 28-38% in cultivars Unit 1 Porto Rico, Triumph, Nancy Hall, and 47442 (Hollinger, 1944). A decline in AA content during storage is common among fruit and vegetables, but is influenced by the commodity, storage conditions, and cultivar (Lee and Kader, 2000). All raw fruits and vegetables undergo a series of postharvest changes, and the key to the stability of ascorbic acid is the enzyme-catalysed oxidation reactions (Davey et al., 2000). A decrease in AA coincided with an increase in the activity of ascorbate oxidase in bell peppers and tomatoes (Yahia et al., 2001), and muskmelon (Mosery and Kanellis, 1994). Differences in ascorbate oxidase activities have been reported among various cucurbits including cucumber, pumpkin, zucchini squash, and four melon cultivars (Bin Saari et al., 1995). Losses

Table 3.1 Water-soluble vitamin content in four sweetpotato cultivars at harvest, after curing, and during storage.

Cultivar	Sampling time	Ascorbic acid (mg/100g)[z]	Thiamin (mg/100g)	Riboflavin (mg/100g)	Vitamin B6 (mg/100g)			
					PL	PN	PM	Total B6[y]
07-146	Harvest	17.4 a-d	0.058 abc	0.030 a	0.027 f	0.128 def	0.073 a	0.21 cd
	Curing	12.9 cde	0.051 a-e	0.020 cde	0.031 ef	0.147	0.071 a	0.23 bc
	3 months	13.5 cde	0.049 a-e	0.023 abc	0.034 def	0.210 b	0.027 de	0.26 b
	6 months	10.2 e	0.041 b-e	0.018 cde	0.039 c-f	0.285 a	0.022 ef	0.34 a
Orleans	Harvest	18.3 abc	0.058 ab	0.029 ab	0.042 cde	0.089 h	0.048 a-	0.17 efg
	Curing	15.5 b-e	0.044 b-e	0.019 cde	0.040 c-f	0.095 gh	0.056	0.18 ef
	3 months	15.3 b-e	0.042 b-e	0.022 bcd	0.057 ab	0.103 fgh	0.032 b-	0.19 de
	6 months	13.7 cde	0.044 a-e	0.019 cde	0.059 a	0.153 cd	0.013 g	0.22 c
Covington	Harvest	24.5 a	0.053 a-d	0.030 a	0.039 c-f	0.08 h	0.066 a	0.17 efg
	Curing	21.3 ab	0.036 de	0.020 cde	0.045 b-e	0.083 h	0.072 a	0.19 de
	3 months	15.2 b-e	0.046 cde	0.023 bcd	0.052 abc	0.08 h	0.019	0.15 g
	6 months	11.6 de	0.032 e	0.020 cde	0.047 a-d	0.172 c	0.030	0.24 bc
Beauregard	Harvest	23.9 a	0.063 a	0.030 a	0.035 def	0.093 gh	0.055 ab	0.17 efg
	Curing	23.1 a	0.047 a-e	0.018 d	0.047 a-d	0.08 h	0.055	0.17 efg
	3 months	21.3 ab	0.040 b-e	0.017 de	0.052 abc	0.079 h	0.023 e	0.15 fg
	6 months	15.3 b-e	0.040 b-e	0.016 e	0.051 abc	0.12 efg	0.013 fg	0.18 de
Source	DF	F-value	F-value	F-value	F-value	F-value	F-value	F-value
Cultivar	3	-	4.50*	5.96*	29.66**	218.62**	8.45**	177.86**
Storage time	3	-	18.67**	62.66**	22.61**	168.7**	116.04**	97.22**
Cultivar*storage time	9	-	0.75ns	1.29ns	2.04*	13.59**	4.96**	15.56**

Mean values with different letter within a column were significantly different ($P<0.05$) according to Tukey's HSD test.

[z]Means were separated by using the Kruskal-Wallis test.

[y]Total vitamin B6 was calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).

ns, *, **, Non-significant, significant at $p\leq0.05$, 0.001, respectively.

of AA in fruits and vegetables during storage have also been influenced by temperature, pH, atmospheric oxygen, light exposure, and the presence of other oxygen scavengers (Shewfelt, 1990). Decreases in AA during storage have been documented in multiple fruits and vegetables, including potatoes (Augustin, 1975; Augustin et al., 1978), strawberry and squash (Watada, 1987), tomatoes (Pantos and Markakis, 1973), apples (Zubeckis, 1962), lima beans (Kramer and Smith, 1947), and others (Lee and Kader, 2000). The AA content of low pH fruits such as citrus fruits is relatively stable (Davey et al., 2000). Leafy vegetables (e.g. spinach) are very vulnerable to spoilage and AA loss, whereas root vegetables (eg. potatoes) retain significant AA content for many months (Davey et al., 2000). In this study, the decrease in AA content of sweetpotatoes after storage for six months followed a similar decrease pattern reported in potatoes. The AA content decreased 56% in Russet Burbank, and 63% in Katahdin potatoes stored at 7 °C for 8 months (Augustin et al., 1978). The results of this study indicate that sweetpotato roots may be subject to AA declines during storage, but the magnitude of the decline is likely to be time and cultivar dependent.

Thiamin

Variable results were observed in thiamin composition in the four cultivars at the different storage times. While thiamin content declined in Beauregard and Covington after six months of storage, it remained similar to at harvest in Orleans and 07-146 (Table 3.1). There was no apparent cultivar superiority in thiamin content (Table 3.1). These results generally agree with Bradbury and Singh (1986), who reported no differences in thiamin content between different sweetpotato cultivars grown in the South Pacific, and Pearson and Luecke (1945) who found no differences in thiamin content between the cultivars Puerto Rico, Nancy Hall, Triumph, and W.M. 3. The results also showed that thiamin content remained mostly stable in all cultivars after

curing. Little research has been published on the effect of storage on thiamin content of sweetpotato. The results of this study contrast with gradual declines reaching 19-29% of thiamin loss in 3 Japanese cultivars after 95 days of storage (Yamamoto and Tomita, 1960). The results obtained for cultivars Orleans and 07-146 showed a similar pattern to results reported by various authors with other vegetables. Thiamin content in Russet Burbank potatoes showed an erratic pattern during storage, but did not significantly change (Augustin, 1975). Thiamin decreased in BelRus but remained stable in Superior potato cultivars during six months of storage (Watada, 1987). Similarly, thiamin content in White Rose potatoes remained similar after storage at 4°C and 10°C for 30 weeks (Yamaguchi et al., 1960). Thiamin content was also stable during storage in green beans, carrots, corn, peas, and spinach (Heinze, 1974). The biosynthesis pathway of thiamin in plants is not well understood (Zhuang and Barth, 2003). An RNA gel-blot analysis of various tissues and developmental stages indicated the gene involved in thiamin synthesis in corn (*Zea mays* L.) was differentially and developmentally regulated (Belanger et al., 1995). The relative stability of thiamine during storage may be due to regulation of biosynthesis by postharvest stresses (Zhuang and Barth, 2003).

Riboflavin

Riboflavin content showed little differences between cultivars, but decreased in all cultivars after curing and then remained mostly stable throughout 6 months of storage. These results contrast with those of Lanier and Sistrunk (1979), who found cultivar differences among Centennial, Jasper, Georgia Jet, and Goldrush sweetpotatoes. These results, however, agree with the generally uniform riboflavin content found in four cultivars from the South Pacific countries (Bradbury and Singh, 1986), and essentially similar riboflavin contents found in Nancy Hall, Triumph, W.M. 3, and Porto Rico (Pearson and Luecke, 1945).

The cause for the significant riboflavin decrease during curing of sweetpotatoes is not clear. No previous reports on the effect of curing on riboflavin content of sweetpotato or other vegetables were found. Besides acting as a coenzyme in multiple physiological processes, riboflavin has shown anti-oxidation and peroxidation properties that affect the production of reactive oxygen intermediates during oxidative stress (Upreti et al., 1991; Dong and Beer, 2000). Curing of sweetpotatoes was conducted at a relatively high temperature (31 °C) for 7 days. The increased tissue metabolism during curing at high temperature may have caused an increase in oxidative activity that affected riboflavin content.

Riboflavin content remained generally stable after curing in Orleans, Covington, Beauregard, and 07-146. These results were in agreement with the riboflavin stability found in potatoes and other vegetables during storage. Riboflavin was stable in the potato cultivar White Rose for 30 weeks at 4°C and 10°C (Yamaguchi et al., 1960). Riboflavin was also stable during 8 month storage of Russet Burbank potatoes (Augustin, 1975). A stable riboflavin content was also found during storage in green beans, carrots, corn, peas, and spinach (Heinze, 1974); and in green beans, bell peppers, and spinach stored at different temperatures (Watada et al., 1987).

Vitamin B6

Vitamin B6 consists of three closely related derivatives of 2-methyl-hydroxypyridine, i.e. pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their 5'phosphate forms. The methodology in this study included the use of acid phosphatase and β-glucosidase to release phosphorylated and glucosylated forms of these vitamers. The results showed an overall higher content of PN relative to PL and PM in all cultivars (Table 3.1). Pyridoxal content ranged between 12% out of the total vitamin B6 in cultivar 07-146 to 27% in Beauregard; PN ranged between 52% in Covington to 69% in 07-146; while PM ranged from 19% in 07-146 to 24% in

Covington. These results contrast with a previous report indicating total vitamin B6 was made of 44% PL, 33% PN, and 23% PM in sweetpotatoes (Kwiatkowska et al., 1989). This discrepancy may be explained by cultivar differences or analytical methodologies.

Significant differences in total vitamin B6 content were found among cultivars (Table 3.1). Cultivar 07-146 contained the highest total vitamin B6 content at all storage times. No differences in total vitamin B6 were found between Orleans, Beauregard and Covington at harvest; however, Orleans and Covington had a higher vitamin B6 than Beauregard after six months of storage. The curing process did not result in changes of total vitamin B6 content in any cultivar. However, with the exception of Beauregard all cultivars showed an increase of vitamin B6 after six months of storage. No previous studies addressing the effect of cultivar, curing and storage time on vitamin B6 content in sweetpotato were found. A similar increase in vitamin B6 was observed in potato during storage (Augustin et al., 1978; Page and Hanning, 1963; Pederson, 1972). Similarly, a slight increase in vitamin B6 was also reported in cabbage during storage (Hounsome et al., 2009). Whether moisture loss during storage played a role in the vitamin B6 increases observed in this study is not clear. Sweetpotato roots from 6 different cultivars averaged 5-12% weight loss after six months of storage at 15.6 °C and 90% RH (Picha, 1986). However, weight losses in this range would not explain the magnitude of the increases. Augustin et al. (1978) hypothesized that vitamin B6 might be bound in some form during the early stages of storage and may not be entirely released by the analytical extraction procedures. However, total vitamin B6 recovery comparing two methods of extraction failed to show differences in potato (Addo and Augustin, 1988). It was concluded that the vitamin B6 increases in potato most likely originated from biosynthesis during storage (Addo and Augustin, 1988). A similar phenomenon may explain the total vitamin B6 increases observed in sweetpotato.

CONCLUSIONS

Ascorbic acid content was mostly similar at harvest and after 6 months of storage in the cultivars Beauregard, Covington, Orleans, and 07-146. Curing did not significantly affect AA, thiamin, and vitamin B6, but it affected riboflavin content in all cultivars. Compared to at harvest, storage for 3 months resulted in lower AA content in Covington, and in similar AA content in 07-146, Orleans, and Beauregard. During the same period, there was a variable content of thiamin, riboflavin and vitamin B6 by cultivar. Storage for 6 months resulted in a decrease in AA content in Beauregard, Covington, and 07-146; and no change in Orleans. There was a gradual increase in vitamin B6 content in the cultivars 07-146, Orleans, and Covington throughout storage; whereas thiamine and riboflavin contents remained mostly stable in all cultivars after curing. Although cultivar 07-146 contained higher vitamin B6 content at every assay point, no cultivar was superior or inferior for all the vitamins throughout 6 months of storage.

