Determination of Sensory Characteristics and Antioxidant Capacity of Pawpaw Pulp

During Frozen Storage

A thesis presented to

the faculty of

the College of Health Sciences and Professions of Ohio University

In partial fulfillment

of the requirements for the degree

Master of Science

Dane E. Salabak

December 2012

© 2012 Dane E. Salabak. All Rights Reserved.
This thesis titled

Determination of Sensory Characteristics and Antioxidant Capacity of Pawpaw Pulp
During Frozen Storage

by

DANE E. SALABAK

has been approved for
the School of Applied Health Sciences and Wellness
and the College of Health Sciences and Professions by

Robert G. Brannan
Associate Professor of Applied Health Sciences and Wellness

Randy Leite
Dean, College of Health Sciences and Professions
Abstract

SALABAK, DANE, E., M.S., December 2012, Food and Nutrition

Determination of Sensory Characteristics and Antioxidant Capacity of Pawpaw Pulp During Frozen Storage

Director of Thesis: Robert G. Brannan

Pawpaws (*Asimina triloba*) are the largest wild fruit native to North America. A tropical fruit grown in a temperate climate, pawpaws are typically found on the east coast namely within the Appalachian region. The fresh fruits themselves have never been successfully commercialized because of their rapid post-harvest ripening and perishability. This study was multifaceted, focusing both on the antioxidant capacity of frozen pawpaw pulp and the characterization of sensory attributes using a trained descriptive sensory analysis panel and an untrained consumer panel. Previous research has shown that there are extractable antioxidants within pawpaw pulp. Findings in this study show that total phenolic content was significantly higher in pawpaw pulp extracted with methanol, compared to chloroform, and remained constant during storage. Total Ferric Reducing Antioxidant Power (FRAP) was four-fold greater in pawpaw pulp extracted in methanol, compared to chloroform, for all months.

Consumer and descriptive sensory analyses were performed on pawpaw pulp. Consumer sensory analyses showed that mango was preferred compared to the pawpaw, but that only one-third of those who preferred the mango were able to identify it correctly. Consumer analyses generated 41 flavor descriptors for pawpaw pulp which was used in the development of the descriptive sensory lexicon. Descriptive sensory analysis
was performed on pawpaw pulp that was stored frozen in the presence or absence of air with and without heat treatment. The sensory panel detected differences in color; however, no differences in any of the sensory attributes were detected during 12 months of frozen storage, suggesting that the flavor of pawpaw pulp is stable during frozen storage. The comprehensive analysis of the sensory characteristics and quality of pawpaw pulp, including the development of a standardized sensory lexicon, was an important step in the field of pawpaw research. Findings that show measures of antioxidant capacity as stable during frozen storage could allow food manufacturers to use the fruit pulp as a flavor additive or as a source of natural antioxidants. Future studies should focus on finding a pawpaw that is preferred by consumers as well as producing pawpaws that are less subjective to the post-harvest challenges. These over time would help commercialize the pawpaw and bring a large benefit to Appalachia.
Acknowledgments

I would like to acknowledge my friends, classmates, and professors at Ohio University who have supported me directly and indirectly throughout my master’s studies in food and nutrition sciences, my brief hiatus teaching, and in the final push to complete this thesis.

My mom, dad, sisters, and girlfriend who are always there for me.

Finally, my committee. Dr. Brannan, Dr. Holben, and Dr. Faik for helping me all the way to the finish.

Thank you all for making me the person that I am today.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>5</td>
</tr>
<tr>
<td>List of Tables</td>
<td>9</td>
</tr>
<tr>
<td>List of Figures</td>
<td>11</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>12</td>
</tr>
<tr>
<td>Overview and Background</td>
<td>12</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>14</td>
</tr>
<tr>
<td>Research Questions</td>
<td>15</td>
</tr>
<tr>
<td>Significance of the Research Study</td>
<td>15</td>
</tr>
<tr>
<td>Limitations</td>
<td>16</td>
</tr>
<tr>
<td>Glossary</td>
<td>18</td>
</tr>
<tr>
<td>Chapter 2: Review of Critical Literature</td>
<td>20</td>
</tr>
<tr>
<td>Background and Growing the Pawpaw Tree</td>
<td>20</td>
</tr>
<tr>
<td>Cultivation and Germplasm</td>
<td>23</td>
</tr>
<tr>
<td>Harvesting</td>
<td>25</td>
</tr>
<tr>
<td>Post Harvest Changes and Physiology, and Storage of the Pawpaw</td>
<td>26</td>
</tr>
<tr>
<td>Uses of the Pawpaw</td>
<td>27</td>
</tr>
<tr>
<td>Pawpaw as an anticancer agent</td>
<td>27</td>
</tr>
<tr>
<td>Pulp as a food ingredient</td>
<td>28</td>
</tr>
<tr>
<td>Characteristics of the Pawpaw Fruit</td>
<td>29</td>
</tr>
</tbody>
</table>
Chapter 1: Sensory and Nutritional Quality

Sensory analysis.......................................................................................................................... 29
Nutritional quality–proximate analysis......................................................................................... 31

Chapter 2: Plant Based Antioxidants to Control Lipid Oxidation

Lipid oxidation................................................................................................................................. 35
Antioxidants and the control of lipid oxidation............................................................................. 36
Characterization of antioxidants.................................................................................................. 41
Antioxidant capacity of the pawpaw............................................................................................ 42
Effect of processing and storage on antioxidants ........................................................................... 43
Conclusion.................................................................................................................................... 45

Chapter 3: Methodology

Experimental Design..................................................................................................................... 46
Sample Preparation....................................................................................................................... 46
Pawpaw Extraction......................................................................................................................... 47
Antioxidant Capacity Assays......................................................................................................... 47
Total phenolics.............................................................................................................................. 47
Total flavonoids............................................................................................................................. 48
Ferric reducing antioxidant power (FRAP) .................................................................................. 48
Quality Indicators......................................................................................................................... 49
Sensory Analysis............................................................................................................................. 49
Statistical Analysis........................................................................................................................ 51

Chapter 4: Results

Antioxidant Capacity..................................................................................................................... 53
List of Tables

Table 1: Research Questions ...........................................................................................15

Table 2: Comparison of Nutritional Composition of Pawpaw and Other Commonly
Referenced Fruits ............................................................................................................33

Table 3: Participant Demographics, Mean Rankings for Three Tropical Fruit Purees From
a Three-Way Forced Choice Consumer Ranking Test, and Percentage of Participants who
Correctly Identified Pawpaw ..........................................................................................58

Table 4: Consumers (n = 98) Free Choice Identification of Tropical Fruit Flavors When
Presented Pawpaw Pulp in the Order of Their Perceived Intensity ................................60

Table 5: Frequency of Fruit Puree Selected as the Favorite (i.e., Ranked First) Compared
to the Other Two Fruits in a Three-Way Forced Choice Consumer Ranking Test .......61

Table 6: Description and Anchored References of Sensory Attributes Generated by
Descriptive Analysis of Pawpaw Pulp ............................................................................62

Table 7: P-values for the Main Effects of Month of Storage (0, 2, 4, 6, 8, 10, 12),
Packaging Condition (Vacuum, Air), and Heat Treatment (Raw, Cooked), Two-Way
Interactions, and Three-Way Interactions on Sensory and Quality Attributes of Pawpaw
Pulp .....................................................................................................................................65

Table 8: Mean Values ± Standard Deviations of Sensory Body (n=4), Sensory Color (n =
4), and CIE L*, a*, and b* Values (n = 6) for Pawpaw Pulp Stored Frozen Raw or Heat
Treated (Cook), in the Absence (Vac) or Presence (Air) of Air in Package .................68
Table 9: Mean Values ± Standard Deviations of Descriptive Sensory Flavor Attributes (n = 4) for Pawpaw Pulp Stored Frozen Raw or Heat Treated (Cook), and in the Absence (Vac) or Presence (Air) of Air in the Package.................................................................69
List of Figures

Figure 1: The pawpaw tree .............................................................................................21
Figure 2: The pawpaw blossom ......................................................................................22
Figure 3: The pawpaw and its seeds ...............................................................................23
Figure 4: Chemical structures for the three main annonaceous acetogenin compounds, asimicin, bullatacin, and bullatalicin, identified in ripe pawpaw pulp .........................28
Figure 5: Phenols ............................................................................................................37
Figure 6: Structures and examples of primary classes of flavonoids .........................39
Figure 7: Experimental design .......................................................................................46
Figure 8: Total phenolic content in pawpaw pulp extracts from different solvents after frozen storage ..................................................................................................................54
Figure 9: Total flavanoid content of pawpaw pulp extracts from different solvents after frozen storage .................................................................................................................55
Figure 10: Ferric reducing antioxidant power (FRAP) of pawpaw pulp extracts from different solvents after frozen storage ........................................................................56
Chapter 1: Introduction

Overview and Background

Pawpaws are the largest wild fruit native to North America (McGrath & Karahadian, 1994b). The pawpaw, *Asimina triloba*, is one of eight different species from the genus *Asimina*, which is the only temperate representative of the tropical *Annonaceae*, or custard apple family (Pomper, Crabtree, et al., 2003). Other members of this family include the sweetsop (*Annona squamosa*), soursop (*Annona muricata*), and the custard apple (*Annona atemoya*) (McGrath & Karahadian, 1994b).

In 1916, the American Genetics Association held a contest to find a superior pawpaw. This contest led many to believe that with some careful planning and intelligent breeding the fruit would be able to reach mainstream commercialization (Peterson, 2003). After nearly 100 years, the pawpaw has yet to reach the prominence of other cultivated crops native to North America like blueberry and pecan. Pawpaws as a niche crop are not widely known because of their lack of mass-distribution (Peterson, 2003), and many of the same setbacks that growers faced in the early 1900’s still present problems for growers today. These problems include, but are not limited to, becoming overripe in two to three days (Archbold, Koslanund, & Pomper, 2003), having many imbedded seeds, and having a lack of consistency of flavor between cultivars (Pomper et al., 2008). Additionally, there is no consistent lexicon of sensory descriptors used to describe this fruit. It is necessary for both producers and consumers to have a definitive sensory lexicon to describe this unique fruit.
Because of their rapid climacteric peak and short ripening period, one way pawpaws could reach a broader market would be to exploit the antioxidative compounds in the fruit and utilize them as an additive in the food industry. The word “antioxidant” has become extremely marketable in recent history, because antioxidants play important roles in both human health and in food production. Research has shown that antioxidants protect cells from free radical damage (Kanner & Rosenthal, 1992), and may prevent heart disease and certain forms of cancer in humans (Baron, Berner, Skibsted, & Refsgaard, 2005). Antioxidants are reducing substances that inhibit free radical initiation or stop the chain reaction of free radical formation (Johnson, 1979). Almost all living things contain antioxidants. They are also used in the food industry to prevent lipids from oxidation via free radicals (Murano, 2003).

There are three different ways of measuring the antioxidant capacity. Prior to testing, an extract is prepared by combining a fruit or seed with an organic solvent processing the mixture, filtering off any solids, and then using the remaining liquid to perform necessary tests. First, one can measure the quantity of antioxidative compounds in the extract. Next, one can measure the antioxidant capacity in a lab assay or model system. However, these first two measures of antioxidant capacity only give the researcher one small piece of the metabolic puzzle. The final method, which is also the best and truest measurement of antioxidant effectiveness, is performed in the actual system of concern, i.e., cells, food, or the body (Moure et al., 2001).

It has been shown in model systems that pawpaws are antioxidative. The seeds contain phenolic compounds and have been shown to reduce oxidation in omega-3 long-
chain polyunsaturated fatty acid model systems (Brannan & Salabak, 2009). The pulp has been shown to contain phenolics (Kobayashi, Wang, & Pomper, 2008) and flavonoids (Harris & Brannan, 2009). The pulp also inhibits oxidation in chicken muscle homogenate (Harris, 2008). Significant changes were observed in the antioxidative compounds after 300 days of frozen storage including a four-fold increase of phenolics and a six-fold increase of flavonoids, however, the kinetics of these increases were not studied (Harris & Brannan, 2009).

Statement of the Problem

A number of reasons including, but not limited to, quickly ripening over a short period of time, high susceptibility to bruising, and a single harvest in early fall have prevented the pawpaw from being commercialized. These reasons force the pawpaw to be seen seasonally at farmer’s markets or for sale frozen, because it is commonplace for the pawpaw fruit pulp to be harvested, processed, and stored. Because of this, an assessment of compositional changes that occur during frozen storage would be beneficial. Previous research has shown that changes in the antioxidant capacity occur during frozen storage (Harris & Brannan, 2009); however, quality indicators including color and sensory evaluation were not investigated. Additionally, the complex flavor profile of the pawpaw fruit has never been assessed with a trained sensory panel. The assessment of fresh and frozen pawpaw fruit will allow for an elaborate sensory lexicon to be established for this unique fruit. The proposed work will characterize the changes in antioxidant capacity of the pawpaw fruit pulp during frozen storage, and develop a
sensory lexicon to be used on the pawpaw fruit pulp, as well as an evaluation of the sensory quality and quality in frozen storage.

**Research Questions**

Table 1 shows the attempted research questions along with approaches and hypotheses.

Table 1

<table>
<thead>
<tr>
<th>#</th>
<th>Question</th>
<th>Approach</th>
<th>Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>What are the dynamics of antioxidant capacity (with regards to change and polarity) during frozen storage?</td>
<td>Evaluate antioxidant capacity at two month intervals for six months while under frozen storage.</td>
<td>Antioxidant capacity will decrease over time during frozen storage, and be affected by different solvents.</td>
</tr>
<tr>
<td>2</td>
<td>How does the average consumer describe the sensory attributes of the pawpaw fruit pulp?</td>
<td>Consumer sensory analysis with untrained panelists</td>
<td>The average consumer will likely have difficulty describing the complex flavor of the fruit pulp.</td>
</tr>
<tr>
<td>3</td>
<td>What changes occur in the sensory quality of pawpaw pulp during frozen storage and can they be controlled by cooking or packaging?</td>
<td>Sensory analysis with a trained panel (Train sensory panel for tropical fruit tasting, develop sensory lexicon, evaluate samples)</td>
<td>Sensory quality will decline during frozen storage with air sealed pawpaw pulp declining faster than vacuum sealed pawpaw pulp.</td>
</tr>
</tbody>
</table>

**Significance of the Study**

The pawpaw is a very unique fruit, and the literature shows a multitude of descriptors that have been used to describe the pawpaw including specific ones relevant
to its skin, seeds, and pulp. There is often disagreement among previous research about the pawpaw because no definitive sensory lexicon exists. It already is plausible to use the pureed pawpaw pulp as an additive for flavor enhancement or enhanced nutritional quality in a commercial food item; however, an assessment of processing and storage and their effects on quality indicators and antioxidant capacity can lead producers in the best direction of storing the pulp and consumers the best time frame of using frozen pawpaw pulp. This research focused on the possibility of using a component of the pawpaw because the pawpaw’s limitations make it unlikely that it would ever be a “lunch box” fruit. By removing the pulp and using it as a fat replacer, a flavor enhancer, or source of antioxidative compounds could give the pawpaw a much wider market, as opposed to the current small market appeal of farmer’s markets and high end restaurants. This could be a great benefit to many pawpaw producers on the eastern United States including those throughout the Appalachian region where pawpaws thrive.