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CHAPTER 4. EFFECT OF TISSUE TYPE ON THE ASCORBIC ACID, THIAMIN, RIBOFLAVIN, AND VITAMIN B6 CONTENTS OF SWEETPOTATO

INTRODUCTION

Water-soluble vitamins (WSV), including ascorbic acid (AA) and B vitamins are essential compounds for adequate functioning of the human body. They play important roles as coenzymes in a wide variety of metabolic reactions that sustain life (Kawasaki and Egi, 2000; Nielsen, 2000; Padayatty et al., 2003; Ubbink, 2000). Ascorbic acid also has antioxidant properties that may protect the human body against free radicals and other oxidized molecules species (Padayatty et al., 2003). Sweetpotato is considered a good source of ascorbic acid and a moderate source of certain B vitamins in human diets. Besides the root, other sweetpotato plant tissues including young leaves and petioles, are edible and high in nutritional value (Johnson and Pace, 2010). Sweetpotato foliar tissues are predominantly consumed in African and Asian countries, functioning as a source of protein, essential amino acids, antioxidants, vitamins, minerals, and dietary fiber (Johnson and Pace, 2010). Sweetpotato foliar tissues have been shown to contain significantly higher contents of certain WSV compared to roots (Ishida et al., 2000). Despite the availability of general reference values for sweetpotato WSV content in root pith and leaves, little is known on the distribution of these vitamins in other sweetpotato root and foliar edible tissues.

Limited research has been conducted on the WSV content of different sweetpotato root tissues. An earlier study reported no gradient in thiamin and riboflavin contents from proximal to distal ends of the root. However, thiamin was found more concentrated at 3mm below the skin compared to the center pith area (Bradbury and Singh, 1986). It is unknown whether other sweetpotato root tissues differ in their WSV content. The objective of this study was to

determine the ascorbic acid, thiamin, riboflavin, and vitamin B6 content in a wide range of edible sweetpotato tissues.

MATERIALS AND METHODS

Reagents

Thiamin hydrochloride, riboflavin, L-ascorbic acid, vitamin B6 vitamers (pyridoxine hydrochloride, pyridoxamine dehydrochloride, and pyridoxal hydrochloride), taka-diastase, acid phosphatase beta-glucosidase, meta-phosphoric acid (MPA), sodium acetate, potassium ferricyanide, potassium phosphate monobasic, 1-octane sulfonic acid, triethylamine, sodium phosphate monobasic, sodium hexane sulfonate, and tris(2carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Sigma Aldrich (St Louis, MO). All reagents were analytical grade, unless otherwise stated. Hydrochloric acid and orthophosphoric acid were supplied by Fisher Scientific (Pittsburgh, PA). Acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All standards were prepared daily for the respective analyses.

Tissue origin

Sweetpotato tissue types analyzed included mature leaves, (the tenth fully- expanded leaf from the apical meristem), mature petioles from sampled mature leaves, young leaves (most recent fully-expanded leaf from the apical meristem), young petioles from sampled young leaves, buds (3 cm from the apical meristem), vine section (15 cm from the apical meristem), and root (central pith tissue). The tissues were sampled on October 28, 2012 from a Beauregard sweetpotato plot at the Horticulture Hill Farm Teaching Facility at Louisiana State University and immediately processed for WSV analysis. The experiment was repeated (second experiment) with tissue samples collected on September 2, 2013 from a Beauregard sweetpotato plot at the LSU AgCenter Burden Research Station, Baton Rouge, LA. All tissues were then frozen with

liquid nitrogen and finely crushed with mortar and pestle for WSV extraction. For AA, thiamin, and riboflavin, a total of 6 and 5 replicates per tissue type were used in the first experiment and second experiment, respectively. For vitamin B6, a total of 4 replicates were used in the first experiment. Vitamin B6 was not analyzed in the second experiment.

A third experiment was conducted to study WSV content among different sweetpotato root tissues. Sweetpotato root material was collected from the LSU AgCenter Sweetpotato Research Station in Chase, LA. Roots from cultivars Beauregard and 07-146 were harvested on August 24, 2012, cured at 29 °C for six days and stored at 14 °C and 85% RH for 5 months before sampling for WSV analyses. Tissues sampled included skins, cortex, pith at the proximal end, pith at the distal end, and pith at the central region of the root. Skin samples were collected by gently rubbing the sweetpotato root surface with a knife. The cortex tissue was sampled from roots that had previously been removed the skin; it was collected by careful tissue excision with a knife. To sample pith tissue, the root was peeled (any left skin and cortex tissue were removed), and a root piece from the indicated areas was obtained with a knife. The pith pieces were then finely chopped into smaller pieces. All root tissues were then frozen with liquid nitrogen and finely crushed with mortar and pestle. A replicate in each tissue type was representative of an individual root. A total of 6 roots/replications were sampled for all tissues.

Vitamin extraction and analysis procedures

All extraction procedures were conducted using amber vials to prevent photo-degradation of the WSV analytes.

Ascorbic acid. The total ascorbic acid (AA) extraction methodology was adapted from Chebrolu et al. (2012). Depending on tissue type, 1.0 g of sweetpotato skins or 3 g of other tissues were placed in an amber vial, and 9 ml of 3% (w/v) meta-phosphoric acid was added. The

tissue was homogenized at 3,000 rpm for 30 s with a 1 cm diameter VirtiShear homogenizer (The Virtis Co., Gardiner, NY), and then the content was transferred to a 15 ml test tube. The sample was centrifuged at 12,857 *x g* for 10 minutes. About 3 ml of the supernatant was carefully filtered through a Phenex 25 mm, 0.45 µm nylon membrane syringe filter, (Phenomenex Inc., Torrance, CA). Then, 0.5 ml of the filtered sample was transferred to an amber 8 x 40 mm (1ml) HPLC vial and then mixed with 0.5 ml of 5 mmol/L of tris(2carboxyethyl)phosphine hydrochloride (TCEP). The sample was manually agitated for 15 s and then allowed to remain at room temperature (21 °C) for 30 min for complete reduction of dehydroascorbic acid to ascorbic acid. The AA determination was conducted with the same methodology described in Chapter 2.

Thiamin and riboflavin. Tissues were frozen with liquid nitrogen and finely crushed with mortar and pestle. For sweetpotato skins, 1.0 g of tissue was placed in a 25 ml Erlenmeyer flask and 10 ml of HCl were added. For all other foliar or root tissues, 5 g were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl were added. The mixture was autoclaved for 30 min at 121 °C. It was allowed to cool and the pH was adjusted to 4.5±0.1 with 2M sodium acetate. In each individual sample, 100 mg of taka-diastase were added, followed by gentle manual stirring for 10 s. All samples were then put in an incubator (Innova™ 4000 Incubator shaker, New Brunswick Scientific Co, Inc., Enfield, CT) at 37° C, with agitation speed of 60 rpm for 12 hr. The volume was then brought to 25 ml (sweetpotato skins) or 100 ml (all other sweetpotato tissues) with distilled water and filtered through Whatman #4 paper (GE Healthcare Co., Buckinghamshire, UK). Exactly 300 µl of 0.03M potassium ferricyanide were added to 5.0 ml of sample extract, followed by 15 s of vigorous manual stirring. The sample extract was placed in the dark for 10 min in order to reduce thiamin to thiochrome. To prevent degradation of

analytical column performance the pH of the sample was then adjusted to 7.0 with a 17% (w/v) orthophosphoric acid solution, followed by filtration through a Phenex 0.45 μm nylon membrane syringe filter (Phenomenex Inc., Torrance, CA) and injected in the HPLC system for simultaneous determination of thiamin and riboflavin. The chromatographic separation of thiamin and riboflavin was conducted by HPLC with the same methodology described in Chapter 2.

Vitamin B6. Tissues were frozen with liquid nitrogen and finely crushed with mortar and pestle. For sweetpotato skins, 1.0 g of tissue was placed in a 25 ml Erlenmeyer flask and 10 ml of HCl were added. For all other foliar or root tissues 5 g were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl were added. The mixture was autoclaved for 30 min at 121 °C. It was allowed to cool at room temperature for 15 min and the pH was adjusted to 4.5±0.1 with 2M sodium acetate. The volume was then brought to 25 ml (sweetpotato skins) or 100 ml (all other foliar and root tissue types) with distilled water, and then vigorously agitated for 15 s. Twenty five ml were transferred to a 50 ml test tube. The sample was then centrifuged at 12,857 *x g* for 10 minutes. An aliquot of 15 ml of supernatant was transferred to a 25 ml volumetric flask, and 1 ml of 25 Units/ml acid phosphatase solution plus 3 ml of 45 Units/ml of the β-glucosidase solution were added. Samples were incubated at 37° C, with gentle stirring for 18 hr. To stop the incubation, the samples were cooled to room temperature and added 5 ml of 1M HCl solution, and the flask were filled to the line (25 ml) with 0.1M HCl. An aliquot of the sample was filtered with 0.45 μm nylon membrane syringe filter and transferred to an HPLC vial. Vitamin B6 analyses were conducted by HPLC using the same chromatographic conditions as described in Chapter 3.

Statistical analyses

A completely randomized design was used to analyze the experiments. Due to little variability between the first and second experiment, the data was combined and analyzed as a single experiment with a total 11 replicates per treatment. An analysis with 6 replicates per treatment was conducted for the third experiment studying the WSV content in different sweetpotato root tissue types. The data was analyzed with SAS program, PROC GLM procedure (SAS Institute, Cary, NC, USA). Treatment means were separated with Tukey's HSD test.

RESULTS AND DISCUSSION

Ascorbic acid

Differences in total AA content were found between tissue types. Young leaves contained the highest AA content (118.6 mg/100g), followed by old leaves (89.9 mg/100g). Buds also contained significantly higher AA content relative to root, vine, and petiole tissues (Table 4.1). A wide range in sweetpotato leaf AA content has been reported in previous studies. Our results are similar to those reported for various cultivars grown in Malaysia (Villareal et al., 1979), but less than the levels reported by Mosha et al., (1995). The AA content obtained in old leaves was also similar to the amounts found by Ishida et al., (2000) in the cultivar Beniazuma. The higher AA found in young leaves is consistent with previous reports that indicated AA is higher in young actively metabolizing tissues. Typically, AA content is lowest in dormant or quiescent cells, it markedly increases under conditions that favor rapid growth and metabolism (Pastori et al., 2003). The higher AA content in leaves compared to other vegetative tissues was associated with its importance as a free radical quencher in the high oxidative environment that accompanies photosynthesis (Foyer et al., 1994).

The results confirm previous studies that sweetpotato foliar tissues are a good source of AA (Johnson and Pace, 2010; Maeda and Salunkhe, 1981), and that young leaves have the highest foliar AA content. The AA content (118.6 mg/100 g fresh weight) in young sweetpotato leaves compares favorably with other vegetables, including spinach (31.6 mg), green beans (15.8 mg), peas (30.9 mg) (Favell, 1998), fresh broccoli (96.7 mg), fresh cabbage (42.3 mg), fresh collards (92.7 mg), potatoes (11.0 mg), which are all considered good sources of AA in the diet (Vanderslice et al., 1990).