Limitations

As for apples, the pawpaw has a number of different varieties which have similarities and differences in their flavor profiles. While this could be seen as a benefit because pawpaws could be selected to meet individual taste preferences, this is also a limitation of this study. Research has shown that variations in flavor can exist among cultivars, and even among individual fruit on a given tree. A limitation of this study simply lies in the fact that some fruit display more complex flavor profiles (Pomper, Layne, & Peterson, 1999), which makes standardization of flavors difficult. When looking at characterizing the flavor profile of the pawpaw with a trained sensory panel,
even slight variations in the pawpaw pulp can cause the data to be skewed. To best control this, all pawpaws used in the study were collected from a single tree in Athens, Ohio.

Descriptive sensory analysis also can be very complex, and this is further compounded by the complexity of the fruit and standards to which the flavor attributes are compared. Some of the standards are straightforward (e.g., a black teabag steeped for one hour); however, some variation will always exist (e.g., fresh papaya) with the standards that are used. Individual variances in taste perception due to personal illnesses or perceptions of flavors can impact data.

Antioxidant capacity assays also vary with the information they provide. Unless the antioxidant effectiveness is measured in cells, food, or the body, antioxidant capacity assays only give researchers part of a big picture. While the Folin-Ciocalteu assay is a simple assay to use because it can quickly identify phenolic antioxidants in a solution, it has limitations because it can overestimate the total number of phenolic compounds due to the fact that it can react with any reducing substances (i.e., sugars) amino acids, vitamins, inorganic ions (Swain & Hillis, 1959). Many assays, including the Ferric reducing antioxidant power (FRAP) assay, rely on trace amounts of solutions being mixed together as well as being time sensitive. Without proper controls, minute differences can have a significant impact on results. Additionally, antioxidative compounds themselves may be affected by frozen storage, therefore, it should be noted that comparisons in this study using “zero” months (no frozen storage, or fresh fruit pulp) to treatments receiving frozen storage could be subjected to changes due to the freezing
and thawing of the fruit pulp. Rather, zero should still be subjected to a single freeze thaw cycle after a few hours to a single day during frozen storage. A final limitation of many antioxidant capacity assays is that antioxidant effectiveness is measured in an aqueous environment, not the lipophilic environment of when lipid oxidation occurs.

**Glossary**

*Autogamy*. Self-fertilization that occurs in hermaphroditic organisms where the two gametes fused in fertilization come from the same individual.

*Climacteric*. A stage of fruit ripening associated with ethylene production and cell respiration rise. Apples, bananas, tomatoes, and others are climacteric fruits.

*Emetic*. Something that causes emesis, (vomiting).

*Membranaceous*. Thin and rather soft or pliable, as the leaves of the rose, peach tree, and aspen poplar.

*Mesic hardwood forests*. Areas where hardwoods grow in cool, moist soils that fall between wetlands and drylands.

*Phenology*. The study of the relationships between the climate of any place and the annual periods of plants and animals.

*Protogynous*. Of or relating to a flower in which the stigma is receptive before the pollen is shed from the anthers of the same flower.

*Sensory lexicon*. A vocabulary, including words and expressions, used to describe a given item during descriptive sensory analysis.

*Soluble solids*. Material that is capable of being dissolved or mixed which is measured in BRIX, or the percent sucrose in a solution.
*Sphagnum*. Any of an order of atypical mosses that grow only in wet acid areas where their remains become compacted with other plant debris to form peat

*Subcoriaceous*. Somewhat or approaching leathery in texture

*Top note*. The initial flavor sensation of a food.
Chapter 2: Review of Critical Literature

Background on Growing the Pawpaw Tree

Pawpaws grow on large trees in the eastern United States in a range that covers parts of northern Florida to southern Ontario and as far west as Nebraska, (Pomper et al., 1999), comprising all of Appalachia. The suitable growing climate for pawpaw trees is the USDA growing zone 5 in *mesic hardwood forests* (Pomper et al., 1999).

The trees, which often grow anywhere from 5 to 10 meters in height, have a straight trunk and long, drooping leaves (Pomper et al., 1999) as seen in Figure 1. The large, tropical-like leaves increase the pawpaw tree’s ornamental appearance, which in turn increases its use for landscaping purposes (Pomper, Crabtree, et al., 2003). The leaves of the *Asimina triloba* are usually *membranaceous*, which differ from the leaves of many other species of *Asimina* which are *subcoriaceous* (Kral, 1960). This difference is likely to be related to ecological factors of the mesic woodlands where *Asimina triloba* tend to be found compared to physiologically drier habitats of other species.
The trees have dark maroon-colored blossoms that appear during the middle of the spring. Studies have observed variances in time of appearance (between April and May), but it is unknown whether the changes in appearance of blossoms are accompanied by changes in intrafloral phenology (Willson & Schemske, 1980). The flower is strongly protogynous (Kral, 1960) and therefore it is believed that autogamy is unlikely (Willson & Schemske, 1980). The pawpaw blossoms can be seen in Figure 2. The pollen grains are too large for the wind to be an agent for pollination (Zimmerman, 1941), therefore researchers have speculated the blossoms are fertilized by either flies or beetles, however, exact agents are uncertain (Kral, 1960). Hand pollination can be also used to increase likelihood of fruit production (Willson & Schemske, 1980).
Figure 2. The pawpaw blossom  

The life of the flower itself has been observed to range from 5 to more than 19 days, with the average between 15 and 16 days. Flower petals are initially green then turn from pink to maroon (Willson & Schemske, 1980). Once the blossoms are pollinated they then produce the carpels on which the fruit eventually grows.

The structure of all *Asimina* species is a multi-seeded, uneven-sided (tetrate), and pulpy berry (Kral, 1960). Fruit can grow singularly or in clusters like the hands of a banana (Pomper et al., 1999) and has been documented numerous times to have been eaten as a staple food of Indian and early settlers of eastern North America (Kral, 1960). Within the fruit are often two rows of seeds. These dark brown (chestnut) seeds vary in both size and quantity per berry, are probably best described as "bean-shaped," (Kral, 1960), and are often the size of a kidney bean, lima bean, or an almond. The seed has a tough coat and is coated by the endosperm, a close series of white plates perpendicular to the long axis of the seed (Kral, 1960). The pawpaw fruit and its seeds can be seen in Figure 3. Additionally, alkaloids can be found within the endosperm of the seeds which
are emetic. This can affect mammalian digestion if seeds are chewed, however if swallowed whole seeds can pass through the digestive tract intact. This is important for wild pawpaws where the primary fruit consumers and seed dispersers are raccoons, foxes, and opossums (Peterson, 1991).

**Figure 3.** The pawpaw fruit and its seeds.

*Note.* Picture one. Pawpaw from http://www.ars.usda.gov/is/graphics/photos/<br> Image Number K7575-8<br>, Photo by Scott Bauer. {{PD-USGov-USDA-ARS}}.


**Cultivation and Germplasm**

Under cultivation, seeds can be cleaned, sterilized, and stored in a self-closing polyethylene bag with moist sphagnum for several years under refrigeration. Because of this stability, seeds can be helpful in the germination and cultivation of pawpaw trees (Layne, 1996). The earliest record of pawpaw development dates back to 1905 (Peterson, 2003). Neal Peterson noted the importance of collecting, cultivating, and breeding the pawpaw in the late 1970’s because, while pawpaw flourished in the wild, the lore and knowledge of the fruit faded as generations that had worked with the pawpaw in the first half of the 20th century were passing away. Neal Peterson and Dr. Harry Swartz played a
pivotal role in the creation of a pawpaw germplasm by tracking down a number of different historical pawpaw collections. Pawpaws were recovered from the Buckman collection of Farmingdale, Illinois, which were believed to be the earliest surviving collection of pawpaws (Peterson, 1986). Collections also came from Dr. G. A. Zimmerman, the Blandy Experimental farm near Winchester, Virginia, the Hershey collection from Dowington, Pennsylvania, and from Dr. H. A. Allard of Arlington, Virginia. Dr. Allard’s pawpaws were of particular interest as they had a unique gene line separate from the Buckman-Zimmerman Line (Peterson, 1986).

Collected trees were cultivated at the University of Maryland along with a number of other *Asimina* species in an attempt to promote hybridization of species. Hybridization of species is important for the commercialization of the pawpaws desirable characteristics from multiple species can be incorporated into domesticated pawpaws (Peterson, 1986). Ideal characteristics from *Asimina* include thick skin (for improved handling), small seeds, precocious flowering, shorter stature, adaptability to dry and exposed sites, and greater pollination success which intern leads to greater yields (Kral, 1960).

In the 1990’s, the inception of the Kentucky State University (KYSU) comprehensive pawpaw research program focused research on developing cultural recommendations, improving propagation, expanding culinary development, collecting germplasm specimens, and molecular characterization in existing cultivars (Pomper, Layne, Peterson, & Wolfe, 2003). In 1991, a germplasm was started with the seeds collected from a contest not unlike the one in 1916. Over 400 entries from 14 states were received and in 1994 KYSU was designated the official *Asimina* satellite repository of
the U.S. Department of Agriculture (USDA) National Clonal Germplasm Repository in Corvallis, Oregon and as of 2003 was documented to contain over 1700 accessions (over 40 varieties) from over 16 states (Peterson, 2003).

**Harvesting**

Traditionally, pawpaws are only found for sale at small farmers markets and plant stands. This is because there are no obvious indicators of ripening other than a decline in fruit firmness. Because this is the only obvious indicator, fruits are harvested by hand when they begin to soften to the touch. Once harvested, research has shown pawpaws average between 2 and 3 days of shelf life, however, if picked at the earliest stage of ripening they can reach 5 to 7 days (Archbold et al., 2003). Because of this lack of stable shelf life, pawpaws must be sold quickly to overcome the risk of over ripening. Some researchers believe alternative methods of identifying the ripe fruit must be developed to maximize the shelf life of the pawpaw.

When harvesting the pawpaw, various challenges arise. One of the biggest challenges is that the fruit on a given tree do not ripen at the same time. In fact, harvest for a single tree can take over 2 weeks. It is likely that this occurs because of the staggered bloom period and subsequent development of fruit clusters from individual flowers. Even though fruit clusters develop from the same flower, they may still ripen at different times. This uneven ripening can become laborious as repeated visits to and touching of individual fruits is often required. Some ethylene regulation can be used to manipulate and concentrate the harvest period; however, the aforementioned challenges make a once-over harvest of a pawpaw tree impossible (Archbold et al., 2003). Peterson
26

(2003) notes that maintaining quality during harvest is challenging due to the pawpaws high susceptibility to bruising, the difficulty in spotting pawpaws below dense foliage as they are easily missed due to their green color, and that pawpaw trees grow 5 to 7 meters high so a device to pick and collect pawpaws must be used. Ladders are unsuitable for this task because of the inherent weakness and flexibility of the tree itself (Peterson, 2003).

**Post-Harvest Changes and Physiology, and Storage of the Pawpaw**

Pawpaws are *climacteric* fruits, and this causes problems during post-harvest ripening and storage. Climacteric fruits are defined by three main characteristics. The fruit goes through an initial autocatalytic increase in ethylene gas ($\text{C}_2\text{H}_4$), followed by an increase in respiration in the fruit that is often referred to as the “respiratory climacteric.” Phenotypic and genetic changes in the fruit occur at the same time as the first two characteristics and indicate ripeness (Iannetta et al., 2006). Ethylene and respiratory climacteric peaks are clearly evident in the pawpaw within three days after harvest (Archbold & Pomper, 2003). The ripeness of the pawpaw when it is picked will have an impact on the final quality of the fruit (McGrath & Karahadian, 1994b), because it has been found that the pawpaw will go from ripe to overripe in a matter of days at room temperature and up to 3 weeks while under refrigeration (Templeton, Marlette, Pomper, & Jones, 2003). Because of the limitations in their perishability, pawpaws are not commonly used for processing or found in fresh markets.

Generally, ripening pawpaws show an increase in *soluble solids* concentration (up to 20%), softening of the flesh, increased production of volatiles, and some genotypes
demonstrate a decline in green color (McGrath & Karahadian, 1994b). The volatiles produced during ripening are mostly ethyl and methyl esters (Shiota, 1991). Most researchers believe that color change cannot be accepted as a reliable indicator of pawpaw fruit ripeness. Although, in some pawpaws, a decline in green color during pawpaw ripening was observed (McGrath & Karahadian, 1994b), this change is not consistent among genotypes (Archbold & Pomper, 2003). If a color change is seen, it often occurs later in ripening if at all, and is difficult to identify with the untrained eye (Archbold & Pomper, 2003).

**Uses of the Pawpaw**

**Pawpaw as an anticancer agent.** Research has found annonaceous acetogenins in the twigs, unripe fruit, seeds, roots, and bark tissues of the pawpaw tree and these components are reported to have antitumor, pesticidal, antimalarial, anthelmintic, piscicidal, antiviral, and antimicrobial effects (Pomper, Lowe, Crabtree, et al., 2009). Other compounds have been discovered in the pawpaw bark that cause the deterioration of cancer cells and tumors. Three specific acetogenin chemicals have been isolated: asimin, asiminacin and asiminecin, which are all structural isomers of the compound asimicin (see Figure 4).
Assessment of ripe pawpaw pulp extracts for acetogenin compounds and acetogenin activity was undertaken. Findings showed that extracts of ripe fruit and several additional pawpaw cultivars had bioactivity with three prominent acetogenins: asimicin, bullatacin, and bullatalicin (see Figure 4) found in pawpaw pulp extracts (Pomper, Lowe, Lu, et al., 2009).

**Pulp as a food ingredient.** Research shows that pawpaw fruit pulp has the potential to be added to various consumer goods. The intense, tropical-fruit-like flavor makes it a potential source of natural fruit flavor (McGrath & Karahadian, 1994a) in products like blended juice drinks, baby food, and ice cream (Layne, 1996). Pawpaw pulp may be a viable option for fat replacement in baked goods. Researchers have utilized the pawpaw as a partial fat-reducing agent in muffins (Duffrin, Holben, & Bremner, 2001) and shortened cake (Wiese & Duffrin, 2003). Because of the highly perishable nature of
the pawpaw fruit, incorporation of the pawpaw puree into food formulations may give the pawpaw greater potential to reach consumer markets.

**Characteristics of the Pawpaw Fruit**

**Sensory analysis.** Sensory testing of foods is grouped into two general categories. One type measures sensory responses to the product (discrimination, acceptance, and preference tests), the other deals with characterization of the product (Moskowitz, Beckley, & Resurreccion, 2006). Consumer testing is used to determine consumer acceptance of a given product using trained or untrained panelists. For example, acceptance tests would employ consumers of the particular product while discrimination testing includes both users and nonusers of the product (Stone & Sidel, 2004). The biggest benefit is that one can receive direct results about consumer preferences. However, these tests require a large number of participants, from 25 to more than 100 (Moskowitz et al., 2006; Stone & Sidel, 2004). Descriptive sensory analysis involves a longer, more rigorous training of a smaller panel (Stone & Sidel, 2004). The panel is trained to generate a sensory lexicon of important characteristics of the product, and are then required to evaluate these attributes with an analytical and objective approach (Moskowitz et al., 2006).

Descriptive analysis involves the discrimination as well as the qualitative and quantitative description of a given product (Meilgaard, Civille, & Carr, 1999). It can be conducted by a panel anywhere between 5 and 100 judges but generally falls between 5 and 10. Panelists are selected based on the ability to detect and describe the perceived sensory attributes including appearance, aroma, flavor, and texture of a product.
Additionally, panelists must be able to quantify differences in quantities of each attribute when comparing two or more products (Meilgaard et al., 1999). Once selected, the training of a panel often takes between 40 and 120 hours of training, depending on the complexity of the product. Initial sessions focus on the development of a sensory lexicon where the product is sampled and attributes, flavors, and aromas are identified. Next a ballot is developed often using a 15-centimeter scale based on intensity, where a zero is no detectible amount of an attribute, and a 15 is a very large amount (Civille, 1979). Standards are then anchored on the ballot based on intensity perceived by the panel or previously conducted research. Through practice and calibration of the panel, the goal is to have all judges sample and rank sensory attributes of a given product with the same precision and accuracy.

Many studies mention that sensory characteristics, health considerations, and pleasure-seeking factors are the main reasons humans consume fruit and continue to consume fruit (Tuorila & Cardello, 2002). Past studies have demonstrated that education level, gender, age group, and income level also can affect frequency and amount of fruit intake. In addition, people with positive beliefs and attitudes towards healthy eating generally have an increased intake of fruits. Familiarity towards a product or fruit can also help or hinder a consumer’s purchase (Kamphuis et al., 2006). In an assessment of consumer responses to an off-flavor in fruit juices, it was revealed that taste perception is a significant factor affecting the level of consumption, and that consumption might discontinue if the first taste impression of the fruit is poor (Tuorila & Cardello, 2002).
From a sensory standpoint, the literature shows a wide range of tastes, aromas, and other descriptors being used to describe the pawpaw fruit pulp. The flesh has a smooth, custard-like texture (Pomper, Lowe, Crabtree, et al., 2009a) that has been described to be reminiscent of an avocado (Kral, 1960). The color of the pulp ranges from creamy white to bright yellow or even shades of orange in color (Pomper et al., 1999). A list of descriptors used to describe the flavor of the pulp consisted of apple, banana, mango, melon, fresh, raw, and *top note* (Duffrin & Pomper, 2006). Most commonly, the flesh of the fresh fruit is described as having a tropical aroma and flavor that is often compared to a mixture of mango, banana, and pineapple (Duffrin & Pomper, 2006). However, according to an untrained panel, frozen pawpaw has been described as tasting sour, bitter and resembling melon (Duffrin & Pomper, 2006). The similarity of aroma and tastes of the pawpaw to other tropical fruits may make it difficult to differentiate (Shiota, 1991). Poor-quality pawpaws have a mushy texture, lack sweetness, have an overly strong flavor, and bittersweet aftertaste. High-quality pawpaws that have a firm texture, a delicate blend of flavors, and no bitter aftertaste (Duffrin & Pomper, 2006). It is also known that variations in flavor can exist among cultivars, with some fruit displaying more complex flavor profiles, and that the flavor can intensify as the fruit over-ripen (Pomper et al., 1999)

**Nutritional quality--proximate analysis.** The pawpaw fruit has been shown to have high nutritional quality compared to other temperate fruit crops including the apple and peach. It was first reported in 1982 that the pawpaw and the banana are similar in overall nutritive composition (Peterson, Cherry, & Simmons, 1982). When comparing the
banana to the pawpaw, the pawpaw has less saturated fat (32% compared to 52%), monounsaturated fat (40% compared to 15%), and nearly the same polyunsaturated fatty acids (28% and 34%, respectively) (Peterson et al., 1982). Much of the nutritional information on the pawpaw is of the fresh fruit with skin and not limited to the edible portion making direct comparisons of the edible portions of other fruits difficult. Table 2 shows a comparison of the nutrient information of the pawpaw with a number of fruits to which the pawpaw is often compared.
Table 2

Comparison of Nutritional Composition of Pawpaw and Other Commonly Referenced Fruits

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Pawpaw $^2$</th>
<th>Banana $^1$</th>
<th>Mango $^1$</th>
<th>Papaya $^1$</th>
<th>Soursop $^1$</th>
<th>Cherimoya $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>75.3</td>
<td>74.91</td>
<td>81.71</td>
<td>88.83</td>
<td>81.16</td>
<td>79.39</td>
</tr>
<tr>
<td>Fat</td>
<td>1.2</td>
<td>0.33</td>
<td>0.27</td>
<td>0.14</td>
<td>0.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Protein</td>
<td>1.2</td>
<td>1.09</td>
<td>0.51</td>
<td>0.61</td>
<td>1.0</td>
<td>1.65</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>18.8</td>
<td>22.84</td>
<td>17.0</td>
<td>9.81</td>
<td>16.84</td>
<td>17.7</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.6</td>
<td>2.6</td>
<td>1.8</td>
<td>1.8</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7</td>
<td>0.82</td>
<td>0.5</td>
<td>0.61</td>
<td>0.7</td>
<td>0.64</td>
</tr>
<tr>
<td>Food energy</td>
<td>80.0</td>
<td>89.0</td>
<td>65.0</td>
<td>39.0</td>
<td>66.0</td>
<td>74.0</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$^6$</td>
<td>66.0</td>
<td>64.0</td>
<td>764.0</td>
<td>1094.0</td>
<td>2.0</td>
<td>NL</td>
</tr>
<tr>
<td>C</td>
<td>7.6</td>
<td>8.7</td>
<td>27.7</td>
<td>61.8</td>
<td>20.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.01</td>
<td>0.031</td>
<td>0.058</td>
<td>0.027</td>
<td>0.07</td>
<td>0.091</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.09</td>
<td>0.073</td>
<td>0.057</td>
<td>0.032</td>
<td>0.05</td>
<td>0.119</td>
</tr>
<tr>
<td>Niacin</td>
<td>1.1</td>
<td>0.665</td>
<td>0.584</td>
<td>0.338</td>
<td>0.9</td>
<td>0.574</td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>53.0</td>
<td>5.0</td>
<td>10.0</td>
<td>24.0</td>
<td>14.0</td>
<td>8</td>
</tr>
<tr>
<td>Potassium</td>
<td>314.0</td>
<td>358.0</td>
<td>156.0</td>
<td>257.0</td>
<td>278.0</td>
<td>269.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>109.0</td>
<td>27.0</td>
<td>9.0</td>
<td>10.0</td>
<td>21.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>47.0</td>
<td>22.0</td>
<td>11.0</td>
<td>5.0</td>
<td>27.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Iron</td>
<td>7.0</td>
<td>0.26</td>
<td>0.13</td>
<td>0.1</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.9</td>
<td>0.15</td>
<td>0.04</td>
<td>0.07</td>
<td>0.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Copper</td>
<td>0.5</td>
<td>0.078</td>
<td>0.11</td>
<td>0.016</td>
<td>0.086</td>
<td>0.073</td>
</tr>
<tr>
<td>Manganese</td>
<td>2.5</td>
<td>0.27</td>
<td>0.027</td>
<td>0.011</td>
<td>NL</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Fatty Acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>20.7</td>
<td>30.9</td>
<td>19.3</td>
<td>22.9</td>
<td>13.3</td>
<td>NL</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>8.3</td>
<td>3.0</td>
<td>17.8</td>
<td>14.3</td>
<td>1.3</td>
<td>NL</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>31.5</td>
<td>6.7</td>
<td>20.0</td>
<td>12.9</td>
<td>28.3</td>
<td>NL</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>8.5</td>
<td>14</td>
<td>5.2</td>
<td>4.2</td>
<td>23.0</td>
<td>NL</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>19.5</td>
<td>8.2</td>
<td>13.7</td>
<td>17.9</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td><strong>Sugars</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.2</td>
<td>2.39</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
<td>1.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>2.6</td>
<td>4.85</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.9</td>
<td>4.98</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td><strong>Essential amino acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.8</td>
<td>2.6</td>
<td>3.5</td>
<td>1.3</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.7</td>
<td>6.2</td>
<td>6.0</td>
<td>2.6</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.0</td>
<td>4.6</td>
<td>8.0</td>
<td>4.0</td>
<td>6.0</td>
<td>NL</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.2</td>
<td>0.7</td>
<td>1.0</td>
<td>0.3</td>
<td>0.7</td>
<td>NL</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.4</td>
<td>0.8</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
</tr>
</tbody>
</table>
Table 2 (Continued)

<table>
<thead>
<tr>
<th></th>
<th>4.3</th>
<th>4.5</th>
<th>3.3</th>
<th>1.4</th>
<th>NL</th>
<th>NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>3.8</td>
<td>2.6</td>
<td>3.7</td>
<td>1.8</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.8</td>
<td>0.8</td>
<td>1.6</td>
<td>1.3</td>
<td>1.1</td>
<td>NL</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.0</td>
<td>2.6</td>
<td>2.0</td>
<td>0.8</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>Valine</td>
<td>4.9</td>
<td>4.3</td>
<td>5.0</td>
<td>1.6</td>
<td>NL</td>
<td>NL</td>
</tr>
</tbody>
</table>

*Note.*

1 All fruits, USDA National Nutrient Database.
2 Raw unpeeled fruit (Peterson et al., 1982).
3 g/100g.
4 Kcal/100g.
5 mg/100g.
6 IU/100g.
7 Percent composition of lipids and protein.
ND indicates no data, or numbers have not been determined.
NL indicates numbers were not listed on USDA National Nutrient Database.
Plant Based Antioxidants to Control Lipid Oxidation

Lipid oxidation. In food, lipids are compounds that often contribute to aroma or aroma precursors, which can be degraded to form aromatic compounds. Some lipids have important roles in the retention of fat or oil soluble pigments and as solvents for numerous taste and odor substances. Lipids exhibit hydrophobicity and, therefore, are soluble in organic solvents but not in water. While different types of lipids exist and some lipids are amphiphilic (polar) molecules, most lipids are neutral (nonpolar). Phospholipids are the building blocks of biological membranes, which surround cells and smaller subcellular organelles. These lipids have a high reactivity and can greatly affect the organoleptic quality of the food (Belitz, Grosch, & Schieberle, 2009). This occurs through lipid oxidation.

The reaction of molecular oxygen with organic molecules has undergone much scrutiny. A number of organic molecules are susceptible to oxidative damage, however, lipids have undergone extensive research because the oxidation of lipids can cause damage to membranes, hormones and other components required for normal cell activity (Kanner, & Rosenthal, 1992). Oxidation can also damage DNA, which could lead to mutations in the DNA (Matsushita, Suzuki, Nara, Yokoyama, & Miyashita, 2000). Free radical damage has been linked to aging in addition to several other diseases such as atherosclerosis, cancer and rheumatoid arthritis (Baron et al., 2005). When occurring in food, oxidation reactions due to the breakdown of vitamins, pigments, and lipids leading to a decrease in nutritional quality and increase of off flavors (Lindsay, 1985). The texture, color, and water holding capacity of a food can also be compromised (Brannan &
Lipid oxidation can lead to decomposition of other cellular components including vitamins such as alpha tocopherol, essential fatty acids and other functional proteins (Brannan, Connolly, & Decker, 2001).

The oxidation of lipids is a chain reaction which occurs through three steps; initiation, propagation and termination. Initiation starts when a hydrogen atom is abstracted from a fatty acid chain and causes a free radical to form. The first few free radicals formed indicate a lipid has begun to oxidize. Once a hydrogen is abstracted from a fatty acid, the fatty acid then reacts with oxygen and forms peroxide. This free radical looks to replace the hydrogen it lost by abstracting one from another fatty acid. Peroxide becomes hydroperoxide when it finds a new hydrogen, however the second fatty acid has now become a free radical (Murano, 2003). Free radicals that contain oxygen are known as reactive oxygen species, or ROS, and include superoxide radicals, hydroxyl radicals and hydrogen peroxide (Aqil, Ahmad, & Mehmood, 2006). The cycle of free radical quenching and creation of free radicals is called the propagation phase (Murano, 2003). The termination phase occurs when two free radicals react with one another, or when an antioxidant sacrifices itself to a free radical, thus stopping the chain reaction (Murano, 2003).

**Antioxidants and the control of lipid oxidation.** Antioxidants are substances that can delay the onset or slow the rate of oxidation of autoxidizable materials (Nawar, 1985). The term antioxidant is used to refer to anything that prevents an oxidation reaction, which is the removal of electrons from an atom or group of atoms (Lindsay, 1985). In food, antioxidants act to inhibit the oxidation of fats and pigments by
molecular oxygen to prevent product rancidity and altered color (Murano, 2003). Antioxidants also perform tasks essential to people’s health in the neutralization or elimination of free radicals in their bodies.

Antioxidants come from a variety of natural sources such as fruits, vegetables, herbs (Aqil et al. 2006), spices (Madsen & Bertelsen, 1995), honey (Beretta, Granata, Ferrero, Orioli, & Facino, 2005), wine, green and black teas, seeds, and a number of vitamins and minerals (Moure et al., 2001). Antioxidant research focuses on their role in humans as well extending the shelf life of food. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) (Moure et al., 2001) have come under scrutiny in recent years because consumers are demanding the use of natural antioxidants.

Phenols are an important building block of dietary antioxidants. (Rickman, Barrett, & Bruhn, 2007). Phenolic compounds range from very simple phenols and phenolic acids to the more complex flavonoids (Pedersen et al., 2000). The phenolic ring structure is shown in Figure 5.

![Phenol chemical structure](image)

*Figure 5. Phenol chemical structure.*
Flavonoids, a class of plant polyphenols, are the most important water soluble antioxidant. Flavonoids are found in nearly all plant parts, particularly plant cells responsible for photosynthesis, and are responsible for color, taste, prevention of lipid oxidation, and protection of vitamins and enzymes. (Yao et al., 2004). The quantity of flavonoids in a given food is dependent on species, environmental conditions such as light, ripeness, and postharvest treatments. Flavonoids are the most consumed secondary plant product ingested by humans, however, it is difficult to determine average dietary intake of flavonoids, due to the sheer quantity of total available flavonoids and the subsequent distribution in various plants. Estimates of dietary intake of vary from 100 to 1000 mg/day (Yao et al., 2004). Flavonoids differ in their properties and are categorized according to the number and position of hydroxyl groups and sugar substitutions (Justesen, Knuthsen, & Leth, 1998). More than 4,000 flavonoids have been discovered, and are categorized into: flavonols, flavones, flavonones, flavan-3-ols (catechins, and epicatechins), and anthocyanidins (USDA, 2003). These five primary classes are shown in Figure 6.
Most, if not all, fruits and vegetables contain flavonoids. Generally, each of the subclasses are predominant nutrient sources or pigment providers for a number of...
commonly eaten food items. Flavonols are the most abundant flavonoids and are found in citrus fruits, black tea, red wine, apples, and hops. Quercetin, kaempferol, and myricetin are the three most common flavonols, and research has shown that quercetin is absorbed four times as readily from onions than from apples or tea (Yao et al., 2004). The most common food sources of flavonols in the United States are tea, onions, and apples (Aherne & O’Brien, 2002). Both pineapple and papaya contained < 1.1 mg/kg flavonols, as well as flavones the following subclass of flavonoids, in the edible portion of the fruit (Franke, Custer, Arakaki, & Murphy, 2004).