Table 4.1 Water-soluble vitamin content in different sweetpotato tissues.[z]

Tissue type	Ascorbic acid (mg/100 g)	Thiamin (mg/100 g)	Riboflavin (mg/100 g)	Vitamin B6 (mg/100 g)[y]			
				PL	PN	PM	B6[x]
Bud	58.1 c	nd	0.142 c	0.138 a	0.102 b	0.033 c	0.27 c
Vine	20.5 d	nd	0.059 d	0.038 c	0.044 d	nd	0.082 e
Young petiole	24.7 d	nd	0.137 c	0.056 b	0.043 d	nd	0.099 e
Young leaf	118.6 a	nd	0.215 b	0.117 a	0.065 c	0.078 b	0.249 c
Old petiole	9.0 e	nd	0.029 e	0.011 d	0.364 a	0.001 d	0.376 b
Old leaf	88.9 b	nd	0.313 a	0.134 a	0.280 a	0.17 a	0.560 a
Root	21.4 d	0.042	0.028 e	0.047 bc	0.112 b	0.005 d	0.164 d

[z]Mean values for ascorbic acid, thiamin, and riboflavin represent the average of 11 replicates.
[y]Mean values for vitamin B6 vitamers represent the average for 4 replicates. Means with different letter within the same column were significantly different ($P<0.05$) according to Tukey's HSD test. All units were calculated in mg/100g fresh weight.
[x]Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).
The coefficient of variation (CV) of AA content ranged from 0.041 in old leaves to 0.28 in roots; riboflavin, 0.11 in vines to 0.45 in roots; vitamin B6, 0.04 in young leaves to 0.17 in buds.
nd: not detected.

Thiamin

No thiamin (vitamin B1) was detected in foliar tissues. This contrasts with other authors, who have reported variable amounts in sweetpotato leaves (Ishida et al., 2000; Mosha and Gaga, 1999; Mosha et al., 1995). The lack of thiamin in our results might be explained by cultivar differences. Thiamin content was particularly low in sweetpotato foliage of the cultivar TU-82-

155 (mean=0.02±0.017 mg/100 g dry weight basis) in greenhouse beds (Almazan et al., 1997). Additional thiamin analyses in various cultivars are necessary to explain the wide variability in thiamin content reported in foliar tissues.

Riboflavin

Riboflavin content differed with tissue type, but was consistently higher in leaves. Old leaves contained higher amounts of riboflavin than young leaves and other plant tissues, including roots (Table 4.1). These results agree with previous reports indicating leaves are a good source of riboflavin. A portion of 85 g of cooked sweetpotato leaves can provide 15% of the daily intake requirements for an adult and nearly 30% for a child (Woolfe, 1989). Our results indicate that riboflavin in sweetpotato leaves compare favorably with other fruit and vegetables including cassava leaves (0.33 mg), sweet basil (0.33 mg), and papaya (0.30 mg) (Caldwell and Enoch, 1972); and with spinach (0.15 mg), potatoes (0.05 mg) and carrots (0.05 mg) (Hanif et al., 2006). The riboflavin content found in the old leaves was similar to values previously reported Caldwell and Enoch (1972). The riboflavin content in young leaves was slightly lower, while vines contained similar contents to results obtained by Ishida et al., (2000).

Riboflavin has been previously found to be more concentrated in green leafy tissues than other plant parts. Riboflavin concentration was at least 3.5 times higher in cassava leaves than roots (Montagnac et al., 2009). The reason for the higher concentration of riboflavin in leaf tissues in this study might be related with the biological roles of this vitamin in plants. In addition to being the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), riboflavin is an essential cofactor for many enzymes in multiple cellular processes, such as the citric acid cycle and cellular redox reactions (Jordan et al., 1999). Riboflavin has also been

found involved in light dependent processes such as photosynthesis and phototropism (Massey, 2000).

Vitamin B6

Leaf tissue also contained higher total vitamin B6 content compared to other tissues. Mature leaves contained 3.4 times higher vitamin B6 than roots, while mature petioles contained 2.3 times more than roots. Bud tissue and young leaves also contained higher B6 levels than roots; while the vine and young petiole tissue contents were lower than roots (Table 4.1). Differences in vitamin B6 content in various sweetpotato tissues have been previously reported, with the extent of these differences dependant on cultivar. Leaf tissue of the cultivar Koganesengan contained 9.1 times higher vitamin B6 content than root tissue, while leaf tissue of the cultivar Beniazuma contained only 1.1 times more than root tissue (Ishida et al., 2000). Vitamin B6 content in mature petioles in this study was higher than the value reported by Ishida et al., (2000), however, the vitamin B6 content found in young petioles was lower.

The contents of individual B6 vitamers were variable among foliar tissues. Pyridoxal (PL) was found in higher amounts in old leaves and old petioles, and lower in young petioles and vines. Pyridoxine (PN) content was higher in old petioles and old leaves and lower in vines. Meanwhile, pyridoxamine (PM) was higher in old leaves and lower in roots. No previous reports were found on vitamin B6 vitamers in sweetpotato leaves. Total vitamin B6 composition of roots consisted of 29% PL, 68% PN, and 3% PM. These results contrast with a previous report indicating total vitamin B6 of sweetpotato roots was 44% PL, 33% PN, and 23% PM (Kwiatkowska et al., 1989). This discrepancy may be due to cultivar differences.

The reason for the higher concentration of vitamin B6 found in leaves may be associated with its important physiological role in actively photosynthesizing tissues. Vitamin B6 has been

implicated in photo-oxidative protection in *Arabidopsis thaliana* by limiting O_2 accumulation under high light conditions and by preventing O_2 mediated oxidative damage (Havaux et al., 2009).

The results of this study indicate old and young leaves could provide significant amounts of vitamin B6 to the diet. The vitamin B6 content found in sweetpotato leaves compares favorably with other fruits and vegetables, including raw broccoli (0.17 mg), fresh avocados (0.44 mg), raw carrots (0.170 mg), bananas (0.31 mg/100 g), and cauliflower (0.16 mg) (Kabir et al., 1983).

Water-soluble vitamin content in root tissues

Limited studies have been conducted on the AA content in different sweetpotato root tissues, although tissue differences in other fruits and vegetables have been widely reported (Erdman and Klein, 1982). The AA content among root tissues was similar between the cortex and all of the pith tissues in this study (Table 4.2). The AA content was lower in skin tissue. No differences in AA content were observed between small and large sweetpotato roots (Reddy and Sistrunk, 1980). Proximal end tissue contained lower AA content than the central section and the distal end in roots stored at 15.5° C, but this difference disappeared in roots exposed to 29° C for 8 and 15 days (Ezell et al., 1952). The results for two cultivars in this study indicated skin tissue contained lower amounts of AA compared to cortex, and all of the pith locations (Table 4.2). The lower AA content found in skin might be due to the skin (periderm) cells structure and function. The sweetpotato skin is composed of three layers: phellem, phelloderm, and phellogen (cork cambium). The phellogen is a lateral meristem that is responsible for secondary growth, producing the phelloderm (living parenchyma cells) towards the inside of the root and the phellem (cells which die to form protective tissue) towards the outside (Firon et al., 2009). The

Table 4.2 Water-soluble vitamin content in different sweetpotato root tissues.

Cultivar	Tissue/part	Ascorbic acid (mg/100 g)	Thiamin (mg/100 g)	Riboflavin (mg/100 g)	Vitamin B6 (mg/100 g)			
					PL	PN	PM	B6[z]
07-146	Skin	3.2 c	0.012 c	0.06 a	0.040 a	0.032 c	0.0053 b	0.08 c
	Cortex	16.0 ab	0.016 c	0.014 d	0.030 bc	0.131 b	0.0573 a	0.21 b
	Proximal	17.0 a	0.035 b	0.024 b	0.034 ab	0.173 a	0.0506 a	0.25 a
	Distal	15.5 b	0.046 a	0.019 c	0.026 c	0.178 a	0.0349 a	0.23 ab
	Pith	16.0 ab	0.05 a	0.022 bc	0.029 bc	0.158 ab	0.0533 a	0.23 ab
Beauregard	Skin	3.8 b	0.031 bc	0.122 a	0.052 a	0.023 c	0.0078 a	0.081 c
	Cortex	13.4 a	0.056 a	0.035 c	0.050 a	0.152 a	0.0065 a	0.21 a
	Proximal	19.4 a	0.025 c	0.050 b	0.061 a	0.105 b	0.0069 a	0.17 ab
	Distal	17.6 a	0.031 bc	0.040 bc	0.053 a	0.090 b	0.0047 a	0.15 b
	Pith	15.0 a	0.038 b	0.041 bc	0.047 a	0.112 ab	0.0047 a	0.16 ab

All results are presented in mg/100 g of fresh weight basis.
Means within the same cultivar and column followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test. All vitamin content represent the average of six individual roots.
[z]Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).
The coefficient of variation (CV) in AA content ranged between 0.036 in central root pith (cultivar 07-146) to 0.377 in skin (Beauregard); thiamin, 0.088 in central root pith (07-146) to 0.27 in the proximal pith (Beauregard); riboflavin, 0.019 in cortex (Beauregard) to 0.19 in distal pith (07-146); vitamin B6, 0.057 in proximal pith (07-146) to 0.21 in proximal pith (Beauregard).

outer skin cells are devoid of starch granules, become partly lignified during growth and are progressively sloughed off (Villavicencio et al., 2007). The phellogen layer remains active until harvest, with its activity maintaining a constant thickness of the periderm layer (Firon et al., 2009). The relatively lower metabolic activity of the cells in the phellem may contribute to the lower skin AA content. Lower AA was also found in potato peels relative to the inner flesh (Augustin et al., 1979).

Thiamin differences were observed among root tissues, but the differences were not consistent across cultivars. Thiamin content was higher in the distal and central pith regions of the root and lowest in cortex and skin in cultivar 07-146. Thiamin was higher in cortex and lowest in the proximal end and skin in Beauregard roots. Information on thiamin distribution within root tissues is limited. A previous report found thiamin content to be twice as high 2-3 mm below the skin in comparison to the center portion of the root in sweetpotato cultivars from the South Pacific (Bradbury and Singh, 1986). Our results are consistent with the lower thiamin content found in potato peel tissue compared to the flesh (Augustin et al., 1979). Since thiamin is a coenzyme involved in multiple metabolic reactions, the lower thiamin content found in skin tissue may be related to the relatively low amount of metabolically active cells.

Riboflavin content was consistently higher in skin tissue relative to the other root tissues in both cultivars. Riboflavin content was lowest in cortex in cultivar 07-146, while in Beauregard the cortex tissue was not lower in riboflavin compared to the distal end and central pith tissue. Riboflavin content was also found to be similar within different internal root locations of other sweetpotato cultivars (Bradbury and Singh, 1986). Riboflavin content was also found to be higher in potato peel tissue compared to flesh tissue (Augustin et al., 1979). The higher of riboflavin in skin tissue could be related to its role in the synthesis of lignin and anti-microbial

compounds. Riboflavin functions as an essential component of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Numerous flavoprotein enzymes present in plant tissue are known to bind FAD and FMN (Zhuang and Barth, 2003). These flavoenzymes include many oxidases, dehydrogenases, and enzymes involved in the synthesis of aromatic amino acids, quinones, lignin, flavonoids, and alkaloids (Zhuang and Barth, 2003). Although riboflavin was higher in skin tissue relative to other root tissues, it is unlikely skin tissue would be a good source of riboflavin in diet due to the lack of consumption of sweetpotato skin tissue.

Vitamin B6 content was consistently lower in skin tissue in both sweetpotato cultivars. The vitamin B6 content in the other tissue types varied with cultivar. Among the vitamin B6 vitamers, PL and PN contents were different among root tissues, but these differences were not consistent across cultivars. Except for a lower PM content in skin tissue in cultivar 07-146, the PM content was mostly similar across tissues of cultivar 07-146 and Beauregard. In general, PN was predominant in the pith of the different root locations and in the cortex. No previous reports were found comparing the vitamin B6 content between root tissue types. The lower vitamin B6 content found in skin tissue might be due to similar reasons as discussed for AA and thiamin. Vitamin B6 is a coenzyme component of numerous larger enzymes, the majority of which are involved in amino acid metabolism (Zhuang and Barth, 2003). General types of enzymatic reactions catalyzed by vitamin B6 include transamination, decarboxylation, transulfhydration and desulfhydration, cleavage, and racemization (Zhuang and Barth, 2003).

CONCLUSIONS

Among the different sweetpotato tissues, the AA, riboflavin, and vitamin B6 contents were higher in leaf tissue. No thiamin was found in sweetpotato foliar tissues, but leaves compared favorably with other vegetables in their AA, riboflavin, and vitamin B6 contents. The

result of this study confirmed that leaf tissue is a potentially good source of multiple WSV in human diet. Sweetpotato root tissues showed a variable distribution of WSV. In general, skins contained a lower level of AA, thiamin and vitamin B6, and higher riboflavin content. All other differences in WSV found in root tissues were cultivar dependent.

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CHAPTER 5. EFFECT OF COOKING METHOD ON THE ASCORBIC ACID, THIAMIN, RIBOFLAVIN, AND VITAMIN B6 CONTENT OF SWEETPOTATO

INTRODUCTION

Water-soluble vitamins (WSV), including ascorbic acid (vitamin C) and the B vitamins, are sources of co-factor molecules that are required for many enzymes to be catalytically active in plant and animal tissues. Ascorbic acid and the B vitamins are not able to be synthesized by humans and are essential compounds for preventing specific diseases and sustaining human health (Kawasaki and Egi, 2000; Nielsen, 2000; Padayatty et al., 2003; Ubbink, 2000). B vitamins help the body obtain energy from food and are important for normal appetite, good vision, healthy skin, nervous system, and red blood cell formation (Anderson and Young, 2012). Ascorbic acid (AA) is widely known as an antioxidant, important for collagen formation, and has been associated with the reduction of certain degenerative diseases such as cataract, cancer, and cardiovascular diseases (Bendich and Langseth, 1995; Salonen et al., 1997).

Sweetpotato is considered a good source of AA and a moderate source of several B vitamins in human diets. Various cooking methods, including microwaving, baking, boiling, and frying are used to prepare sweetpotatoes for consumption. Previous reports have shown a variable effect of cooking method on sweetpotato AA and B vitamin content. A loss of 85% AA was reported in baked sweetpotatoes, but a 126% gain in AA was obtained in boiled sweetpotatoes (Scoular and Eakle, 1943). Baked sweetpotatoes showed a higher AA content in relation to microwaved and canned roots, but not significantly higher than boiled and steamed roots (Lanier and Sistrunk, 1979). Losses of 52%, 62% and 71% AA were reported in baked, fried and boiled sweetpotatoes, respectively (Babalola et al., 2010). More recently, no significant differences were observed in AA content between fried vs. raw sweetpotato samples; however

there was a significant increase AA in boiled tissue compared to raw tissue (Chukwu et al., 2012).

Limited research has been conducted comparing the effects of cooking method on B vitamin content in sweetpotato. The few reports available have reported differences in the content of thiamin, niacin and pantothenic acid between cooking methods (Lanier and Sistrunk, 1979; Mosha et al., 1995).

The recent advances in vitamin analytical methodologies offer opportunities to advance the information on the effect of cooking method on nutritional content of sweetpotatoes. The objective of this study was to determine the effect of different cooking methods, including baking, boiling, microwaving and frying on the AA, thiamine, riboflavin, and vitamin B6 composition of sweetpotato.

MATERIALS AND METHODS

Reagents

Thiamin hydrochloride, riboflavin, L-ascorbic acid, vitamin B6 vitamers (pyridoxine hydrochloride, pyridoxamine dehydrochloride, and pyridoxal hydrochloride), taka-diastase from *Aspergillus oryzae*, acid phosphatase from potato, β-glucosidase from almonds, meta-phosphoric acid (MPA), sodium acetate, potassium ferricyanide (III), potassium phosphate monobasic, 1-octane sulfonic acid, triethylamine, sodium phosphate monobasic, sodium hexane sulfonate, and tris(2carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Sigma Aldrich (St Louis, MO). All reagents were reagent grade unless otherwise stated. Hydrochloric acid and orthophosphoric acid were supplied by Fisher Scientific (Pittsburgh, PA). Acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All standards were prepared daily for the respective analyses.

Root origin and management

Sweetpotato roots cv. Beauregard and 07-146 were harvested on August 22, 2012 from the same field at the LSU AgCenter Sweetpotato Research Station, Chase, LA. The roots were cured at 30° C and 85-90% relative humidity (RH) for 7 days, and then placed in storage at 14°C and 85% RH until the analyses were conducted.

Comparison of cooking methods

Six individual sweetpotato 'Jumbo' size roots from cultivar Beauregard were sliced into five transversal pieces of 175 g. A section from each root was randomly assigned to each of five preparation method treatments including microwaving, boiling, baking, frying, and raw (control). The regions at the distal and proximal ends from the root were trimmed off and not used in these procedures to limit variability of results, based on root regions WSV composition results presented in Chapter 4.

The conditions used in each preparation method were as follows. For microwaving, the sweetpotato pieces were folded in a wet paper towel to limit desiccation. Three sweetpotato pieces were microwaved for 10 minutes in each run. The microwave was a Frigidaire 1000W output with a microwaving frequency of 2450 MHz. Boiling was conducted for 35 minutes in 2.0 L of water at 100 °C. For baking, individual root pieces were folded in aluminum foil to limit desiccation, and then baked at 218 °C for 50 minutes. For frying, the sweetpotato pieces were cut into French fry sections about 8 cm long and 1 cm wide. Frying was conducted at149 °C for 3.5 min in canola oil. Following each preparation method, the individual root pieces were analyzed for AA, thiamin, riboflavin, and vitamin B6. To correct for weight gain in French fries due to oil uptake during the frying process, French fries were analyzed for fat content based on the AOCS method Ai 3-75 (AOCS, 2010) and the resulting fat percent in French fries subtracted from the

sampled tissue weight before WSV content calculation. A control treatment consisting of raw sweetpotato pieces was also sampled for WSV analysis. The experiment was initially conducted with roots stored for three months and then repeated with roots stored for six months.

Boiling treatments

Sweetpotato jumbo size roots cv. Beauregard were cut in five 175 g pieces. The pieces from each root were randomly assigned among four boiling treatments and a control (raw). The treatments included boiling for 25 min in 1.5 L of water, 45 min in 1.5 L of water, 25 min in 3 L of water, and 45 min in 3 L of water. Boiled pieces were sampled for AA, thiamin and riboflavin analyses. The experiment was initially conducted with 5 replicates, and then repeated with 4 replicate roots per treatment.

Microwaving treatments

Three pieces of 175 g each were obtained from sweetpotato Beauregard jumbo roots. The pieces from each root were randomly assigned among two microwaving treatments and a control (raw). Each piece was folded in a wet paper towel to limit tissue desiccation. Microwaving was conducted for 10 min and 15 min. Microwaved pieces were cooled and then sampled for AA, thiamin, and riboflavin analysis. The experiment was conducted twice with 5 replicate roots per treatment.

Baking treatments

Sweetpotato jumbo size roots cv. Beauregard were cut into 175 g pieces. The pieces from each root were randomly assigned among five baking treatments and enclosed in aluminum foil to limit desiccation during the conventional oven baking process. Baking treatments included three different temperatures, 177 °C, 204 °C and 232 °C for 45 min; and three baking durations, 45, 60, and 75 min at 204 °C. After baking, the sections were cooled to room temperature and

then sampled for AA, thiamin, riboflavin, and vitamin B6 analysis. The experiment was initially conducted with 6 replications and later repeated with 5 replications per treatment.

Frying treatments

Six jumbo size roots were cut into French fry sections. An equal number of 15 fries from each root were assigned to each of the frying treatments. Frying treatments included three temperatures, 149° C, 166° C, and 182° C for 120 seconds; and three frying times, 120, 165 and 210 seconds at 166 °C. Fried tissue was sampled for AA, thiamin, riboflavin and vitamin B6 analysis. To correct for weight gain in French fries due to oil uptake during the frying process, French fries were analyzed for fat content based on the AOCS method Ai 3-75 (AOCS, 2010), and the fat content % in French fries was subtracted from the sample tissue weight prior to WSV content calculation. The experiment was initially conducted with roots of cultivar Beauregard and later with roots of 07-146. A total of 6 individual root replications per treatment were used.

Vitamin extraction and analysis procedures

Ascorbic acid. The AA extraction methodology was adapted from Chebrolu et al. (2012). Three g of finely mashed pith tissue (cooked pieces) or finely grated pith tissue (raw pieces) were placed in an amber vial, and 6 ml of 3% (w/v) meta-phosphoric acid were added. The tissue was homogenized at 3,000 rpm for 30 s with a 1.0 cm diameter VirtiShear homogenizer (The Virtis Co., Gardiner, NY), and then the homogenate was transferred to a 15-ml polypropylene test tube. The sample was centrifuged at 12,857 *x g* for 10 minutes. About 3 ml of the supernatant was carefully filtered through a Phenex 25 mm, 0.45 µm nylon membrane syringe filter, (Phenomenex Inc., Torrance, CA). Exactly 0.5ml of the filtered sample was transferred to an amber 8 x 40mm (1ml) HPLC vial and then mixed with 0.5ml of 5mmol/L of tris(2carboxyethyl)phosphine hydrochloride (TCEP). The sample was manually agitated for 15 s

and then allowed to remain at room temperature (21 °C) for 30 min for complete reduction of dehydroascorbic acid to AA. The HPLC analytical procedure for AA was based on the methodology described in Chapter 3.

Thiamin and riboflavin. Thiamin and riboflavin were extracted based on the methodology of Barrera and Picha (2013, unpublished). Five g of finely mashed tissue from the central pith region of the pieces (cooked pieces) or finely grated tissue (raw pieces) were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl was added. The mixture was autoclaved for 30 min at 121 °C. It was allowed to cool and the pH was adjusted to 4.5±0.1 with 2M sodium acetate. In each individual sample, 100 mg of taka-diastase was added, followed by gentle manual stirring for 10 s. All samples were then put in an incubator (Innova™ 4000 Incubator shaker, New Brunswick Scientific Co, Inc., Enfield, CT) at 37° C, with agitation speed of 60 revolutions per minute (rpm) for 12 hr. The volume was then brought to 100 ml with distilled water and filtered through Whatman #4 paper (GE Healthcare Co., Buckinghamshire, UK). Exactly 300 µl of 0.03M potassium ferricyanide were added to 5.0 ml of sample extract, followed by 15 s of vigorous manual stirring. The sample extract was placed in the dark for 10 min in order to reduce thiamin to thiochrome. To prevent degradation of analytical column performance the pH of the sample was then adjusted to 7.0 with a 17% (w/v) orthophosphoric acid dilution, followed by filtration through a 0.45 µm nylon membrane syringe filter (Phenex, Phenomenex Inc., Torrance, CA) and injected in the HPLC system for simultaneous determination of thiamin and riboflavin. The analytical procedures for thiamin, riboflavin were conducted based on the HPLC methodology described in Chapter 2.

Vitamin B6. The extraction and analysis methodology for vitamin B6 was adapted from Kall (2003). Five g of finely mashed tissue (cooked pieces) or finely grated tissue (raw pieces)

from the pith of the central region of the pieces were transferred into a 250 ml Erlenmeyer flask and 50 ml of 0.1M HCl were added. The mixture was autoclaved for 15 min at 121° C. It was then allowed to cool and the pH was adjusted to 4.5 with 2M sodium acetate. The volume was then brought to 100 ml with distilled water, and then vigorously agitated for 15 s. Forty ml were transferred to a 50 ml polypropylene test tube. The sample was then centrifuged at 12,857 *x g* for 10 minutes. An aliquot of 15 ml of supernatant was transferred to a 25-ml volumetric flask, to which was added 1 ml of 25 Units/ml acid phosphatase solution plus 3 ml of 45 Units/ml of the β-glucosidase solution. Samples were incubated at 37° C, with gentle stirring, for 18 hr. Following incubation, the samples were cooled to room temperature for 15 minutes and 5 ml of 1M HCl was added. The flask was made up to final volume of 25 ml with 0.1M HCl. An aliquot of this solution was filtered through a 0.45 μm nylon membrane syringe filter and transferred into an HPLC amber vial. The vitamin B6 analysis and expression of results was conducted based on the methodology described in Chapter 3. All results from WSV analyses were calculated in mg/100 g of fresh weight (FW) and dry weight (DW).