Often, reported dietary flavonoid content is based only on two common flavones—apigenin and luteolin (Aherne & O’Brien, 2002). These flavones are found in many herbs as well as citrus fruits, cereals, and flowers (Yao et al., 2004). Although flavones are not found in high concentrations in citrus fruits, they have been found to be powerful antioxidants and free radical scavengers (Benavente-García, Castillo, Marin, Ortuño, & Rio, 1997). Of fruits commonly eaten in Hawaii, both onions and blueberries were found to have the highest total flavone content (Franke et al., 2004).

Flavanones are the most abundant flavonoid found in citrus fruits (Benavente-Garcí et al., 1997) to which they contribute color (Yao et al., 2004). Flavanones are also found in celery, cumin and peppermint (Yao et al., 2004). Flavanones, do not have a uniform distribution throughout citrus fruits as demonstrated by a specific flavanone naringin, which has been isolated from the juice, albedo, and peel. In the juice, naringin content of grapefruit juice was reported between 295-377mg/L, while the albedo
contained 13-16 g/kg\(^{-1}\), peels contained 1-16 g/kg\(^{-1}\), and dry peel exhibited concentrations up to 70g/kg\(^{-1}\) (Tomás-Barberán & Clifford, 2000).

Flavan-3-ols, or catechins, are the predominant flavonoid in green and black teas, they are also found in red wine, kiwifruit and black chocolate. Common catechins that are found in tea include gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), and epicatechin (Yao et al., 2004). Catechins are the only type of flavonoids that do not occur in plants as aglycones, which are flavonoid molecules without an attached sugar. All other flavonoid molecules that contain a sugar moiety are called glycosides (Aherne & O’Brien, 2002). Research has shown that peaches are a rich source of procyanidins, a polymer of catechins, and similar to those found in cocoa, apples, wine and tea (Asami, Hong, Barrett, & Mitchell, 2003).

Anthocyanins are found in strawberries and other berries, teas, wines, and flowers (Yao et al., 2004). These compounds often produce red, blue, and purple hues and occasionally black colors in fruits (Clifford, 2000). Color in flowers is often due to anthocyanins, with blue flower often due to anthocyanins based on delphinidin, and red or magenta flowers based on cyanidin (Harborne & Williams, 2000). While flavonoids are very common in citrus fruits, anthocyanins are only found in blood oranges (Benavente-Garcí et al., 1997).

**Characterization of antioxidants.** According to Buettner (1993), antioxidants are primarily categorized into three types, based their involvement in the lipid oxidation chain reaction. Preventive antioxidants prevent the initiation of free radical chain reactions by eliminating the species that cause these preliminary reactions to occur. There are a number of important antioxidant enzymes in this category including superoxide...
dismutase, catalase, and the glutathione peroxidases. The second type of antioxidants, chain–breaking antioxidants, includes ascorbate, the tocopherols, urate, and glutathione. They work during the propagation phase, and repair oxidizing radicals by sacrificing electrons of their own. The third type is the synergistic antioxidant, and these antioxidants function together to protect membranes from lipid damage. For example, after tocopherol sacrifices itself to stop the lipid oxidation chain reaction, it is reduced, i.e., regenerated by its interaction with ascorbic acid (Buettner, 1993), which then becomes oxidized. There is circumstantial evidence that glutathione can reduce ascorbic acid in a similar manner (Brannan & Erickson, 1996).

**Antioxidant capacity of the pawpaw.** Research has shown that the antioxidant content of pawpaws, while similar to values for strawberry and orange, is greater than values for banana and apple (Galli, Archbold, & Pomper 2007). The phenolic content of the pawpaw is affected by ripening and cold storage (Galli et al., 2007). Phenolic compounds and antioxidant capacity decreased with ripening of fruit in ‘PA-Golden (#1)’ and the “Taylor” pawpaw fruit cultivars (Kobayashi et al., 2008), with the highest antioxidant capacity found in the semiripe ‘PA-Golden (#1)’. For the “Taylor” cultivar, the greatest phenolic compounds and antioxidant capacity were observed in ripe fruit. Other results suggest that phenolic components of pawpaw pulp have a major effect on antioxidant capacity, as a positive correlation was found between phenolic compounds and antioxidant capacity of both cultivars, which is similar respect to other fruits and vegetables (Kobayashi et al., 2008). Work done at Ohio University agreed with previous research. Total phenolics were affected by ripeness (underripe = ripe > overripe) while
reducing potential was found to be the highest in ripe pulp and lowest in overripe pulp. However, flavonoids were affected inversely by ripeness (ripe < underripe < overripe) (Harris & Brannan, 2009). These pawpaw pulp extracts also inhibited oxidation of chicken muscle homogenates (Harris, 2007). Pawpaw seed extracts effectively inhibited lipid oxidation biomarkers in 22:6 phosphatidylcholine liposome suspensions during storage. Pawpaw seed extracts also inhibited lipid oxidation biomarkers in chicken muscle homogenates (Brannan & Salabak, 2009).

**Effect of processing and storage on antioxidants**. Cooking pawpaw pulp increased reducing potential and radical scavenging and did not have a significant affect on either total phenolics or flavonoids in underripe pulp, while and flavonoids increased in both ripe and overripe pawpaws (Harris & Brannan, 2009). Similarly, processing clingstone peaches for 40 minutes at 213 °F did not cause a significant loss in total phenolics (Asami et al., 2003). A decrease in the measures of antioxidant capacity in the pawpaw was shown by a loss of ascorbic acid (Harris & Brannan, 2009). In blueberries, heat processing for 18 minutes at 190 °C caused nearly one fifth to one half (17-46%) of the resveratrol to be degraded (Lyons et al., 2003).

Harris and Brannan (2009) found that storing pawpaws at 4 °C for 7 days prior to extraction caused an increase in total phenolic levels, ascorbic acid, and total flavonoids. Underripe pawpaws showed a five-fold increase in total phenolics, and ripe and overripe pawpaws showed an increase in total flavonoids. These changes however, did not contribute to antioxidative capacity of the pulp (Harris & Brannan, 2009). Similarly, antioxidant levels in refrigerated red raspberries also undergo changes in antioxidant
activity with no apparent effect on the antioxidant capacity (Türkben, Sarıburun, Demir, & Uylaşer, 2009). Cold storage of clingstone peaches at 4 °C for 14 days did not cause a loss in total phenolics (Asami et al., 2003). Conversely, storage of strawberry juice and strawberry puree for 11 weeks at 8 °C caused a decrease in antioxidant activity (loss of ascorbic acid, anthocyanins and polyphenols) (Hartmann, Patz, Andlauer, Dietrich, & Ludwig, 2008).

Storing processed pawpaw pulp for 300 days at -18 °C prior to extraction caused a greater than four-fold increase in total phenolics and flavonoids, an increase in reducing potential, and a slight decrease in radical scavenging when compared to pawpaw pulp that had been extracted immediately after processing from skin and seeds (Harris & Brannan, 2009). In blackberries, anthocyanins and phenols were shown to have been retained after 12 months of frozen storage, which was aided by addition of sugar solutions (Kopjar, Tiban, Pilizota, & Babic, 2009). Findings show that blueberries stored frozen at -20 °C up to 3 months showed no difference in total anthocyanins when compared to fresh samples as well as dried samples (Lohachoompol, Srzednicki, & Craske, 2004). Frozen storage of clingstone peaches at -12 °C for 3 months did not cause a loss in total phenolics (Asami et al., 2003). Unlike the pawpaw, at −23 °C total anthocyanins content in Bing cherries decreased 66% after 3 months and nearly 87% after 6, while at −70 °C, 90% of total anthocyanin content remained after three months and 88% remained after 6 (Chaovanalikit & Wrolstad, 2004). Similarly, freezing for 6 months at -18 °C for months caused the antioxidant activity to increase in green beans,
zucchini, and peas, while it caused a decrease in carrots, tomatoes, and yellow pepper (Dansei & Bordoni, 2008).

**Conclusion**

In summary, the pawpaw is an underutilized fruit crop that even with much effort over the past century has yet to reach mainstream commercialization. While research is still in its early stages, great strides have been made in the characterization of pawpaw sensory descriptors, pawpaw quality indicators during storage, and antioxidant capacity of the fruit pulp and seeds. However, much work that needs to be done with this unique fruit for it to reach the commercial recognition of such fruits as the pomegranate or the acai berry. A definitive sensory lexicon and a characterization of the dynamic quality indicators and antioxidant capacity during frozen storage would benefit pawpaw consumers, growers, and the region where pawpaws are grown.
Chapter 3: Methodology

Experimental Design

Shown in Figure 7 is the experimental design employed in this study.

![Diagram of experimental design]

Figure 7. Experimental design.

Sample Preparation

Pawpaws were collected from a single tree in Athens Ohio. This tree consistently produces pawpaws that earn top honors in the “Best Pawpaw Contest” at the yearly Pawpaw Festival based on their weight, appearance, skin surface, aroma, skin thickness, flavor, texture, aftertaste, and number of seeds. The pawpaws were separated into the three parts of the fruit: skin, seeds and pulp. The pulp was pooled and divided into 100 g portions. Each portion was put into a polyethylene/nylon 27.94-cm bags (FoodSaver, Jarden Corp., Rye, NY) with an oxygen transmission rate of 6.7 cc/m²/24 h/23°C/0% RH.
Some of the bags were heat treated (cooked) in boiling water, until they reached an internal temperature of 165 °F. The bags were randomly selected prior to labeling. Once the bags were filled, they were either vacuum sealed (vacuum) or sealed without attempting to remove air prior to sealing (air). All bags were immediately transferred into frozen storage at -18 ºC. At 2-month intervals, pawpaw samples were either analyzed or transferred from -18 ºC to a freezer at -40 ºC to maintain pawpaw quality until analysis.

**Pawpaw Extraction**

Pawpaw fruit pulp (10 g) was extracted in 50 ml of either methanol or methanol/chloroform (1:1, v/v) using a Waring blender followed by agitation for one hour. Methanol-extracted samples were filtered and stored in glass screw top vials in which the headspace had been flushed with N₂ gas. Methanol/chloroform extracts were filtered, centrifuged, mixed with NaCl solution. The chloroform layer was collected and separated using a separatory flask (Company). The chloroform was then flashed off under N₂ gas and the extractables were rehydrated with 50 ml of methanol. Both the methanol and chloroform-extracts were stored in glass screw top vials, in which the headspace had been flushed with N₂ gas, at -40 ºC

**Antioxidant Capacity Assays**

**Total phenolics.** The measurement of total phenolics was determined using the Folin–Ciocalteu (FC) assay (Swain & Hillis, 1959). FC reagent was diluted with deionized water and the diluted reagent (750 μl) then mixed with aliquots of either the chloroform or methanol pawpaw pulp extract (100 μl) and 7.5% bicarbonate solution (750 μl). After incubation for 120 min in the absence of light, absorbance was measured
at 750 nm using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). Total phenolics were quantified according to a standard curve prepared from gallic acid and expressed as μmol gallic acid equivalents.

**Total flavonoids.** Total flavonoids were measured spectrophotometrically using a method developed by Bor, Chen, & Yen, (2006). Chloroform or methanol pawpaw extracts (0.5 ml) were mixed with methanol (1.5 ml), to which 10% aluminum chloride solution (0.1 ml), 1 M potassium acetate (0.1 ml), and deionized water (2.8 ml) were added. After vortexing and sitting for 40 min at 25 ºC, the absorbance was measured at 415 nm using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). The total flavonoid content was quantified according to the standard curve prepared for rutin and the concentrations of flavonoids were reported as μmol rutin equivalents.

**Ferric reducing antioxidant power (FRAP).** The ferric reducing capacity was measured using the FRAP assay (Benzie & Strian, 1996). The FRAP reagent (1.5 ml) consists of sodium acetate buffer (pH 3.6) mixed with 10 mM (2,4,6,-tri(2-pyridyl)-S-triazine; TPTZ) in 40 mM HCl and ferric chloride in a ratio of (10:1:1). This buffer was mixed with 100 μl of the chloroform or methanol pawpaw pulp extract. After 4 min at room temperature, absorbance was monitored at 593 nm using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). The reducing capacity was quantified according to a standard curve prepared from Trolox solution and the concentrations were then expressed in Trolox equivalents.
Quality Indicators

Pulp color was characterized by using a Konica Minolta Colorimeter based on the CIELAB system. This model provides three measures: lightness is represented by $L^*$, and chromaticity is represented by redness $a^*$, greenness $-a^*$, yellowness $b^*$, and blueness $-b^*$. Prior to each use, the meter was calibrated against a standard white plate. Three measurements were taken and results were presented as the mean of three readings for each $a^*$, $b^*$ and $L^*$.

Sensory Analysis

Sensory analysis utilizes different types of testing to obtain information on the sensory characteristics of a given product. When developing or using new food products, sensory analysis affects the decisions involved in the creation of a final product and often has a great effect on its success. Up to 80-90% of new food products fail within a year of production, often as a result of flawed process of product development that did not include adequate sensory analysis (Moskowitz, 2006).

Descriptive sensory analysis is a complex, comprehensive, and informative tool used in sensory analysis. It can be used to provide accurate and precise sensory descriptions of products, analyze how ingredient or processing changes could affect product characteristics, and identify important sensory attributes that could be used to promote product acceptance.

Prior to descriptive testing, sensory testing was performed using a convenience sample of 98 randomly selected consumers in Grover Center at Ohio University. Testing used protocols approved by the Ohio University Institutional Review Board for the
protection of human subjects, as seen in Appendix A. Ninety eight people participated in the consumer study. First, demographic data were collected from each participant. Next, each participant evaluated a randomly coded sample of pureed pawpaw pulp. Participants were asked to taste the tropical fruit pulp, identify as many tropical fruit flavors as they could, and write them in the order of their intensity. Participants were asked to submit at least one, and were given the option to provide five responses. Finally, participants were presented with three cups of pureed fruit (mango, papaya, pawpaw) and asked to rank them in the order of most to least liked (1-3), and then to identify each of the fruits.

Descriptive sensory analyses were conducted with Ohio University’s trained sensory panel. Approval for sensory analysis was performed in accordance with Ohio University’s Institutional Review Board for the protection of human subjects in research, seen in Appendix C. Prior to this study, the descriptive sensory panel was trained for 17 hours on recognition of basic tastes, oral perceptions including texture and mouthfeel, and on how to develop a sensory lexicon prior to sampling for research (Mah, 2008). Then, approximately 26 hours were utilized to train the sensory panel to perceive flavors within the complex flavor profile of the pawpaw. Testing using complex solutions, as well as fresh and processed pawpaw pulp was utilized in the training of the panel.

The panel developed a lexicon and ballot containing 13 different attributes that included basic tastes (sweet and sour), common fruits (mango and honeydew melon), as well as oral sensations and mouthfeel (astringency) within the pawpaw. Attributes were
based on results from the consumer study, as well as aroma descriptors (McGrath & Karahadian, 1994b) and the “pawpaw flavor wheel” (Duffrin & Pomper, 2006).