Statistical analyses

The data from each experiment was analyzed as a completely randomized design. Each root was considered one replication. The data was analyzed with SAS program, PROC GLM procedure (SAS Institute, Cary, NC, USA). Treatment means were compared with Tukey's HSD test.

RESULTS AND DISCUSSION

Ascorbic acid

Differences in sweetpotato root tissue AA content were found between the different cooking methods (Tables 5.1 and 5.2). On a fresh weight basis, frying resulted in higher AA

Table 5.1 Water-soluble vitamin content (fresh weight basis) of Beauregard sweetpotatoes cooked under different methods and at different storage times.

Preparation method	Ascorbic acid (mg/100 g FW)	Thiamin (mg/100 g FW)	Riboflavin (mg/100 g FW)	Vitamin B6 (mg/100 g FW)			
				PL	PN	PM	Total B6^{z}
Three-month stored roots							
Raw	23.5 a	0.045 bcd	0.017 abc	0.061	0.088 de	0.009 fg	0.157 de
Microwaved	6.3 fg	0.056 abc	0.019 abc	0.017	0.118 cde	0.042 b	0.171 cd
Boiled	12.2 de	0.041 cd	0.014 c	0.020	0.080 e	0.022 de	0.119 f
Baked	15.8 bcd	0.034 d	0.019 abc	0.014	0.128 c	0.022 de	0.161 cde
Fried	23.7 a	0.059 ab	0.020 ab	0.021	0.197 b	0.036 bc	0.249 b
Six-month stored roots							
Raw	16.9 bc	0.039 d	0.016 bc	0.037	0.122 cd	0.006 g	0.164 cd
Microwaved	4.7 g	0.047 abc	0.017 abc	0.022	0.147 c	0.029 cd	0.194 c
Boiled	9.8 ef	0.038 d	0.017 bc	0.021	0.108 cde	0.016 ef	0.143 e
Baked	12.1 cde	0.037 d	0.021 ab	0.019	0.124 cd	0.024 de	0.163 cd
Fried	17.1 b	0.064 a	0.023 a	nd	0.377 a	0.12 a	0.479 a

FW, fresh weight basis.
Values for each treatment represent the mean of six replicates. Mean values within the same column followed by a different letter are significantly different ($P<0.05$) according to Tukey's HSD test.
^{z}Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).
nd: not detected.

Table 5.2 Water-soluble vitamin content (dry weight basis) of Beauregard sweetpotatoes cooked under different methods and at different storage times.

Preparation method	Ascorbic acid (mg/100 g DW)	Thiamin (mg/100 g DW)	Riboflavin (mg/100 g DW)	Vitamin B6 (mg/100 g DW)			
				PL	PN	PM	Total B6[z]
Three-month stored roots							
Raw	117.4 a	0.23 ab	0.087 ab	0.306 a	0.442 d	0.043 d	0.78 b
Microwaved	26.3 e	0.23 ab	0.080 ab	0.070 cd	0.492 bcd	0.174 b	0.71 b
Boiled	72.0 bcd	0.25 a	0.083 ab	0.119 c	0.471 cd	0.127 c	0.70 b
Baked	75.3 bc	0.16 b	0.092 a	0.065 d	0.614 bc	0.106 c	0.77 b
Fried	74.0 bc	0.19 ab	0.063 b	0.062 d	0.589 bcd	0.109 c	0.74 b
Six-month stored roots							
Raw	85.6 b	0.20 ab	0.082 ab	0.190 b	0.620 bc	0.030 d	0.84 b
Microwaved	19.6 e	0.19 ab	0.072 ab	0.089 cd	0.604 bc	0.117 c	0.79 b
Boiled	57.6 cd	0.22 ab	0.098 a	0.122 c	0.632 b	0.019 c	0.77 b
Baked	61.3 cd	0.18 ab	0.099 a	0.093 cd	0.595 bcd	0.114 c	0.78 b
Fried	53.5 d	0.20 ab	0.072 ab	nd	1.129 a	0.359 a	1.43 a

DW, fresh weight basis.

Values for each treatment represent the mean of six replicates. Mean values within the same column followed by a different letter are significantly different ($P<0.05$) according to Tukey's HSD test.

[z]Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).

nd: not detected.

content relative to baking, boiling, and microwaving. As shown in Table 5.1, French fries and raw sweetpotato tissues had similar AA contents, while lower amounts of AA were found in baked, boiled and microwaved root sections; respectively. However, the results obtained for each cooking method were affected by moisture loss during cooking. Expressed on a dry weight basis (dwb), all cooking methods resulted in a significant decrease in AA content compared to raw tissue (Table 5.2). No differences were found between baked, boiled, and fried sweetpotatoes, while microwaved root sections contained the lowest amount of AA. Differences in sweetpotato tissue AA content (fwb) by cooking method have been previously reported. Baked sweetpotatoes contained more AA than microwaved roots, but were similar to boiled roots (Lanier and Sistrunk, 1979). The reduction in AA content was 50% in baked sweetpotatoes and 65% in boiled roots compared with raw tissue (Bradbury et al., 1988). The amount of AA loss during cooking depended on the degree of heating, amount of leaching into the cooking medium, root surface area exposed to water and oxygen, pH, presence of transition metals, and other factors that facilitate oxidation (Eitenmiller and Laden, 1999). Losses in AA have also been associated with enzymatic oxidation, especially during the initial cooking stage when the tissue temperature is not high (Burg and Fraile, 1995). Microwaving has been shown to have a variable effect on AA content in other vegetables. Microwave cooking results from the simultaneous production of heat throughout the food product when polar molecules, under the influence of a rapidly oscillating electromagnetic field are induced to undergo rapid reorientation, thus, rapidly converting electromagnetic energy to thermal energy (Lassen and Ovesen, 1995). Conventional cooking, on the other hand, involves surface absorption of thermal energy from external sources by radiation and conduction, thus necessitating a longer cooking time to obtain a given internal product temperature (Chung et al., 1981). Microwaving did not significantly reduce AA content

in mini-potatoes (Navarre et al., 2010). Similarly, little or no decrease in AA was reported in peppers microwaved or stir-fried (Chuah et al., 2008). On the other hand, Augustin et al. (1978) found microwaving had a similar effect with baking in AA retention in potato. Microwaving was the most detrimental domestic processing method for vitamin C content in broccoli florets, with respect to conventional boiling, steaming, or pressure cooking (Vallejo et al., 2002). The wide variability in loss of AA during microwaving has been attributed to specific microwaving conditions and the type of commodity. Microwaving for short times allowed a higher retention of AA by limiting thermal and enzymatic degradation (Burg and Fraile, 1995). The overall lower AA found in microwaved sweetpotato tissue in this study might be explained by the relatively longer microwaving time used compared with other studies, a low amount of tissue (3 pieces of 175 g each), and a relatively high microwave oven cooking power (1000W). The results of this study suggest that a holding time in the microwave oven beyond the necessary cooking time could cause higher AA losses. Microwaving sweetpotatoes for 15 minutes resulted in a further decrease in AA content compared with tissues microwaved for 10 minutes (Table 5.3).

Losses in AA during boiling have been widely reported in sweetpotato and other vegetables. The AA loss (fwb) in sweetpotatoes was 70% if the water was discarded after 30 minutes of boiling (Bradbury and Singh, 1986a). Babalola et al., (2010) reported 63% AA losses (fwb) in boiled sweetpotatoes compared to raw. These results generally agree with the AA decrease observed in boiled sweetpotatoes in this study. Losses during boiling have been mainly associated with leaching into the boiling water and chemical degradation due to heat (Bradbury et al., 1988). Additional contributing factors are the volume of boiling water used (Ryley and Kajda, 1994) and the boiling time (Bradbury and Singh, 1986a). In this study, however, sweetpotatoes boiled for 25 and 45 min in 1.5 L and 3 L did not show AA content differences

(Table 5.4). It is possible that AA retention during boiling of sweetpotatoes was related to the integrity of the sampled tissue. Samples obtained for water-soluble vitamin analysis were collected approximately 3 mm below the boiled tissue surface. Sweetpotatoes boiled for 45 min in 1.5 L and 3 L were noticeably less firm in texture compared with sweetpotatoes boiled for 25 min in the same water volumes. However, the edible structural integrity of the sampled root pieces was not compromised.

Table 5.3 Water-soluble vitamin content of Beauregard sweetpotatoes after two different microwaving intervals.

Preparation method	Ascorbic acid (mg/100 g)		Thiamin (mg/100 g)		Riboflavin (mg/100 g)	
	FW	DW	FW	DW	FW	DW
Raw	18.7 a	89.1 a	0.051 a	0.24 a	0.020 a	0.09 a
Microwaving for 10 min	10.5 b	43.1 b	0.054 a	0.22 a	0.019 a	0.08 a
Microwaving for 15 min	4.70 c	17.0 c	0.049 a	0.18 b	0.020 a	0.07 a

FW, fresh weight basis; DW, dry weigh basis.
Values for each treatment represent the mean of 10 replicates. Means followed by different letter were significantly different ($P<0.05$) based on Tukey's HSD test.

Table 5.4 Water-soluble vitamin content in boiled sweetpotatoes during different intervals and water volumes.

Preparation method	Ascorbic acid (mg/100 g)		Thiamin (mg/100 g)		Riboflavin (mg/100 g)	
	FW	DW	FW	DW	FW	DW
Raw	20.4 a	103.7 a	0.048 a	0.24 a	0.020 a	0.10 a
Boiling 25 min-1.5 L	13.0 b	70.6 b	0.051 a	0.27 a	0.018 a	0.10 a
Boiling 25 min-3 L	12.4 b	73.1 b	0.049 a	0.25 a	0.018 a	0.10 a
Boiling 45 min-1.5 L	12.8 b	75.0 b	0.047 a	0.24 a	0.020 a	0.11 a
Boiling 45 min-3 L	13.0 b	64.9 b	0.044 a	0.23 a	0.020 a	0.10 a

FW, fresh weight basis; DW, dry weight basis.
Values for each treatment represent the mean of 9 replicates. Means followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.

The results of this study were in agreement with previous reports indicating AA losses during conventional oven baking of sweetpotato. The AA losses previously reported due to

baking were 51.5% (fwb) (Babalola et al., 2010), and 24.3% (fwb) (Hollinger, 1944). The AA retention in this study (64-72% on dwb) was slightly lower compared to recoveries of 69-79% found in baked potato (Augustin et al., 1978). The main cause of AA losses during baking has been associated with a long residence time at high temperatures (Augustin et al., 1978), which is inherent in convection oven baking. The results of this study nonetheless, indicated that baking sweetpotatoes at higher temperatures and increasing baking time did not result in an additional AA decrease (Table 5.5 and 5.6). In potato, the activity of oxidative enzymes explained the rapid AA loss during the first stage of the baking process when the internal temperatures were still low; then, enzyme deactivation resulting from the temperature increase was associated with lowering of the vitamin C destruction rate (Burg and Fraile, 1995). This phenomenon may have played an important role in AA decrease in sweetpotatoes during baking and other thermal

Table 5.5 Water-soluble vitamin content (fresh weight basis) in sweetpotatoes subjected to different baking temperatures and times.

Baking method	Ascorbic acid	Thiamin	Riboflavin	Vitamin B6 (mg/100 g FW)			
	(mg/100 g FW)			PL	PN	PM	Total B6[z]
Baking temperature for 45 min							
149 °C	10.8 a	0.054 a	0.026 a	0.045 a	0.125 a	0.014 a	0.18 a
166 °C	10.4 a	0.052 a	0.026 a	0.043 a	0.120 a	0.014 a	0.17 a
182 °C	12.4 a	0.053 a	0.025 a	0.043 a	0.132 a	0.015 a	0.19 a
Baking time at 166 °C							
45 min	9.5 a	0.057 a	0.025 a	0.042 a	0.130 a	0.015 a	0.18 a
60 min	9.9 a	0.056 a	0.026 a	0.039 a	0.130 a	0.015 a	0.18 a
75 min	11.0 a	0.054 a	0.026 a	0.038 a	0.136 a	0.016 a	0.19 a

FW, fresh weight basis.
Values for each treatment represent the mean of 11 replicates. Baking temperature treatments means followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.
[z] Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).

Table 5.6 Water-soluble vitamin content (dry weight basis) in sweetpotatoes subjected to different baking temperatures and times.

Baking method	Ascorbic acid	Thiamin	Riboflavin	Vitamin B6 (mg/100 g DW)			
	(mg/100 g DW)			PL	PN	PM	Total B6[z]
Baking temperature for 45 min							
149 °C	52.8 a	0.26 a	0.12 a	0.215	0.594	0.067	0.87 a
166 °C	50.8 a	0.26 a	0.13 a	0.211	0.588	0.071	0.86 a
182 °C	59.0 a	0.25 a	0.12 a	0.207	0.633	0.072	0.90 a
Baking time at 166 °C							
45min	47.9 a	0.28 a	0.12 a	0.208	0.633	0.073	0.90 a
60min	51.8 a	0.28 a	0.13 a	0.194	0.655	0.076	0.91 a
75min	55.8 a	0.25 a	0.12 a	0.181	0.641	0.075	0.88 a

DW, dry weight basis.
Values for each treatment represent the mean of 10 replicates. Means followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.
[z]Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).

cooking methods used in this study.

Although no decrease in AA content was found in French fry tissue compared to raw tissue on a fresh weight basis, losses were observed on a dry weight basis. Previous reports in the literature have presented results on the effect of frying on a fresh weight basis. The results of this study (fwb) are similar to the results reported by Chukwu et al., (2012), who found a comparable AA content in fried sweetpotatoes for 10 and 15 minutes compared to raw tissue. Similarly, vitamin C in potato French fries has been reported as high as in raw potatoes (Fillion and Henry, 1998). An insignificant decrease in AA was also observed in stir-fried green peppers during 5 minutes at "medium" heating capacity (Chuah et al., 2008). Frying at higher temperatures and extending the frying time did not result in an additional decrease in AA content (Table 5.7 and 5.8). This may have been explained by rapid enzymatic deactivation due to high temperature and lowering of the oxidative AA destruction rates.

Table 5.7 Water-soluble vitamin content (fresh weight basis) in sweetpotatoes subjected to different frying temperatures and times.

Cultivar/frying method	Ascorbic acid (mg/100 g FW)	Thiamin (mg/100 g FW)	Riboflavin (mg/100 g FW)	Vitamin B6 (mg/100 g FW)			
				PL	PN	PM	Total B6[z]
Beauregard							
Frying temperature for 120 s							
149 °C	17.8 b	0.054 ab	0.030 ab	nd	0.219 f	0.064 a	0.27 f
166 °C	19.8 ab	0.055 ab	0.031 ab	nd	0.320 ef	0.092 a	0.40 ef
182 °C	19.1 ab	0.045 b	0.030 ab	nd	0.469 d	0.085 a	0.54 cde
Frying times at 166 °C							
120 s	20.5 ab	0.065 ab	0.030 ab	nd	0.301 ef	0.102 a	0.39 ef
165 s	23.6 a	0.066 ab	0.024 b	nd	0.375 de	0.088 a	0.45 def
210 s	23.2 a	0.055 ab	0.025 b	nd	0.458 d	0.103 a	0.54 cde
07-146							
Frying temperature for 120 s							
149 °C	18.0 b	0.047 ab	0.035 a	0.044 a	0.594 c	0.024 b	0.66 bcd
166 °C	17.5 b	0.054 ab	0.035 a	0.039 a	0.664 bc	0.021 b	0.72 abc
182 °C	18.6 ab	0.046 ab	0.037 a	0.031 a	0.757 ab	0.017 b	0.80 ab
Frying times at 166 °C							
120 s	16.5 b	0.069 a	0.030 ab	0.042 a	0.685 bc	0.020 b	0.74 abc
165 s	16.6 b	0.059 ab	0.033 ab	0.039 a	0.723 ab	0.019 b	0.78 ab
210 s	18.6 ab	0.067 ab	0.037 a	0.038 a	0.816 a	0.022 b	0.87 a

Values for each treatment represent the mean of 6 replicates. Treatments means within the same column followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.

[z]Vitamin B6 calculated as PN, HCl: $PN+(1.01\times PL)+(0.85\times PM)$.

nd: not detected.

Table 5.8 Water-soluble vitamin content (dry weight basis) in sweetpotatoes subjected to different frying temperatures and times.

Cultivar/frying method	Ascorbic acid (mg/100 g DW)	Thiamin (mg/100 g DW)	Riboflavin (mg/100 g DW)	Vitamin B6 (mg/100 g DW)			
				PL	PN	PM	Total B6[z]
Beauregard							
Frying temperature for 120 s							
149 °C	60.3 abc	0.20 a	0.112 abc	nd	0.829 f	0.243 a	1.03 e
166 °C	69.6 abc	0.19 a	0.107 bc	nd	1.122 de	0.323 a	1.40 cd
182 °C	65.8 abc	0.16 a	0.102 cd	nd	1.616 b	0.292 a	1.86 b
Frying times at 166 °C							
120 s	68.5 abc	0.22 a	0.092 b-e	nd	1.007 ef	0.340 a	1.30 de
165 s	78.4 a	0.22 a	0.081 de	nd	1.247 cd	0.291 a	1.49 bcd
210 s	72.8 ab	0.17 a	0.077 e	nd	1.437 bc	0.324 a	1.71 bc
07-146							
Frying temperature for 120 s							
149 °C	68.1 abc	0.18 a	0.134 a	0.167 a	2.246 a	0.093 b	2.49 a
166 °C	61.3 abc	0.19 a	0.122 abc	0.118 ab	2.327 a	0.074 b	2.51 a
182 °C	64.1 abc	0.16 a	0.129 ab	0.108 b	2.612 a	0.059 b	2.77 a
Frying times at 166 °C							
120 s	55.3 c	0.23 a	0.100 cde	0.140 ab	2.286 a	0.065 b	2.48 a
165 s	55.3 c	0.20 a	0.110 abc	0.129 ab	2.405 a	0.064 b	2.59 a
210 s	58.2 bc	0.21 a	0.117 abc	0.118 ab	2.561 a	0.069 b	2.73 a

Values for each treatment represent the mean of 6 replicates. Treatments means within the same column followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.
[z]Vitamin B6 calculated as PN, HCl: PN+(1.01×PL)+(0.85×PM).
nd: not detected.

Thiamin

Thiamin is generally considered more stable than ascorbic acid, but less stable than other B vitamins during cooking. The degradation of thiamin during thermal processing has been associated with the cleavage of its pyrimidine and thiazole moieties (Dwivedi and Arnold, 1973). Thiamin degradation is enhanced by oxygen, metal ions, and neutral or alkaline conditions (Beetner et al., 1974; Borenstein, 1975; Ritter, 1976). No consistent differences in thiamin content due to cooking method were observed in this study. Raw sweetpotato tissue contained similar amounts of thiamin to baked, boiled and microwaved tissues (Table 5.1 and 5.2). Limited research has been published on the effect of cooking method on thiamin content in sweetpotato. The results of this study contrast with a 20% thiamin loss (fwb) observed after baking sweetpotatoes for 30 min (Bradbury and Singh, 1986b); but are similar to results in boiled, baked, and microwaved potatoes (Augustin et al., 1978).

Little or no information is available on the effect of microwaving on thiamin content in sweetpotato. Microwaving has been shown to have a variable effect on thiamin content in other vegetables. Thiamin content was lower compared to raw tissue in microwaved peas (Chung et al., 1981), chick peas (Alajaji and El-Adawy, 2006) and other vegetables (Lešková et al., 2006). The results of this study agree with Augustin et al., (1978), who found mostly similar thiamin contents (dwb) in uncooked and microwaved potatoes. Stability of thiamin depends on the extent of heating and on the food matrix properties (Lešková et al., 2006). Thiamin is relatively stable in an acidic pH, but losses become significant when pH rises above 6 (Ranhotra and Bock, 1988). The relatively high thiamin content obtained in microwaved sweetpotatoes in this study might be explained by the comparatively short microwaving time to get a cooked product (10 minutes) and a low pH (5.8 for juice and 5.6 for baked tissue). These factors may have played an

important role in limiting thiamin thermal degradation and oxidation. Although an increase in thiamin content in microwaved tissue relative to raw tissue was observed in roots stored for 6 months (fwb), this was due to moisture loss, as shown by the same results expressed on a dry weight basis. Significant moisture losses have been observed during microwaving of foods (Cross et al., 1982). The results of this study showed that increasing the microwaving time from 10 to 15 minutes caused a significant decrease in sweetpotato thiamin content on a dry weight basis, although this was not evident on a fresh weight basis most likely due to moisture loss and concentration of this vitamin in tissue (Table 5.3).

Boiled sweetpotato tissue contained similar thiamin content to uncooked root tissue. Various reports with variable results have been published on the effect of boiling on thiamin content in sweetpotato and other vegetables. An average of 20% thiamin, riboflavin and niacin content (fwb) was lost in boiled sweetpotatoes (Bradbury and Singh, 1986b). Our results are mostly similar to a study that reported boiled sweetpotatoes averaged 92% of the thiamin content (fwb) found in raw sweetpotatoes (Pearson and Luecke, 1945). Similarly, the results were comparable to other reports indicating no differences in thiamin content (fwb) between boiled and uncooked potatoes (Augustin et al., 1978; Augustin et al., 1979). The source of differences between results may be attributed to the specific cooking conditions used in each study. Some authors have suggested that increased boiling times and water volumes resulted in higher water-soluble vitamin losses (Bradbury and Singh, 1986b; Ryley and Kajda, 1994). However, in this study doubling the amount of water (from 1.5 L to 3 L) and increasing the boiling time (from 25 minutes to 45 minutes) did not result in significant differences in thiamin content (Table 5.4). Other factors, including sweetpotato section size and the integrity of the sampled tissue may have had an effect on thiamin leaching into the water medium. As indicated with AA, unless

substantial deterioration of the boiled tissue occurs, thiamin leaching into the water may be minimal.

Baking of sweetpotato sections in a conventional oven did not result in significant changes in thiamin content compared to raw tissue. However, a previous report indicated thiamin losses averaged 23% (fwb) in sweetpotato tissue after baking for 30 minutes (Bradbury and Singh, 1986b). The amount of thiamin retention after baking at 204 °C averaged 75% (fwb) (Pearson and Luecke, 1945). Our results for baked sweetpotatoes are similar to those obtained by Augustin el al., (1978) and Augustin et al., (1979) in potato, who found no differences in thiamin between raw and baked potatoes (results expressed on fwb). Although baking involves a relatively long time at high temperatures, thiamin may have not been affected due to root physical and chemical properties. Thiamin is relatively stable at an acidic pH, with losses becoming significant when pH rises above 6 (Ranhotra and Bock, 1988). In this study, the pH of the juice and baked tissue was below 6, as previously indicated. A delayed internal tissue temperature increase may have further contributed to maintaining thiamin levels. Internal temperature measurements at the central core of the roots indicated it took nearly 30 minutes to exceed 80 °C during baking at 175 °C (Damir, 1989). The results of this study also indicated that baking for longer periods (60 and 75 min) and higher temperatures (204 °C and 232 °C) did not result in significant thiamin changes (Tables 5.5 and 5.6).