At the time of the sampling, panelists were supplied with a set of standards for each attribute that they could use throughout the tasting session, water and unsalted crackers. Panelists were also presented with a ballot that contained thirteen, 15-cm lines anchored with standards. At each session, random samples of pawpaw that had been placed into cups labeled with a randomly generated three-digit number were tested one at a time at each sampling session.

**Statistical Analysis**

Antioxidant capacity assays were generated for six trials \( (n = 6) \) while sensory analysis was performed by four trained panelists \( (n = 4) \). Unfortunately, because of the scarcity of the sample, the complete design could not be replicated. Statistical analysis was performed using the PASW Statistics 18 (IBM Corporation, Armonk, New York). Analysis of variance (ANOVA) was used to determine differences between the means. Significance was set at 95 percent level, and a post-hoc test, Duncan’s Multiple Range test, determined where significant differences occurred.

Using the three factors, month of storage \((0, 2, 4, 6)\), packaging condition (vacuum, air), and heat treatment (raw, cook), a \( 4 \times 2 \times 2 \) full factorial design was constructed. Three trials for color and antioxidant capacity measurements were generated. Individual ratings of the four panelists during descriptive sensory analysis were used to generate means for each sample. Analysis of Variance (ANOVA) was used to analyze
differences between treatments and post hoc means separation was achieved using Duncan’s Multiple Range test.
Chapter 4: Results

Antioxidant Capacity

This study looked at the effect of frozen storage for up to 6 months (0, 2, 4, and 6 months of frozen storage) on pawpaw pulp antioxidant quality. Pawpaw pulp was extracted using two different solvents to determine if differences existed between more polar antioxidants (i.e., those extracted in methanol) and less polar antioxidants (i.e., those extracted in chloroform). This was to address research question one, What are the dynamics of antioxidant capacity (with regards to change and polarity) during frozen storage? My hypothesis was that antioxidant capacity will decrease over time during frozen storage, and be affected by different solvents.

Total phenolic compounds from frozen-stored pawpaw pulp extracted in methanol and chloroform are shown in Figure 8. Findings show that total phenolic content was significantly higher in methanol extracts, compared to chloroform extracts, and ranged from 31-53% greater. The phenolic content of methanol and chloroform extracts of pawpaw pulp remained constant during storage.
Figure 8. Total phenolic content in pawpaw pulp extracts (PPPE’s) from different solvents after frozen storage. 
*Note.* Different letters indicate significant differences at $p < 0.05$.

Flavonoid content of methanol and chloroform extracts of frozen-stored pawpaw pulp extracted in are shown in Figure 9. There was a significant difference in flavonoid content between methanol and chloroform extracts at the beginning of the storage period. By 2 months of storage, greater than two-fold increases in both methanol and chloroform extracts were observed. Thereafter, chloroform extracts exhibited no significant change in total flavonoid content over the 6-month period, whereas methanolic extracts exhibited a significant decrease in flavonoid content (see Figure 2).
Figure 9. Total flavanoid content PPPE’s from different solvents after frozen storage. 
*Note.* Different letters indicate significant differences at $p < 0.05$.

Ferric reducing antioxidant power (FRAP) of methanol and chloroform extracted from frozen-stored pawpaw pulp are shown in Figure 10. Total FRAP is four-fold greater in pawpaw pulp extracted in methanol compared to chloroform for all months.
Figure 10. Ferric Reducing Antioxidant Power (FRAP) of PPPE’s from different solvents after frozen storage.  
*Note.* Different letters indicate significant differences at $p < 0.05$.

**Proximate Analysis**

While previous research has indicated the pawpaw fruit has been shown to have high nutritional quality compared to other temperate fruit crops, much of the nutritional information on the pawpaw is of the fresh fruit with skin and not limited to the edible portion making direct comparisons of the edible portions of other fruits difficult. Previous research indicates that the moisture content of the edible portion and the skin was at 75.3% while the lipid content was at 1.2% (Peterson et al., 1982). Findings of this
research indicate that the lipid content of the edible portion was 0.12% while the moisture content was 72.86%.

**Sensory Analysis**

Findings from the sensory analysis portion of this thesis have been published in the article “Sensory analysis of pawpaw (*Asimina triloba*) pulp puree: Consumer appraisal and descriptive lexicon,” by Brannan, Salabak and Holben (2011).

**Consumer study.** The consumer study was used to address research question two, How does the average consumer describe the sensory attributes of the pawpaw fruit pulp? My hypothesis was that the average consumer will likely have difficulty describing the complex flavor of the fruit pulp. Findings from the consumer test were based on a convenience sample of 98 randomly selected subjects. Demographics of these subjects are shown in Table 3. Mango is preferred significantly compared to papaya and pawpaw when comparing the mean ranking for the three fruits across all demographics. With few exceptions, mango was preferred by between one-half and one-full ranking point compared to papaya and pawpaw within all demographic categories. Other results of note showed nearly all subjects were not able to accurately identify pawpaw. Males identified the pawpaw correctly 11% of the time while females identified it correctly only 8% of the time. People over 21 correctly identified the pawpaw 14% of the time and those under 21 only 4% of the time. Those who consumed more fruit identified the pawpaw correctly 17% of the time compared to 8% for those who consume less. Lastly, people who consumed more than four servings of tropical fruit per week were able to identify the pawpaw correctly 19% of the time with both one to three and zero servings at
7%. Education showed a trend that with increased education came increased pawpaw recognition with the highest recognition in participants with a master’s degree or a Ph.D.

Table 3

*Participant Demographics, Mean Rankings for Three Tropical Fruit Purees From a Three-Way Forced Choice Consumer Ranking Test, and Percentage of Participants who Correctly Identified Pawpaw*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>279 Papaya</th>
<th>852 Pawpaw</th>
<th>452 Mango</th>
<th>Correct pawpaw identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant demographics overall</td>
<td>98</td>
<td>2.3 a</td>
<td>2.3 a</td>
<td>1.4 b</td>
<td>9%</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26 (27%)</td>
<td>2.0 b</td>
<td>2.5 a</td>
<td>1.5 c</td>
<td>11%</td>
</tr>
<tr>
<td>Female</td>
<td>72 (73%)</td>
<td>2.4 a</td>
<td>2.2 a</td>
<td>1.4 b</td>
<td>8%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-21</td>
<td>48 (49%)</td>
<td>2.2 a</td>
<td>2.4 b</td>
<td>1.4 b</td>
<td>4%</td>
</tr>
<tr>
<td>Older than 21</td>
<td>50 (51%)</td>
<td>2.4 a</td>
<td>2.2 a</td>
<td>1.5 b</td>
<td>14%</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High School</td>
<td>4 (4%)</td>
<td>1.8 a,b</td>
<td>2.8 b</td>
<td>1.5 b</td>
<td>0%</td>
</tr>
<tr>
<td>Some College</td>
<td>60 (61%)</td>
<td>2.3 a</td>
<td>2.3 a</td>
<td>1.4 b</td>
<td>3%</td>
</tr>
<tr>
<td>Bachelor’s</td>
<td>20 (20%)</td>
<td>2.2 a</td>
<td>2.5 a</td>
<td>1.4 b</td>
<td>10%</td>
</tr>
<tr>
<td>Master’s or Ph.D.</td>
<td>14 (14%)</td>
<td>2.4 a</td>
<td>2.0 a,b</td>
<td>1.6 b</td>
<td>36%</td>
</tr>
<tr>
<td>Servings of fruit consumed per day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5</td>
<td>86 (88%)</td>
<td>2.3 a</td>
<td>2.3 a</td>
<td>1.4 b</td>
<td>8%</td>
</tr>
<tr>
<td>5 or more</td>
<td>12(12%)</td>
<td>2.3 a</td>
<td>2.4 a</td>
<td>1.2 b</td>
<td>17%</td>
</tr>
<tr>
<td>Frequency of consumption of tropical fruit per week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15 (15%)</td>
<td>2.5 a</td>
<td>2.2 a</td>
<td>1.3 b</td>
<td>7%</td>
</tr>
<tr>
<td>1-3</td>
<td>67 (68%)</td>
<td>2.3 a</td>
<td>2.3 a</td>
<td>1.4 b</td>
<td>7%</td>
</tr>
<tr>
<td>More than 4</td>
<td>16 (16%)</td>
<td>2.2 a,b</td>
<td>2.3 a</td>
<td>1.5 b</td>
<td>19%</td>
</tr>
</tbody>
</table>
When asked to identify the tropical fruit flavors in pawpaw pulp and their order of intensity, 41 different flavor attributes were identified in the sample. The five most frequent responses for each intensity are shown in Table 4. By far, the two most common flavors were banana and mango, as evidenced by the fact that 77% of the participants who identified a flavor rated either banana or mango as the most intense flavor and that 68% of participants identified either banana or mango as the second most intense flavor. Orange and papaya, pineapple, grapefruit, and tangerine were commonly identified. It should be noted that pawpaw was identified as a tropical flavor only three times, once as the primary flavor, once as the third most intense flavor, and once as the fifth most intense flavor.
Table 4

Consumers (n = 98) Free Choice Identification of Tropical Fruit Flavors When Presented Pawpaw Pulp in the Order of Their Perceived Intensity

<table>
<thead>
<tr>
<th>Identified as most intense flavor</th>
<th>Identified as 2nd most intense flavor</th>
<th>Identified as 3rd most intense flavor</th>
<th>Identified as 4th most intense flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana (40.8)</td>
<td>Mango (24.4)</td>
<td>Mango (10.2)</td>
<td>Papaya (4.1)</td>
</tr>
<tr>
<td>Mango (24.4)</td>
<td>Banana (13.3)</td>
<td>Pineapple (9.2)</td>
<td>Mango (4.1)</td>
</tr>
<tr>
<td>Orange (7.1)</td>
<td>Papaya (8.2)</td>
<td>Orange (8.2)</td>
<td>Tangerine (3.1)</td>
</tr>
<tr>
<td>Papaya (7.1)</td>
<td>Orange (7.1)</td>
<td>Banana (7.1)</td>
<td>Guava (2.0)</td>
</tr>
<tr>
<td>Pineapple (5.1)</td>
<td>Grapefruit (2.0)</td>
<td>Papaya (4.1)</td>
<td>Pineapple (2.0)</td>
</tr>
<tr>
<td>Did not identify (15.3)</td>
<td>Did not identify (44.9)</td>
<td>Did not identify (61.2)</td>
<td>Did not identify (84.7)</td>
</tr>
</tbody>
</table>

Note. Number in parenthesis indicates percent of consumers who identified the flavor.

The frequency of the fruit puree selected as the favorite (i.e., ranked first) compared to the other two fruits and the percentage of consumers who correctly identified the fruit is shown in Table 5. Mango was most preferred by 70% of subjects, pawpaw preferred by 16% and papaya preferred by 14%. Only 33% of participants who ranked mango first correctly identified it, while 26% identified papaya, and 9% of participants correctly identified pawpaw.
Table 5

*Frequency of Fruit Puree Selected as the Favorite (i.e., Ranked First) Compared to the Other Two Fruits in a Three-Way Forced Choice Consumer Ranking Test*

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Favorite</th>
<th>Correct identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango</td>
<td>70%</td>
<td>33%</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>16%</td>
<td>9%</td>
</tr>
<tr>
<td>Papaya</td>
<td>14%</td>
<td>26%</td>
</tr>
</tbody>
</table>

*Note.* Correct identification refers to the percentage of consumers who correctly identified the fruit puree that they ranked as favorite.

**Descriptive analysis.** Descriptive analysis using a trained panel was performed to address question number three, What changes occur in the sensory quality of pawpaw pulp during frozen storage and can they be controlled by cooking or packaging? My hypothesis was that sensory quality would decline during frozen storage with air sealed pawpaw pulp declining faster than vacuum sealed pawpaw pulp.

This study was the first study that utilized a trained descriptive sensory panel to ascertain sensory aspects of the pawpaw. The panel developed and standardized the pawpaw lexicon that was used for descriptive analysis and is shown in Table 6.
Table 6

*Description and Anchored References of Sensory Attributes Generated by Descriptive Analysis of Pawpaw Pulp*

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description</th>
<th>References</th>
<th>Position (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Color of the top surface of the pulp, detected prior to mixing&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Printed gradient color scale from:</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- yellow, RGB values 255, 221, 0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- brown, RGB values 106, 60, 0</td>
<td></td>
</tr>
<tr>
<td>Fermented odor</td>
<td>The degree of fermented odor, described as a complex combination of cloyingly sweet, fruity, and musty&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Overripe pawpaw pulp that had badly browned</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>Mouthfeel sensation associated with the firmness, cohesiveness, and denseness of the pulp when compressed between the tongue and palate.&lt;sup&gt;2&lt;/sup&gt;</td>
<td>- Applesauce, unsweetened (Great Value brand)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Creamed Wheat Cereal (Malt O Meal, prepared according to package, cooled to room temp.)</td>
<td>14</td>
</tr>
<tr>
<td>Sweet</td>
<td>The amount of sweet taste detected from the sample as it is being chewed before being swallowed or expectorated.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Applesauce, unsweetened (Great Value brand)</td>
<td>4</td>
</tr>
<tr>
<td>Sour</td>
<td>The amount of sour taste detected from the sample as it is being chewed before being swallowed or expectorated.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Applesauce, unsweetened (Great Value brand)</td>
<td>5</td>
</tr>
<tr>
<td>Fruit</td>
<td>Flavor Description</td>
<td>Sample Description</td>
<td>Flavor Value</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Banana</td>
<td>The amount of banana flavor detected from the sample as it is being chewed before being swallowed or expectorated.³</td>
<td>Banana pudding (Kroger brand, prepared according to package)</td>
<td>7.5</td>
</tr>
<tr>
<td>Melon</td>
<td>The amount of melon flavor detected from the sample as it is being chewed before being swallowed or expectorated.²</td>
<td>Honeydew melon, fresh, ½-1 inch cube</td>
<td>8</td>
</tr>
<tr>
<td>Mango</td>
<td>The amount of mango flavor detected from the sample as it is being chewed before being swallowed or expectorated.²</td>
<td>Mango (Del Monte brand, in light syrup)</td>
<td>8</td>
</tr>
<tr>
<td>Papaya</td>
<td>The amount of papaya flavor detected from the sample as it is being chewed before being swallowed or expectorated.²</td>
<td>Papaya, fresh, ½-1 inch strip</td>
<td>7</td>
</tr>
<tr>
<td>Tropical</td>
<td>The amount of tropical flavor detected from the sample as it is being chewed before being swallowed or expectorated.²</td>
<td>Papaya chunk (from Dole Tropical Fruit mix)</td>
<td>9.5</td>
</tr>
<tr>
<td>Bitter</td>
<td>The amount of bitter aftertaste detected from the sample after it is chewed and swallowed or expectorated.³</td>
<td>Black tea (Rose brand, steeped for 1 hour in hot water)</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 6 (continued)

<table>
<thead>
<tr>
<th>Rindy aftertaste</th>
<th>Orange rind, served with flesh and rind</th>
<th>9.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astringent</td>
<td>Black tea (Rose brand, steeped for 1 hour in hot water)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

*Note.*

1 Position on 15-cm line scale.
2 Generated by descriptive analysis panel.
3 Adapted from Meilgaard et al. (1999).

Table 7 identifies *p*-values for the main effects and two and three-way interactions between month of storage (0, 2, 4, 6), packaging (air or vacuum), and heat treatment (cooked or raw). With the exception of a significant difference occurring for the main effect of packaging on the sensory panel’s perception of body, the only other significant changes were observed for color. A significant difference in color was observed by the panel when looking at the main effect of heat treatment.

Significant differences for color (L*, a*, b*), were observed during frozen storage of pawpaw pulp for the main effect of month of frozen storage, packaging condition and heat treatment, as well as all two- and three-way interactions involving month, packaging, and heat. None of the sensory attributes (other than body) were affected by frozen storage over the 12-month storage period.
Table 7

_P-values for the Main Effects of Month of Storage (0, 2, 4, 6, 8, 10, 12), Packaging Condition (Vacuum, Air), and Heat Treatment (Raw, Cooked), Two-Way Interactions, and Three-Way Interactions on Sensory and Quality Attributes of Pawpaw Pulp_

<table>
<thead>
<tr>
<th></th>
<th>Main effects</th>
<th>Packaging condition</th>
<th>Heat treatment</th>
<th>2-way interactions</th>
<th>3-way interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Month of storage (MONTH)</td>
<td>PACK (PACK)</td>
<td>HEAT (HEAT)</td>
<td>MONTH X PACK</td>
<td>MONTH X HEAT</td>
</tr>
<tr>
<td>Color (sensory)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>L*</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.028</td>
<td>0.011</td>
<td>N.S.</td>
</tr>
<tr>
<td>a*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>b*</td>
<td>N.S.</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>N.S.</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body (sensory)</td>
<td>N.S.</td>
<td>0.031</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Fermented odor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Banana flavor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Melon flavor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mango flavor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Table 7 (continued)

<table>
<thead>
<tr>
<th>Flavor Type</th>
<th>N.S.</th>
<th>N.S.</th>
<th>N.S.</th>
<th>N.S.</th>
<th>N.S.</th>
<th>N.S.</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaya flavor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Tropical flavor</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sweet taste</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sour taste</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Bitter aftertaste</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Rindy aftertaste</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Astringent</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Note: N.S. = Not significant at p < 0.05.
Table 8 shows the means for the three-way interactions of month of storage, packaging, and heat treatment for the sensory evaluations of body and color, and the objective analysis of color. These results produced 28 distinct samples. According to the sensory evaluations by the panel, there were no significant differences, however, all three CIE color measurements (L*, a*, b*) yielded significant findings.

The biggest changes in the L* value were observed in the heat treated/vacuum stored sample set, which increased significantly during the first four months of storage (53.8 to 68.9), where it remained constant for the remaining month. The means of the a* values were grouped into many subsets so specific interpretations of the data were difficult. However, the same general changes were observed in the a* value where heat treated/vacuum stored samples decreased during storage (4.9 to 5.9) over the first 6 months of storage. Typically uncooked samples were significantly higher than cooked samples for most, but not all samples. The means of pawpaw pulp for the b* values were grouped many post hoc subsets, and findings showed the lowest b* values were exhibited by the 6 month air samples, however no general trends seemed to exist. Findings showed a significant difference between the months in the a* value with month 0 < month 2 < months 4 and 6.
Table 8

Mean Values ± Standard Deviations of Sensory Body (n = 4), Sensory Color (n = 4), and CIE L*, a*, and b* Values (n = 6) for Pawpaw Pulp Stored Frozen Raw or Heat Treated (Cook), in the Absence (Vac) or Presence (Air) of Air in Package.

<table>
<thead>
<tr>
<th>Mo.</th>
<th>Treatment</th>
<th>Color L*</th>
<th>a*</th>
<th>b*</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Raw vac</td>
<td>6.3 ±1.8</td>
<td>67.9&lt;sup&gt;a&lt;/sup&gt; ± 0.1</td>
<td>5.4&lt;sup&gt;fg&lt;/sup&gt; ± 0.6</td>
<td>30.0&lt;sup&gt;ef&lt;/sup&gt; ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>5.6 ±2.0</td>
<td>67.5&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
<td>7.0&lt;sup&gt;bc&lt;/sup&gt; ± 0.2</td>
<td>33.2&lt;sup&gt;abcd&lt;/sup&gt; ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>5.6 ± 0.6</td>
<td>53.8&lt;sup&gt;b&lt;/sup&gt; ± 1.4</td>
<td>4.9&lt;sup&gt;g&lt;/sup&gt; ± 0.7</td>
<td>33.6&lt;sup&gt;abc&lt;/sup&gt; ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>5.6 ± 1.8</td>
<td>65.5&lt;sup&gt;a&lt;/sup&gt; ± 0.6</td>
<td>5.5&lt;sup&gt;fg&lt;/sup&gt; ± 0.2</td>
<td>30.3&lt;sup&gt;ef&lt;/sup&gt; ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>Raw vac</td>
<td>5.3 ± 0.8</td>
<td>63.4&lt;sup&gt;a&lt;/sup&gt; ± 1.8</td>
<td>6.6&lt;sup&gt;cd&lt;/sup&gt; ± 0.1</td>
<td>34.5&lt;sup&gt;ab&lt;/sup&gt; ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>6.5 ±1.0</td>
<td>64.8&lt;sup&gt;a&lt;/sup&gt; ± 1.2</td>
<td>7.5&lt;sup&gt;ab&lt;/sup&gt; ± 0.5</td>
<td>32.4&lt;sup&gt;bcd&lt;/sup&gt; ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>5.8 ± 0.9</td>
<td>65.4&lt;sup&gt;a&lt;/sup&gt; ± 0.7</td>
<td>5.2&lt;sup&gt;g&lt;/sup&gt; ± 0.4</td>
<td>32.3&lt;sup&gt;bcd&lt;/sup&gt; ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>3.9 ± 0.8</td>
<td>64.2&lt;sup&gt;a&lt;/sup&gt; ± 0.3</td>
<td>5.1&lt;sup&gt;g&lt;/sup&gt; ± 0.5</td>
<td>30.6&lt;sup&gt;def&lt;/sup&gt; ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>Raw vac</td>
<td>5.9 ± 1.2</td>
<td>66.1&lt;sup&gt;a&lt;/sup&gt; ± 0.3</td>
<td>6.9&lt;sup&gt;c&lt;/sup&gt; ± 0.1</td>
<td>34.6&lt;sup&gt;ab&lt;/sup&gt; ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>6.1 ± 0.7</td>
<td>67.6&lt;sup&gt;a&lt;/sup&gt; ± 1.5</td>
<td>7.7&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
<td>35.9&lt;sup&gt;a&lt;/sup&gt; ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>5.9 ± 1.0</td>
<td>68.9&lt;sup&gt;a&lt;/sup&gt; ± 1.6</td>
<td>5.2&lt;sup&gt;g&lt;/sup&gt; ± 0.2</td>
<td>29.6&lt;sup&gt;ef&lt;/sup&gt; ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>5.7 ± 1.5</td>
<td>63.2&lt;sup&gt;a&lt;/sup&gt; ± 0.5</td>
<td>6.1&lt;sup&gt;de&lt;/sup&gt; ± 0.1</td>
<td>28.0&lt;sup&gt;1&lt;/sup&gt; ± 1.8</td>
</tr>
<tr>
<td>6</td>
<td>Raw vac</td>
<td>5.9 ± 1.7</td>
<td>65.3&lt;sup&gt;a&lt;/sup&gt; ± 0.6</td>
<td>7.6&lt;sup&gt;ab&lt;/sup&gt; ± 0.5</td>
<td>34.7&lt;sup&gt;ab&lt;/sup&gt; ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>6.8 ± 1.7</td>
<td>62.1&lt;sup&gt;a&lt;/sup&gt; ± 0.3</td>
<td>6.7&lt;sup&gt;cd&lt;/sup&gt; ± 0.5</td>
<td>32.3&lt;sup&gt;bcd&lt;/sup&gt; ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>5.5 ± 0.9</td>
<td>65.6&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
<td>5.9&lt;sup&gt;ef&lt;/sup&gt; ± 0.5</td>
<td>30.5&lt;sup&gt;def&lt;/sup&gt; ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>6.0 ± 1.3</td>
<td>53.7&lt;sup&gt;b&lt;/sup&gt; ±14.7</td>
<td>5.5&lt;sup&gt;fg&lt;/sup&gt; ± 0.3</td>
<td>31.4&lt;sup&gt;cd&lt;/sup&gt; ± 1.3</td>
</tr>
</tbody>
</table>

Note. Different superscripts within a column denote significant differences at p < 0.05.

Descriptive sensory data for pawpaw flavors (banana, melon, mango, papaya, and tropical), odor (fermented), tastes (sweet, sour, and bitter and rindy aftertastes), and mouthfeel (astringent), are shown on Table 9. No significant differences were observed for any of these attributes.
Table 9

Mean Values ± Standard Deviations of Descriptive Sensory Flavor Attributes (n = 4) for Pawpaw Pulp Stored Frozen Raw or Heat Treated (Cook), and in the Absence (Vac) or Presence (Air) of Air in the Package

<table>
<thead>
<tr>
<th>Mo.</th>
<th>Trtmt.</th>
<th>Banana</th>
<th>Melon</th>
<th>Mango</th>
<th>Papaya</th>
<th>Tropical</th>
<th>Fermented odor</th>
<th>Sweet taste</th>
<th>Sour taste</th>
<th>Bitter aftertaste</th>
<th>Rindy aftertaste</th>
<th>Astringent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Raw vac</td>
<td>5.1 ± 1.1</td>
<td>4.4 ± 2.1</td>
<td>5.0 ± 1.2</td>
<td>5.0 ± 2.0</td>
<td>4.8 ± 0.5</td>
<td>1.2 ± 0.4</td>
<td>5.2 ± 3.2</td>
<td>4.3 ± 1.4</td>
<td>2.2 ± 1.1</td>
<td>2.7 ± 1.6</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>5.1 ± 1.8</td>
<td>4.8 ± 0.8</td>
<td>5.6 ± 0.7</td>
<td>5.6 ± 1.2</td>
<td>5.4 ± 0.8</td>
<td>0.7 ± 0.4</td>
<td>5.1 ± 0.5</td>
<td>4.8 ± 1.3</td>
<td>2.2 ± 1.7</td>
<td>2.6 ± 1.5</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>6.5 ± 2.0</td>
<td>4.1 ± 1.8</td>
<td>4.6 ± 1.5</td>
<td>4.6 ± 2.1</td>
<td>5.4 ± 1.9</td>
<td>1.5 ± 0.7</td>
<td>5.2 ± 1.4</td>
<td>4.5 ± 1.8</td>
<td>2.1 ± 1.4</td>
<td>2.9 ± 1.5</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>5.0 ± 1.5</td>
<td>4.6 ± 1.3</td>
<td>4.5 ± 1.1</td>
<td>4.5 ± 2.0</td>
<td>4.3 ± 0.7</td>
<td>1.1 ± 0.7</td>
<td>4.6 ± 1.9</td>
<td>5.1 ± 1.3</td>
<td>2.1 ± 1.2</td>
<td>2.3 ± 1.0</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>Raw vac</td>
<td>5.3 ± 1.4</td>
<td>4.3 ± 1.5</td>
<td>4.9 ± 1.5</td>
<td>4.9 ± 2.1</td>
<td>5.7 ± 1.1</td>
<td>1.5 ± 0.9</td>
<td>4.9 ± 0.5</td>
<td>4.4 ± 1.5</td>
<td>1.6 ± 1.0</td>
<td>2.9 ± 1.9</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>5.1 ± 1.7</td>
<td>4.5 ± 0.9</td>
<td>5.6 ± 1.3</td>
<td>5.6 ± 1.4</td>
<td>5.9 ± 0.4</td>
<td>1.3 ± 0.8</td>
<td>4.6 ± 1.6</td>
<td>5.0 ± 1.1</td>
<td>2.7 ± 1.2</td>
<td>3.2 ± 1.8</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>5.9 ± 0.9</td>
<td>4.3 ± 1.9</td>
<td>4.5 ± 1.0</td>
<td>4.5 ± 1.9</td>
<td>5.8 ± 0.6</td>
<td>1.0 ± 0.7</td>
<td>4.9 ± 0.9</td>
<td>4.7 ± 1.1</td>
<td>1.9 ± 1.2</td>
<td>2.8 ± 1.8</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>6.0 ± 0.9</td>
<td>5.1 ± 1.6</td>
<td>5.0 ± 1.2</td>
<td>5.0 ± 1.9</td>
<td>5.0 ± 0.4</td>
<td>1.2 ± 0.8</td>
<td>5.3 ± 1.4</td>
<td>5.0 ± 0.3</td>
<td>2.6 ± 1.9</td>
<td>3.4 ± 1.8</td>
<td>3.6 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>Raw vac</td>
<td>5.5 ± 1.1</td>
<td>4.3 ± 1.9</td>
<td>4.1 ± 1.6</td>
<td>4.1 ± 2.0</td>
<td>4.9 ± 1.3</td>
<td>1.6 ± 0.9</td>
<td>5.3 ± 0.8</td>
<td>4.4 ± 1.6</td>
<td>2.0 ± 1.0</td>
<td>2.1 ± 1.3</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>5.9 ± 0.9</td>
<td>4.6 ± 1.1</td>
<td>4.8 ± 1.3</td>
<td>4.8 ± 1.9</td>
<td>5.3 ± 0.8</td>
<td>1.3 ± 0.5</td>
<td>5.6 ± 1.1</td>
<td>5.1 ± 1.1</td>
<td>2.0 ± 1.2</td>
<td>3.1 ± 1.9</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>4.8 ± 1.5</td>
<td>5.1 ± 1.4</td>
<td>4.7 ± 1.1</td>
<td>4.7 ± 2.0</td>
<td>4.6 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>4.6 ± 1.6</td>
<td>4.7 ± 1.1</td>
<td>2.1 ± 1.5</td>
<td>2.2 ± 1.8</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>4.9 ± 1.4</td>
<td>4.7 ± 1.3</td>
<td>4.7 ± 1.0</td>
<td>4.7 ± 1.9</td>
<td>6.0 ± 1.3</td>
<td>1.0 ± 0.4</td>
<td>4.4 ± 0.8</td>
<td>5.1 ± 0.8</td>
<td>2.2 ± 1.5</td>
<td>2.9 ± 2.1</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>Raw vac</td>
<td>5.5 ± 1.3</td>
<td>4.9 ± 1.5</td>
<td>4.5 ± 1.0</td>
<td>4.5 ± 1.8</td>
<td>4.7 ± 1.0</td>
<td>1.0 ± 0.9</td>
<td>4.9 ± 1.2</td>
<td>5.2 ± 0.5</td>
<td>3.7 ± 2.4</td>
<td>2.6 ± 1.5</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Raw air</td>
<td>5.6 ± 0.8</td>
<td>4.2 ± 1.6</td>
<td>4.7 ± 1.3</td>
<td>4.7 ± 2.0</td>
<td>5.5 ± 2.5</td>
<td>1.3 ± 0.4</td>
<td>4.8 ± 2.4</td>
<td>4.7 ± 1.5</td>
<td>1.9 ± 0.9</td>
<td>2.9 ± 1.7</td>
<td>4.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Cook vac</td>
<td>4.5 ± 1.6</td>
<td>4.8 ± 0.9</td>
<td>4.2 ± 1.1</td>
<td>4.2 ± 1.8</td>
<td>5.3 ± 1.7</td>
<td>1.1 ± 0.8</td>
<td>4.9 ± 0.5</td>
<td>4.9 ± 0.9</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 1.3</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Cook air</td>
<td>5.5 ± 0.6</td>
<td>4.4 ± 1.4</td>
<td>4.8 ± 1.2</td>
<td>4.8 ± 2.2</td>
<td>5.2 ± 0.7</td>
<td>1.2 ± 1.1</td>
<td>4.6 ± 1.1</td>
<td>4.2 ± 1.9</td>
<td>3.5 ± 2.5</td>
<td>2.9 ± 1.7</td>
<td>3.1 ± 0.8</td>
</tr>
</tbody>
</table>
Chapter 5: Discussion

Antioxidant Capacity

This research identified levels of phenolic and flavonoid compounds and reducing capacity during frozen storage of pawpaw pulp that was extracted with solvents of different polarity; methanol, with a dielectric constant of 33, and chloroform with a dielectric of 4.8. For comparison, water has a dielectric constant of 80. These solvents were used to determine whether or not different antioxidative compounds in pawpaw pulp could be extracted at different polarities. In other words, highly hydrophobic compounds will be extracted in chloroform as opposed to methanol.