Little or no changes in thiamin were observed due to frying in this study. Although an increase in thiamin was observed (fwb) in French fries from roots stored for 6 months, this increase was caused by moisture loss, as shown by the same results compared on a dry weight basis (Tables 5.1 and 5.2). Little or no research has been conducted on the effect of frying on sweetpotato thiamin content. The results on a fresh weight basis (fwb) of this study are similar

with the thiamin increases observed in potato French fries relative to raw tissue (fwb) (Weaver et al., 1983). Thermal and chemical changes in food during frying involve water transfer from inside the tissue into the oil (followed by steam evaporation), oil migration into the tissue, chemical changes in the tissue due to elevated temperature and water loss, and chemical interaction of the oil with the food matrix components (Fillion and Henry, 1998). Although the tissue surface temperature rises to the oil temperature during frying, the internal tissue temperature does not exceed the water vapor temperature (Farinu and Baik, 2007; Ryley and Kajda, 1994). The amount of vitamin retention depends more on the internal tissue temperature than the oil temperature (Ghidurus et al., 2010). These factors may help explain why frying at higher temperatures and longer times did not result in significant changes in thiamin, and also little or no detrimental changes on other WSV in this study (Tables 5.7 and 5.8).

Riboflavin

The effect of cooking method on riboflavin content in sweetpotato tissue was mostly similar to the results obtained for thiamin. No consistent differences were found between raw, microwaved, boiled, baked, and fried sweetpotato tissue riboflavin content (on a fresh weight basis). This pattern was similar when the results were presented on a dry weight basis (Tables 5.1 and 5.2). Riboflavin is generally considered more stable than thiamin under various cooking conditions. Its degradation is generally enhanced by alkaline pH, light, and heat, while it has shown relative stability at neutral and acid pH, and in the presence of oxygen (Harris, 1971). Microwaved sweetpotatoes were previously reported to have similar riboflavin content (fwb) to boiled, canned, and steamed, but lower than baked sweetpotato tissues (Lanier and Sistrunk, 1979). Microwaving has also been shown to have a limited effect on riboflavin and other nutrients in various foods (Cross et al., 1982). Similar riboflavin content was observed in

microwaved and raw potatoes (Augustin et al., 1978). An additional study with potatoes indicated microwaving resulted in similar riboflavin contents to raw, boiled, and baked tissues (Augustin et al., 1979). Sweetpotato tissue has a slightly acidic pH and riboflavin degradation is thought to be more significant under alkaline conditions. Similarly, microwaving for 10 or 15 minutes did not result in differences in riboflavin content in this study (Table 5.3).

Boiling of food products has often been attributed to leaching of riboflavin and other water-soluble vitamins into the water medium. However, boiling sweetpotatoes did not result in significant riboflavin differences with uncooked root tissue in this study. These results are comparable with Pearson and Luecke (1945), who found similar riboflavin contents in boiled and uncooked sweetpotatoes (fwb). These results may be explained by boiled piece size, the specific tissue sampling location, and the integrity of the sampled tissue. It may be possible that unless a significant deterioration of the boiled tissue section occurs, riboflavin content may not be significantly leached into the surrounding water medium.

Baking of sweetpotato tissue has been found to result in favorable retention of various nutrients, including riboflavin. Baked sweetpotatoes contained higher riboflavin content (fwb) than boiled, microwaved, canned, and steamed sweetpotatoes (Lanier and Sistrunk, 1979). Losses of riboflavin and other B vitamins averaged 23% after baking for 30 minutes (Bradbury and Singh, 1986b). Baked sweetpotatoes retained on average 89% riboflavin (Pearson and Luecke, 1945). The results of this research generally agree with these reports, and suggest that within limits, baking at higher temperatures or longer time intervals does not result in significant riboflavin changes (Tables 5.5 and 5.6).

Frying has not been generally associated with causing significant effects on riboflavin content in foods. However, little or no research has been conducted on the effect of frying on

riboflavin in sweetpotato. The higher riboflavin content observed in fried sweetpotatoes (fwb) stored for 6 months was associated with moisture loss, as indicated when the results are expressed on a dry weight basis (dwb) (Table 5.2). Frying at higher temperatures or longer times did not result in consistent differences in riboflavin content in this study (Tables 5.7 and 5.8).

Vitamin B6

Vitamin B6 is considered to be mostly stable during cooking. However, vitamin B6 losses during boiling can be high, particularly due to leaching (Lešková et al., 2006). Heat degradation of vitamin B6 may be more important in animal tissues, which contain higher amounts of pyridoxal and pyridoxamine; these B6 vitamers have shown lower stability to thermal conditions than pyridoxine (Gregory III, 1985), which is usually found in significant amounts in plants (Eitenmiller and Laden, 1999; Kall, 2003). In general, all cooking methods resulted in a decrease of pyridoxal (PL), relatively unchanged pyridoxine (PN), and an increase in pyridoxamine (PM) contents (dwb). These observations agree with previous reports indicating PL interconversion to PM during thermal processing (Gregory III, 1985); such process is associated with PL transamination to either 4-piridoxic acid or PM (Pingali and Trumbo, 1995). French fry tissue contained a higher vitamin B6 content compared to the other cooking methods (fwb) (Table 5.1). However, this higher content was mainly associated with moisture loss, as indicated by the lack of differences when expressed on dry weight basis (Table 5.2). The vitamin B6 content of French fry tissue prepared from Beauregard roots stored for 6 months showed a significant vitamin B6 increase on a dry weight basis after frying at higher temperatures and longer times (Tables 5.7 and 5.8). Vitamin B6 content remained mostly stable in roots stored for six months of cultivar 07-146 during different frying conditions. No other reports on vitamin B6 increases during frying were found. Whether frying enhances the release of vitamin B6 bound

forms in roots stored for long periods in certain cultivars is unclear. Vitamin B6 is generally recognized as being more stable during cooking than vitamin C and thiamin. Vitamin B6 is generally stable at acid, neutral and alkaline pH, and in the presence of oxygen; it is, however, very sensitive to photo degradation (Harris, 1971). Limited studies have been conducted on the effect of cooking methods on vitamin B6 content of sweetpotato. One previous study found no differences in vitamin B6 content between raw and boiled sweetpotatoes (Kaunda, 2002). Vitamin B6 has been widely quantified in other vegetables, including potato. Boiled, baked, and microwaved potatoes contained similar vitamin B6 content (fwb) to raw tissue (Augustin et al., 1978; Augustin et al., 1979). The higher vitamin B6 content (fwb) observed in microwaved and baked sweetpotatoes relative to boiled in this study was attributed to moisture loss, as indicated by expression of the results on a dry weight basis. The retention of vitamin B6 content (dry weight basis) in boiled, baked, and microwaved sweetpotato tissue generally agreed with the 85-100% recoveries reported in potatoes (Augustin et al., 1978). Although no evaluation of the effect of increasing microwaving and boiling time on vitamin B6 content was attempted in this study, baking at higher temperatures or longer times did not result in differences in vitamin B6 content (Table 5.5 and 5.6). The results suggest that cooking method, including frying, baking, boiling and microwaving, have little or no detrimental effect on sweetpotato vitamin B6 content.

CONCLUSIONS

Among the water-soluble vitamins analyzed, AA was found to be less stable during cooking of sweetpotato tissue compared to thiamin, riboflavin and vitamin B6. All cooking methods resulted in a significant AA loss on a dry weight basis. In contrast, little or no loss of thiamin, riboflavin, and vitamin B6 was found in microwaved, baked, boiled, and fried sweetpotato tissue. Except for the detrimental effect on AA and thiamin from increased

microwaving time, all other cooking method treatments did not result in lower AA, thiamin, and riboflavin contents. Vitamin B6 content increased in French fry tissue from Beauregard roots stored for 6 months, or from higher frying temperatures and longer frying times. The overall results of this study indicate that baking, boiling, microwaving and frying cause a significant loss in AA content of sweetpotato, but little or no loss in thiamin, riboflavin, and vitamin B6.

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CHAPTER 6. EFFECT OF LOW TEMPERATURE STORAGE ON THE ASCORBIC ACID, THIAMIN, RIBOFLAVIN, AND VITAMIN B6 CONTENT OF SWEETPOTATO

INTRODUCTION

Sweetpotato, as many others fruits and vegetables of tropical origin, is susceptible to chilling injury. Chilling injury (CI) is a physiological disorder that occurs when certain susceptible fruits and vegetables are exposed to low temperatures, but above the tissue freezing point. It induces physiological changes that generally lead to rapid product decay and an abbreviated storage life. In sweetpotato, CI occurs when it is stored below 12.5 °C, although it varies between cultivars and curing treatment (Picha, 1987). Typical symptoms include internal discoloration from brown to black, internal tissue breakdown, off-flavor development, increased sucrose levels, hard core internal texture when cooked, and increased susceptibility to pathogens (Paull, 1990). The development of CI is a cumulative event of low temperature and exposure time. Sweetpotatoes exposed for short periods to low temperatures may recover and preserve their regular appearance and edible quality (Saltveit, 2003). Storage temperature abuses are common during postharvest handling of fruits and vegetables and may have an effect on nutrient composition, including water-soluble vitamin contents. Ascorbic acid (AA) content is reduced in tissues exhibiting CI symptoms after 7 weeks of exposure at 7.5 °C (Lieberman et al., 1958). However, little is known about the effect of low temperatures and additional storage durations on the AA and B vitamin contents in sweetpotato.

The objective of this study was to determine the effect of short-term low temperature storage on AA, thiamin, and riboflavin contents in sweetpotato root tissue.

MATERIALS AND METHODS

Reagents

Thiamin hydrochloride, riboflavin, L-ascorbic acid, taka-diastase, meta-phosphoric acid (MPA), sodium acetate, potassium ferricyanide, potassium phosphate monobasic, sodium phosphate monobasic, sodium hexane sulfonate, and tris(2carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Sigma Aldrich (St Louis, MO). All reagents were analytical grade, unless otherwise stated. Hydrochloric acid and orthophosphoric acid were supplied by Fisher Scientific (Pittsburgh, PA). Acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All standards were prepared daily for the respective analyses.

Root origin and storage conditions

Beauregard sweetpotato roots were harvested on August 24, 2012 at the LSU AgCenter Sweetpotato Research Station in Chase, LA. The roots were cured at 30 °C and 85-90% RH for 7 days, and then placed under storage at 14 °C and 85% RH until the beginning of experiments. Roots were exposed for 2 and 4 weeks at 1° C, 6 °C, and 14 °C. Additional roots were held for one week at 14° C following exposure to the various low temperature and time treatments. Six individual root replications were used for each treatment. The experiment was repeated with roots from a different field, and same harvest date.

Vitamin extraction and analysis procedures

All extraction and analytical procedures for AA, thiamin, riboflavin and vitamin B6 were conducted based on the methodologies described in Chapters 2 and 3.

Statistical analyses

A completely randomized design with 6 replications per treatment was used. Each root was considered one replication. The data was analyzed with SAS PROC GLM procedure (SAS

Institute, Cary, NC). Treatment means were separated using Tukey's HSD test. The experiment was conducted twice. Due to data variability between the two experiments, the results were analyzed and presented separately. All results were expressed as mg/100 g of fresh weight .