Findings showed a significantly higher total phenolic content in the methanolic extracts compared to the chloroform extracts for all six months (see Figure 8). This result indicates that it is possible there are more or different phenolic compounds being extracted in methanol. According to previous research at Ohio University, storing pawpaw pulp at -18 °C for 300 days (10 months) prior to solvent extraction caused a four-fold increase in total phenolics (Harris & Brannan, 2009). A significantly lower amount of phenolic compounds extracted in chloroform were observed at 6 months of storage compared to 4 months. This effect was not observed for methanol-extracted samples, where no difference in phenolic compounds was observed over the six month storage period. These findings support a conclusion that there were additional phenolic compounds that were extracted in methanol that had greater stability during storage. It is probable that phenolic acids, due to their increased polarity, are being extracted in methanol but not in chloroform. Other research using the pomegranate peel shows that
methanol (dielectric constant = 33) exhibited a higher extract yields compared to other organic solvents with lower dielectric constants such as ethanol (dielectric constant = 24), acetone (dielectric constant = 21), and ethyl acetate (dielectric constant = 6) (Zhenbin, Zhongli, Haile, & Griffiths, 2011). These results support the finding that higher levels of phenolics are extracted when using methanol in pawpaw fruit pulp. Thus, future work in this area should focus on methanolic extracts unless chloroform extracts contain a different flavonoid profile.

Findings in other fruits showed that blackberries were shown to have retained phenols after 12 months of frozen storage, which was aided by addition of sugar solutions (Kopjar et al., 2009). Frozen storage of clingstone peaches at -12 ºC for three months did not cause a loss in total phenolics (Asami et al., 2003), while frozen storage for six months at -18 ºC caused the antioxidant activity to increase in green beans, zucchini, and peas, but a decrease in carrots, tomatoes, and yellow pepper (Dansei & Bordoni, 2008). In pawpaw, no difference in phenolic compounds was observed in methanolic extracts over the 6-month storage period, which supports the use of frozen storage as a viable option to maintain antioxidant content.

There was no significant difference when comparing flavonoid content in chloroform and methanol extracts at month zero. This supports the conclusion that other non-flavonoid phenols, likely to be phenolic acids, are extracted in methanol. Methanolic extracts showed an increase in flavonoids from month zero to two, then a decline to month six with levels near month zero levels. Chloroform extracts exhibited a steady increase in flavonoids over six months. These increases in flavonoid content could be
caused by hydrolysis of more complex long chain flavonoids into smaller simple flavonoid chains. Although it is also possible that the increase came from the frozen storage that month “2” received as it is possible there was breakdown of cells within the fruit during freezing and then after thawing. because month “0” received no frozen storage.

Additionally, reducing power, as measured by the FRAP assay (see Figure 10) was stable across the storage period for both methanol and chloroform extracts. However, methanolic extractions showed a five-fold higher reducing capacity for all 6 months. This finding is supported by Zhenbin, Zhongli, Haile, and Griffiths (2011) who found that the antioxidant activities of the extracts of pomegranate peel showed a strong correlation between DPPH and total phenolics ($R^2 = 0.98$), but no correlation with flavonoids ($R^2 = 0.05$). Comparing methanol with water as the solvent in antioxidant extraction, the DPPH antioxidant activities were 53.74% and 65.30%, respectively. Research in cranberry juice powder found chloroform extracts were more effective in inhibiting lipid oxidation as compared to extracts prepared using other solvents (Raghavan & Richards, 2006). Since reducing capacity is not the same as inhibiting lipid oxidation via quenching of free radicals, additional tests that measure radical quenching should be performed on pawpaw pulp extracts from both methanol and chloroform extracts.

Overall, these results support previous research in that pawpaw fruit pulp that is frozen and stored over time retains high measures of antioxidant capacity. When analyzed over 6 months of frozen storage, measures seem stable over time, with
flavonoids actually appearing to increase over continued frozen storage, although this trend is not statistically significant. It could be assumed that if the antioxidants in pawpaws are stable during frozen storage that this could be another benefit to the eventual and hopeful commercialization of the pawpaw.

**Sensory Analysis**

The findings of the sensory analysis portion of this thesis are published in the article “Sensory analysis of pawpaw (*Asimina triloba*) pulp puree: Consumer appraisal and descriptive lexicon,” by Brannan, Salabak, and Holben (2011).

**Consumer Study.** Based on personal observation at the 2008 Pawpaw Workshop in Frankfort, Kentucky, pawpaw tasting was conducted for a variety of cultivars within the USDA Germplasm. However, it was noted that this analysis was performed in a manner inconsistent with proper sensory principles and generated descriptors that seemed vague in their interpretation. This in conjunction with several other unrefined attempts at characterization of a sensory lexicon for the pawpaw fruit furthers the need for a properly conducted consumer study.

There have been previous attempts to characterize the pawpaw’s flavor and texture attributes in a variety of different ways. Most commonly pawpaw has been shown to exhibit characteristics that are similar to other tropical fruits (Shiota, 1991). However the Shiota work was a gas chromatographic flavor analysis, not a sensory study. McGrath and Karahadian (1992) confirmed the findings from the Shiota work and added small-scale sensory analysis, characterizing the pawpaw as green, fruity, tropical, and sweet. At Ohio University, a pseudo-descriptive study showed that predominant flavors
were tropical in nature with mango, banana, papaya, and pineapple (Duffrin & Pomper, 2006) being the most common. However, this research was conducted as a class project with inadequately trained students acting as panelists, constrained by the 10-week duration of the course. In the current study, a consumer sensory test utilized 98 subjects of varying degrees of familiarity with tropical fruit to assess familiarity of tropical flavors and to generate a pawpaw flavor lexicon. Then, a highly-trained descriptive sensory panel developed and standardized the pawpaw lexicon found in Table 4.

Results from the consumer study indicated that the demographics of the subjects in this study skew young and female (see Table 3). This may be expected, as the study was performed on a college campus in a building that houses programs which are traditionally high in female enrollment (e.g., nursing and dietetics). From a health and wellness perspective, these results indicated that 86% of panelists consume less than the recommended five daily servings of fruit, but nearly the same number consume at least one tropical fruit per week.

Table 4 shows consumers’ identification of the “tropical” flavors when presented with a randomly coded sample of pawpaw pulp. These results are dominated by banana and mango which were identified as the most intense flavors by 77% of consumers who identified a flavor and the second most intense by 68% of those who responded. Of the 25 other flavors identified in the pawpaw pulp by at least one consumer, flavors of note are citrus (orange, grapefruit, tangerine), papaya, and pineapple. The flavor “pawpaw” was identified only three times.
Although consumers identified tropical fruit flavors in the test described above, when presented with actual mango, papaya, and pawpaw, most of the participants were unable to recognize the flavor of the given fruits (see Table 5). Only one-third of consumers who selected mango as their favorite tropical fruit identified it correctly; only one-fourth of consumers who selected papaya as their favorite identified it correctly; and less than one in ten consumers who selected pawpaw as their favorite could identify it correctly. When comparing Tables 4 and 5, a large percentage of participants were able to name one or more tropical flavors in a single sample, but it seems that most of these participants were not able to actually identify the tropical fruit that they were presented, suggesting that consumers who identify tropical flavors, especially mango and papaya, may not actually be able to identify them.

With respect to preference between pawpaw, mango, and papaya, Table 3 shows that mango is preferred significantly compared to papaya and pawpaw overall and across most of the demographic classifications in this study. The data shown in Table 5 reinforce this conclusion as seven in ten consumers selected mango as their favorite, four-to five-fold higher than either papaya or pawpaw. Interestingly, identification of pawpaw seems to increase with increasing age, education, consumption of fruit, and consumption of tropical fruit (see Table 3).

**Descriptive analysis.** The consumer research led to the development of a standardized lexicon for the pawpaw. The development of a standardized lexicon becomes increasingly important should pawpaw producers ever wish to achieve mainstream status like the aforementioned tropical fruits, as well as allow for analytical
comparison throughout the 80+ known varieties of the pawpaw fruit. Such scientific analysis will further the evolution of the growing field of pawpaw research.

To begin developing a sensory lexicon, the sensory panel at Ohio University used the flavors identified by the consumer study in addition to the aroma descriptors fruity, cut grass, sweet, melon-like, and fermented as determined by, “a group familiar with pawpaw aroma characteristics” (McGrath and Karahadian, 1994b), and flavors (apple, banana, mango, melon, citrus, estery, fresh, raw), textures (viscosity, surface, body), and appearances (viscosity, surface, body, color, intensity) developed by semitrained students participating in a class project (Duffrin & Pomper, 2006).

From the large number of attributes that the descriptive panel started with, the lexicon was pared down to 13 different attributes for sensory analysis (see Table 6). Color (appearance), fermented odor (odor), texture, two mouthfeel descriptors (body, astringency), two basic tastes (sweet, sour), five flavors (banana, melon, mango, papaya, tropical), and two aftertastes (bitter, rindy) were the thirteen attributes used in the descriptive study. Five of the attributes used in the study had published sensory standards (Meilgaard, Ceville, & Carr, 1999); however, the other attributes were standardized by the panel using foods regularly available at any supermarket in the United States. It was the intent to use products that had comparable products found in other countries, and thus make the vocabulary universal. This was of importance because while pawpaws are grown throughout Appalachia they are found across the world, especially in Europe in areas with climates similar to that in the eastern United States. While the list was generated to create a standardized and easily replicable list of
standards, despite the panels’ efforts, three of the standards came from fresh fruit – honeydew melon, papaya, and orange rind. This may create differences for future replications of sensory analysis as “ripe” is a variable term.

When looking at the main effects (see Table 7), very few differences were observed for the main effects of month of storage, packaging condition, and heat treatment. Significance was observed for a* value, which measures the relationship between red (+a*) and green (-a*), for all three main effects. During storage, samples become more red (0 months < 2 months < 4, 6 months). Samples exposed to air during storage also were more red. Cooking affected all three instrumental color variables, as cooked samples were significantly darker (lower L*), less red (lower a*), and less yellow (lower b*) than raw samples. Table 5 also shows that the two-way interactions between packaging and heat treatment were significant for all three instrumental color variables (L*, a*, b*). This result suggests that pawpaw pulp color may be affected by the presence of oxygen and heat treatment. It is likely that the change in color (from yellow to rusty brown) involves the formation of colored pigments via the enzyme polyphenol oxidase, which requires oxygen and is denatured by heat. Polyphenol oxidase has been shown to be present in pawpaw pulp (Fang, Wang, Xiong, & Pomper, 2007) and likely is responsible for discoloration observed during refrigerated storage (Archbold et al.,). The discoloration of pawpaw pulp due to polyphenol oxidase has not been monitored in pawpaw pulp stored frozen.

However, findings from the sensory panel do not support these conclusions, as the panel perceived samples that were not subjected to the cooking process as not
significantly darker, redder, or yellower. This suggests that the impact of storage does affect color but may be slight enough to go unnoticed by the consumer.

Shown in Table 8, three way interactions (month of storage x packaging condition x heat treatment) indicate that no differences were observed for sensory color or sensory body. However, differences were observed for all of the instrumental color measurements. Of note, raw samples that were vacuum sealed were more red (higher a* value) after frozen storage, while cooked samples held in air packaging were significantly darker (lower L* value) at 6 months of storage compared to the first 4 months of storage, and were significantly darker than any of the raw samples at any month of storage, which supports the conclusions for the main effects described previously.

As shown in Table 9, no significant differences were observed for any of the sensory flavor, odor, or taste attributes, which is a surprising result because this study and previous research has shown that phenolic and flavonoid compounds in pawpaw pulp are affected by frozen storage (Harris & Brannan, 2009), and phenols are often components of flavor volatiles in food. Despite this change in the pawpaw, this research seems to demonstrate that the sensory attributes of pawpaw pulp are not affected during extended frozen storage. Findings from a trained sensory panel found that the maximum storage period of unblanched mushrooms was 4 months before significant changes in visual appearance, cell fluid leakage, colour, texture, taste, and aroma were observed. However, frozen products having undergone preliminary processing (blanching or soaking and blanching) in solutions with ascorbic, citric, or lactic acid retained good sensory quality for up to 12 months (Grazyna & Emilia, 2009). A study using four different cultivars of
Spanish raspberries tested frozen raspberries (which were thawed before sampling) after 1, 3, 9 and 12 months of frozen storage. Changes in sensory quality indicated after even the first month of freezing significant losses in acceptability occurred in all of four cultivars (Gonzales, de Ancos, & Cano, 2002). It should be noted that whole raspberries and pawpaw pulp are texturally different, which could account for the changes observed in the raspberries.