RESULTS AND DISCUSSION

Ascorbic acid

No consistent effect of storage temperature on sweetpotato AA content was found. The AA content of roots stored at 1 °C, 6 °C or 14 °C was similar. In the same way, the length of exposure to low temperatures did not result in consistent changes in AA. Nonetheless, with only two exceptions across the two experiments, AA content was lower in roots stored at 1 °C and 6 °C, for 2 and 4 weeks, followed by transfer for 7 days to regular storage temperature (14 °C) (Tables 6.1 and 6.2). Although there were other differences in treatments, these were not consistent across both experiments. Loss of AA was previously reported as a symptom of chilling injury in pineapples (Miller and Heilman, 1952), bananas (Lyons, 1973), sweetpotatoes (Lieberman et al., 1958), and other fruits and vegetables (Lee and Kader, 2000). Sweetpotato roots stored for 10-13 weeks at 7.5 °C showed a decrease in AA content (Lieberman et al., 1958). Additionally, AA content generally decreased in fresh-cut Beauregard sweetpotatoes stored for 14 days at 2 °C and 8 °C (Erturk, 2000). Despite using lower temperatures in this study, exposure for 2 or 4 weeks did not result in a consistent decrease in AA content. These results are most likely explained by the shorter storage and the use of whole roots. Cellular disruption caused by peeling and slicing of the root causes exposure of tissues to air and increases enzyme activity by allowing substrates and enzymes to come in contact (Klein, 1987). Additionally, the "breaking point", a time in which the cell mitochondria underwent severe

damage, phenolics content started to increase, and AA declined was theorized to be between 5 and 6 weeks of storage at 7.5 °C in intact sweetpotato roots (Lieberman et al., 1958).

Low temperature storage abuses are common during postharvest handling of sweetpotatoes. The duration of these events may range from hours to several weeks during on-farm storage, long distance transport, in wholesale and retail facilities, in supermarkets display racks, or/and in consumer refrigerators. These low temperature storage periods may be followed by storage periods at higher temperatures. The decrease of AA in roots transferred to non CI temperature after storage at 1 °C and 6 °C for 2 and 4 weeks might be more likely explained by increased respiration rate and enzymatic oxidative activities due to higher temperatures. Although respiration rate was not measured in this study, a higher respiration rate was reported in sweetpotatoes exposed for longer storage periods at CI temperatures, followed by transfer to non-chilling temperatures (Picha, 1987). The production of radical oxygen species (ROS) is enhanced by a number of environmental stresses, including drought, wounding, high salt, high light, and exposure to pollutants. It is known that AA interacts enzymatically and non-enzymatically with the damaging oxygen radicals and their derivatives during oxidative stress in plants (Davey et al., 2000). These factors may explain why chilling injury symptoms in susceptible fruits and vegetables are usually expressed upon transfer to non-chilling temperatures (Saltveit, 2003). The relatively higher AA content observed in Experiment 2 relative to Experiment 1 was likely explained by root originating from different fields in the same farm. The AA content of fruits and vegetable has been influenced by multiple preharvest and postharvest factors, including cultural practices climatic, and storage conditions (Lee and Kader, 2000).

Table 6.1 Water-soluble vitamin content in sweetpotato roots stored at low temperatures for different time intervals, including transfer to storage at 14 °C (Experiment 1).

Temperature °C	Storage time	Ascorbic acid (mg/100g)	Thiamin (mg/100g)	Riboflavin (mg/100g)
1 °C	2 weeks	11.73 cd	0.043 a	0.022 ab
	2 weeks + 7 days at 14 °C	11.13 cd	0.038 a	0.020 a-d
	4 weeks	13.42 bc	0.040 a	0.025 a
	4 weeks + 7 days at 14 °C	9.44 d	0.045 a	0.021 a-d
6 °C	2 weeks	17.55 a	0.044 a	0.016 cd
	2 weeks + 7 days at 14 °C	13.43 bc	0.048 a	0.023 ab
	4 weeks	15.63 ab	0.040 a	0.020 a-d
	4 weeks + 7 days at 14 °C	11.71 cd	0.041 a	0.021 abc
14 °C	2 weeks	12.81 bc	0.047 a	0.015 d
	4 weeks	11.73 c	0.041 a	0.019 bcd

Values for each treatment represent the mean of 6 single root replications. Mean values within the same column followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.

Table 6.2 Water soluble vitamin content in sweetpotato roots stored at low temperatures for different time intervals, including transfers to storage at 14 °C (Experiment 2).

Temperature °C	Storage time	Ascorbic acid (mg/100g)	Thiamin (mg/100g)	Riboflavin (mg/100g)
1 °C	2 weeks	15.98 bc	0.041 ab	0.019 c
	2 weeks + 7 days at 14° C	12.18 de	0.037 b	0.024 ab
	4 weeks	12.80 cd	0.049 a	0.020 bc
	4 weeks + 7 days at 14° C	9.85 e	0.044 ab	0.018 c
6 °C	2 weeks	20.79 a	0.035 b	0.021 bc
	2 weeks + 7 days at 14° C	18.68 ab	0.038 ab	0.025 a
	4 weeks	16.37 b	0.042 ab	0.024 ab
	4 weeks + 7 days at 14° C	12.81 cd	0.042 ab	0.019 c
14 °C	2 weeks	18.22 ab	0.039 b	0.019 c
	4 weeks	16.21 b	0.039 b	0.021 bc

Values for each treatment represent the mean of 6 single root replications. Mean values within the same column followed by different letters were significantly different ($P<0.05$) based on Tukey's HSD test.

Thiamin and riboflavin

No consistent differences in thiamin and riboflavin content were found among the different low temperature storage treatments across the two experiments (Tables 6.1 and 6.2). Thiamin and riboflavin are generally regarded as relatively more stable than vitamin C during regular storage of fruits and vegetables (Zhuang and Barth, 2003). Despite thiamin's involvement in a wide variety of metabolic processes as the coenzyme thiamin pyrophosphate (TPP) in plants, there is not clear evidence that TPP is affected by postharvest stresses (Zhuang and Barth, 2003). Riboflavin nonetheless is involved in the biosynthesis of flavonoids (Zhuang and Barth, 2003). Biosynthesis and accumulation of anthocyanins is a common response in plants to cold acclimation, but this process is light dependent (Chalker-Scott, 1999). The relative stability of thiamin and riboflavin in this study might have been attributed to the low metabolic activity due to low temperatures and by the relative short periods of transfers to non-chilling conditions.

CONCLUSIONS

Low temperature storage of sweetpotatoes at 1 °C and 6 °C and did not cause a detrimental effect on AA. Similarly the effect of exposure time for 2 or 4 weeks was not consistent on sweetpotato AA content. However, AA generally decreased in sweetpotatoes stored at 1° C and 6 °C for 2 or 4 weeks followed by transfer to 14 °C storage for 7 days. Storage at low temperatures resulted in little or no significant changes in the thiamin and riboflavin contents of sweetpotato.

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CHAPTER 7. SUMMARY

Water-soluble vitamins, including ascorbic acid (AA), thiamin, riboflavin and vitamin B6 play essential roles in multiple metabolic reactions that sustain human health. Sweetpotato is considered a good source of ascorbic acid and a reasonable source of certain B vitamins, including thiamin, riboflavin, and vitamin B6 in the diet. The objectives of this study were to determine the effect of storage, cooking methods and tissue type on water-soluble vitamin content of sweetpotatoes. An additional goal was to improve the analytical procedure for the extraction and quantification of these vitamins.

A new HPLC methodology was developed for simultaneous analysis of thiamin and riboflavin. Compared to previous methods, the developed method included new chromatographic conditions for the separation of thiamin and riboflavin. The methodology was validated with adequate recovery values of 97-100% for thiamin and 97-101% for riboflavin, limits of detection of 1.6 ng/ml for thiamin, and 0.31 ng/ml for riboflavin, and precision (relative standard deviation < 5%). The new method showed adequate resolution and selectivity of thiamin and riboflavin in multiple fruits and vegetables and may be used in a wider variety of food matrixes as an alternative methodology for the analysis of these vitamins.

Various factors can affect the content of water-soluble vitamins in sweetpotato tissue. Limited information on the effect of storage and curing is available, and no studies have been conducted on water-soluble vitamin composition of current US commercial cultivars. The results of this study indicated that AA decreased in cultivars 07-146, Beauregard, and Covington over a 6-month storage period, while Orleans contained a mostly similar AA content to at harvest after the same period. No cultivar differences in AA content were found at harvest and after 6 months of storage. Curing did not result in a significant change in AA, thiamin, and vitamin B6, but

resulted in a decrease in riboflavin content in all cultivars. Thiamin and riboflavin were mostly stable after curing, while AA and vitamin B6 content were cultivar and storage time dependent. Compared to at harvest, stored roots for 3 months resulted in comparable AA content in cultivars 07-146, Orleans, and Beauregard, and a decrease in AA in Covington. In the same period, vitamin B6 content increased in cultivar 07-146, but remained stable in all other cultivars. Storage for 6 months resulted in a decrease in AA in all cultivars, except Orleans. During the same period, vitamin B6 increased in all cultivars, except Beauregard. Although cultivar 07-146 contained higher vitamin B6 content, no cultivar was superior or inferior for all the vitamins throughout 6 months of storage. The overall results of this study suggest that AA in sweetpotato roots decreases, while, thiamin, riboflavin and B6 undergo little or no decline during storage for 6 months after curing. Further research is needed to understand the physiological mechanism that resulted in a decline of riboflavin content during the curing period.

Differences in AA, riboflavin, and vitamin B6 contents were observed in Beauregard sweetpotato foliar tissues. This study identified young leaves as the richest foliar tissue source of AA, and old leaves as the highest sources of riboflavin and vitamin B6 content. Root tissues showed a variable distribution of water-soluble vitamins. In general, skin tissue contained a lower level of AA, thiamin, and vitamin B6, and higher riboflavin content. No thiamin was detected in foliar tissues. This study presented new information and expanded the current knowledge of AA, thiamin, and riboflavin distribution in different sweetpotato tissues. Additionally, it confirmed that foliar tissues can be a good source of multiple B-vitamins in human diets. Further research is needed to explain the wide variability in thiamin content that has been reported in foliar tissues in previous studies.

All preparation methods including baking, boiling, frying, and microwaving resulted in a decrease in AA content on a dry weight basis. However, the changes in thiamin, riboflavin, and vitamin B6 were minimal due to cooking. The AA content was found to be less stable during cooking of sweetpotato tissue compared to thiamin, riboflavin, and vitamin B6. Expressed on a fresh weight basis, fried roots had a similar AA, thiamin and riboflavin, and higher vitamin B6 content compared with raw tissue. Pyridoxin (PN) was generally the most abundant B6 vitamer, in raw and prepared tissue, while pyridoxal (PL), and pyridoxamine (PM) were found in similar proportions to each other. All preparation methods generally resulted in a decrease in PL and an increase in PM. In general, no preparation method was superior or inferior in retention of all vitamins analyzed. The overall results of this study indicated that baking, boiling, microwaving and frying cause a significant loss in AA content of sweetpotato, but cause little or no loss in thiamin, riboflavin, and vitamin B6.

Exposure of sweetpotatoes to chilling injury-inducing temperatures of 1 °C and 6 °C for 2 or 4 weeks did not result in consistent changes in AA. However, transfer of the low temperature-stored roots to 14 °C for an additional 7 days generally resulted in AA decreases. Little changes were observed in thiamin and riboflavin root contents due to low temperature storage, or transfer of low temperature stored roots to 14 °C for an additional 7 days.

THE VITA

Wilmer Barrera was born in Rosario de Mora, El Salvador, in December, 1983. He was raised and attended primary school at this municipality. He then completed high school studies at Instituto Nacional Profesora Berta Filedia Cañas in Planes de Renderos, San Salvador. In 2001, he was given a scholarship by EARTH University in Limón, Costa Rica, to complete a bachelor's degree in agricultural sciences. After his graduation at this university he was an intern for Sakata Seed America Inc., in Fort Myers, Florida. In 2007, he moved to Cocle, Panama, to perform duties as field supervisor for Ramafrut Internacional S.L. In the fall 2008, he was admitted to Louisiana State University to earn a master's degree in the Department of Plant Pathology and Crop Physiology. In the spring 2011 he began his studies to get a doctorate degree at the School of Plant, Environmental and Soil Sciences at Louisiana State University.