**Conclusions**

This research is the presentation of the proximate analysis of the water and the fat content of the fruit pulp, an analysis of the dynamics of antioxidant capacity over 6 months of frozen storage, in addition to a comprehensive analysis of the sensory and quality of pawpaw pulp from both a consumer and descriptive sensory perspective. While mainstream commercialization of the pawpaw fruit itself seems unlikely due to various limitations on the fruit’s perishability, the findings of this research suggest that frozen storage may be a viable option for preservation, storage, and sale. The sensory quality of the fruit, with regards to the flavor profile, is stable over frozen storage regardless of vacuum sealed or stored in the presence of air, and even in the presence of air, as the only attribute that changed over frozen storage was the objective analysis of the color. This approach, processing the pulp, extracting the seeds, and storage of the pulp in a vacuum sealed bag is already being utilized commercially by an entrepreneurial pawpaw processor (Integration Acres, Albany, OH). Future research should focus on validating and refining the pawpaw lexicon of pawpaw pulp by comparing different varieties of the pawpaw. Agriculturally, pawpaws that are less subjective to the post harvest challenges
would be of benefit for the producer. Furthermore, establishing the flavonoid profile and continued analysis of antioxidant capacity over additional frozen storage would be an additional health benefit. These over time may help the pawpaw someday reach mainstream commercialization as a fat replacer, flavor enhancer, or source of natural antioxidants.
References


Available from [http://www.actahort.org/books/290/290_13.htm](http://www.actahort.org/books/290/290_13.htm)


Appendix A: IRB Form – Consumer Study

IRB Number________________
Committee: B S

OHIO UNIVERSITY
INSTITUTIONAL REVIEW BOARD (IRB)
PROJECT OUTLINE FORM

Title of Research Proposal: Tropical Fruit Study

Investigator(s) Information
Primary Investigator
Name Dane Salabak Department Human and Consumer Sciences

Address 11 Station Street apt. A, Athens Ohio, 45701
(If off-campus, include city, state and zip code)

Email ds111904@ohio.edu Phone 330.416.5770
Training Module Completed? X Yes No

Co-investigators
Name Robert C. Brannan, Ph.D. Department Human and Consumer Sciences

Address W324 Grover Center
(If off-campus, include city, state and zip code)

Email brannan@ohio.edu Phone 740.593.2879
Training Module Completed? X Yes No

Name David H. Holben, Ph.D., RD Department Human and Consumer Sciences

Address W339 Grover Center
(If off-campus, include city, state and zip code)

Email holben@ohio.edu Phone 740.593.2875
Training Module Completed? X Yes No

Advisor Information (if applicable)
Name Robert C. Brannan, Ph.D. Department Human and Consumer Sciences

Address W324 Grover Center
(If off-campus, include city, state and zip code)

Email brannan@ohio.edu Phone 740.593.2879
Training Module Completed? X Yes No
Anticipated Starting Date 01.12.2009
(Work, including recruitment, cannot begin prior to IRB approval. This date should never precede the submission date)

Funding Status
Is the researcher receiving or applying for external funding? Yes X No

If yes, list source
If yes, describe any consulting or other relationships with this sponsor.

Is there a payment of any kind connected with enrollment of participants on this study that will be paid to persons other than the research participants?
Yes X No
(if yes, describe.)

Review Level
Based on the definition in the guidelines, do you believe your research qualifies for:
X Exempt Review Category 6
____ Expedited Review Category
____ Full Committee Review

Recruitment/Selection of Subjects
Maximum Number of Human Participants 120

Characteristics of subjects (check as many boxes as appropriate).
___ Minors ___ Physically or Mentally Disabled ___ Elementary School Students
X Adults ___ Legal Incompetency ___ Secondary School Students
___ Prisoners ___ Pregnant Females X University Students
___ Others (Specify)____________________

Briefly describe the criteria for selection of subjects (inclusion/exclusion). Include such information as age range, health status, etc. Attach additional pages if necessary.

Interested subjects will have to meet the following initial criteria:
• At least 18 years old
• In good health
• No food allergies

All interested, potential subjects that have met the initial guidelines will sign a consent form before continuing on with the study. Participants will recruited through announcements in classes within the school of Human and Consumer Sciences in addition to being recruited through flyers. Participants will not be excluded for any reason.
How will you identify and recruit prospective participants? If subjects are chosen from records, indicate who gave approval for the use of the records. If records are "private" medical or student records, provide the protocol, consent forms, letters, etc., for securing consent of the subjects for the records. Written documentation for cooperation/permission from the holder or custodian of the records should be attached. (Initial contact of subjects identified through a records search must be made by the official holder of the record, i.e. primary physician, therapist, public school official.)

Prospective participants will be recruited in two ways: 1) announcements in classes within the School of Human and Consumer Sciences and 2) from the general public through signs posted in Grover Center.

A consent form will be given to all subjects outlining the expectations of the study, any potential risks, and details of compensation (none).

All of the participants personal information will be stored in a locked filing cabinet in Grover Center Room W139, and only study investigators will be given access to this information.

Please describe your relationship to the potential participants, i.e. instructor of class, co-worker, etc. If no relationship, state no relationship.

Potential participants may include students of the investigators. However, participation will not be connected to course grade.

Attach copies of all recruitment tools (advertisements, posters, etc.) and label as APPENDIX B

Performance Sites

List all collaborating and performance sites, and provide copy of IRB approval from that site and/or letters of cooperation or support.

Not applicable
Project Description

Please provide a brief summary of this project, using non-technical terms that would be understood by a non-scientific reader. Please limit this description to no more than one typewritten page.

Study participants will perform two simple taste tests during a single visit. Participants will consume tropical fruit samples in both tests.

The participants will evaluate tropical fruit, and analyze each appearance, taste, flavor, and texture of samples in one test and ranking order of preference of several different fruit samples in the second, as described in the methodology section.

Participants will need no prior training.

Please describe the specific scientific objectives (aims) of this research and any previous relevant research.

Pawpaws are tropical fruits that grow in temperate climates. This fruit is commonly found in the eastern United States, typically throughout the region of Appalachia. The commercialization potential of the pawpaw has been slow to emerge due to rapid post-harvest ripening. The pawpaw will go from ripe to overripe in a matter of days at room temperature and in 2-3 weeks under refrigeration.

From a sensory point of view, a wide range of taste descriptors have been used to describe the pawpaw. Some studies have focused on using semi-trained panelists there have not been studies conducted with the average consumer describing the fruit.

The use of human subjects is essential in conducting sensory analysis. Because all humans are consumers of food products, it is only appropriate to use people in determining what sensory attributes are desirable in tropical fruit. If it is determined the "every day" consumer enjoys the "tropical" taste of the pawpaw fruit, it could bring benefit to the Appalachian area. Ultimately, the sensory attributes determined in this study are essential in developing an acceptable and safe use of pawpaws.
Methodology: please describe the procedures (sequentially) that will be performed/followed with human participants.

1. Tasters be recruited through announcements in classes within the School of Human and Consumer Sciences and from the general public through signs posted in Grover Center.

2. Tasters will sign a consent form on the day of sampling before participating.

3. Tasters will be presented with one coded sample and a blank ballot. Participants will describe the different flavors of the tropical fruit and write these descriptors on their ballot.

4. Ballots will be collected after the first test and saltines and water will be available to the tasters.

5. Tasters will be presented with three coded samples and a second ballot and will be asked to rank the order of preference of the three tropical fruits (the pawpaw, papaya and the mango).

6. Ballots will be collected and tasters will be free to leave.

7. Data will be entered into a statistical software package and analyzed (see below).

Describe any potential risks or discomforts of participation and the steps that will be taken to minimize them.

When serving food product samples to this panel, all measures will be taken in order to protect the participating individuals.

Individuals who self-identify as having food allergies to tropical fruits will be removed from the panel.

Other than the possibility of an allergic reaction, no other risks or discomforts are anticipated.

Describe the anticipated benefits to the individual participants. If none, state that. (Note that compensation is not a benefit, but should be listed in the compensation section on the next page.)

By participating in this study, these individuals will gain a sense of achievement and importance in being a key factor of this study. Many will feel a sense of social responsibility donating their time and supporting a graduate student’s research. Participation in this study could potentially enhance future job skills as some of the students participating in the study may be employed in the area of food and nutrition.
Describe the anticipated benefits to society and/or the scientific community. There must be some benefit to justify the use of human subjects.

The use of human subjects is essential in conducting sensory analysis. Because all humans are consumers of food products, it is only appropriate to use people in determining what sensory attributes are desirable in tropical fruit. If it is determined the “every day” consumer enjoys the “tropical” taste of the pawpaw fruit it could bring benefit to the Appalachian area. Ultimately, the sensory attributes determined in this study are essential in developing an acceptable and safe use of pawpaws for society.

Please discuss the level of confidentiality, if any, honored for the data collected. For example, indicate whether records will be labeled with the subject’s name, or whether they will be labeled with a code number, with a master key that links name and code number maintained in a separate and secure location.

All of the participant’s identities and personal information will be left confidential if/when details of the study are used for other purposes such as marketing and recruiting measures.

All efforts to protect confidential information will be taken. All documents with personal information will be kept in a locked filing cabinet in room W139 in Grover Center. No personal information will be announced or exchanged in the group environment.

With whom will identifiable data be shared outside the immediate research team? For each, explain confidentiality measures.

No identifiable data will be shared.

Will participants be: Audiotaped? Yes
X No
Videotaped? Yes
X No

If so, describe how/where the tapes will be stored (i.e. locked file cabinet in investigator office), who will have access to them, and an estimate of the date they will be destroyed.

n/a

Provide details of any compensation (money, course credit, gifts) being offered to participants, including how the compensation will be prorated for participants who discontinue participation prior to completion.

Participants will receive no compensation for participating in study.
**Instruments**

List all questionnaires, instruments, standardized tests below, with a brief description, and provide copies of each, labeled as APPENDIX C.

**Consent form**

**Sensory ballots**

How will the data be analyzed? If applicable, state the hypothesis and describe how the analysis of the data will test that hypothesis.

The first test will determine the frequency of descriptors used to describe the pawpaw, while the second test will be used to rank the tasters' preference for mango, pawpaw, and papaya.

The quantitative data will be analyzed using SPSS version 14.0 software. Descriptive analysis will be performed in order to obtain the mean and standard deviations of the data.

An analysis of Variance (ANOVA) model will be used to measure differences in specific sensory characteristics. The one-way ANOVA will show the magnitude of differences between products through the F ratio and the corresponding probability value. Higher probability values will indicate a greater difference between products while lower probability values indicate higher level of consistency (which can be an indicator of an individual's performance).

A Post-Hoc test, such as the Tukey test, will be used to determine where significant sensory differences occur between products.
Informed Consent Process

Are you requesting a waiver or alteration of Informed Consent? Yes X No
(if yes, check one, and answer a-e)

Waiver of signature
Deception (incomplete disclosure)
Complete Waiver of consent

a. Provide justification for the waiver.

b. Describe how the proposed research presents no more than minimal risk to participants.

c. Why will a waiver of informed consent not adversely affect the rights and welfare of participants?

d. Why is it impracticable to carry out the research without a waiver or alteration of informed consent?

e. How will pertinent information be provided to participants, if appropriate, at a later date?

Even if waiver of written informed consent is granted, you will likely be required to obtain verbal permission that reflects the elements of informed consent (if appropriate). Please specify below information to be read/given to participants.
Attach copies of all consent documents or text and label as APPENDIX A. Please use the template provided at the end of this document.

Informed consent is a process, not just a form. Potential participants/representatives must be given the information they need to make an informed decision to participate in this research. How will you provide information/obtain permission?

All information will be addressed prior to the sampling with potential subjects, both orally and in written form. Each participant will be allowed to consider continuing on with the study. All continuing participants will sign the consent form and will be provided with a copy upon submission to the investigator.

How and where will the consent process occur? How will it be structured to enhance independent and thoughtful decision-making? What steps will be taken to avoid coercion or undue influence?

The consent process will occur prior to the commencement of the study. It will be stressed that at anytime an individual can drop out of the tasting and all personal information gathered and data collected from that person will be destroyed and thrown out of the usable data. A copy of the consent form will be given to each individual.

Will the investigator(s) be obtaining all of the informed consents? X Yes No
If not, identify by name and training who will be describing the research to subjects/representatives and inviting their participation?

Will all adult participants have the capacity to give informed consent? If not, explain procedures to be followed.

Yes

If any participants will be minors, include procedures/form for parental consent and for the assent from the minor.

Not applicable

Will participants be deceived or incompletely informed regarding any aspect of the study?

Yes X No

If yes, provide rationale for use of deception.

If yes, attach copies of post-study debriefing information and label as APPENDIX D. Additionally, complete the questions related to a consent form waiver or alteration on page 9.
Investigator Assurance

I certify that the information provided in this outline form is complete and correct.

I understand that as Principal Investigator, I have ultimate responsibility for the protection of the rights and welfare of human subjects, conduct of the study and the ethical performance of the project.

I agree to comply with Ohio University policies on research and investigation involving human subjects (O.U. Policy # 19.052), as well as with all applicable federal, state and local laws regarding the protection of human subjects in research, including, but not limited to the following:

- The project will be performed by qualified personnel, according to the OU approved protocol.
- No changes will be made in the protocol or consent form until approved by the OU IRB.
- Legally effective informed consent will be obtained from human subjects if applicable, and documentation of informed consent will be retained, in a secure environment, for three years after termination of the project.
- Adverse events will be reported to the OU IRB promptly, and no later than within 5 working days of the occurrence.
- All protocols are approved for a maximum period of one year. Research must stop at the end of that approval period unless the protocol is re-approved for another term.

I further certify that the proposed research is not currently underway and will not begin until approval has been obtained. A signed approval form, on Office of Research Compliance letterhead, communicates IRB approval.

Principal Investigator Signature: Date 12/02/08

Co-Investigator Signature: Date 12/12/08

(please print name)
Faculty Advisor/Sponsor Assurance

By my signature as sponsor on this research application, I certify that the student(s) or guest investigator is knowledgeable about the regulations and policies governing research with human subjects and has sufficient training and experience to conduct this particular study in accord with the approved protocol. In addition:

- I agree to meet with the investigator(s) on a regular basis to monitor study progress.
- Should problems arise during the course of the study, I agree to be available, personally, to supervise the investigator in solving them.
- I assure that the investigator will report significant or untoward adverse events to the IRB in writing promptly, and within 5 working days of the occurrence.
- If I will be unavailable, as when on sabbatical or vacation, I will arrange for an alternate faculty sponsor to assume responsibility during my absence.

I further certify that the proposed research is not currently underway and will not begin until approval has been obtained. A signed approval form, on Office of Research Compliance letterhead, communicates IRB approval.

Advisor/Faculty Sponsor Signature ___________________________ Date 12/2/08

(please print name) Robert Brannan

*The faculty advisor/sponsor must be a member of the OU faculty. The faculty member is considered the responsible party for legal and ethical performance of the project.
Checklist:

Completed and Signed IRB-1 (this form)

Appendix A - copies of all consent documents (in 12 pt. Font) including
   ___ Informed Consent to Participate in Research (adult subjects)
   ___ Parental Permission: Informed Consent (parents of subjects who are minors or children)
   ___ Assent to Participate in Research (used when subjects are minors or children)

Appendix B - copies of any recruitment tools (advertisements, posters, etc.)

Appendix C - copies of all instruments (surveys, standardized tests, questionnaires, interview topics, etc.).

Appendix D - Copies of debriefing text

Appendix E - Approval from other IRB, School District, Corporation, etc.

Appendix F - Any additional materials that will assist the Board in completing its review

Appendix G - Copies of any IRB approvals

Appendix H - Copies of Human Subjects Research Training Certificates
   (for all key personnel involved in non-exempt research)

All fields on the form must be completed, regardless of review level. If a field is not applicable, indicate by inserting n/a. Incomplete forms will result in delayed processing. Forward this completed form and all attachments to:

   Human Subjects Research
   Office of Research Compliance
   RTEC 117

Questions? Visit the website at www ohio edu/research/compliance/ or email compliance@ohio edu
Ohio University Consent Form

Title of Research: Tropical Fruit Study

Researchers: Dane Salabak, Robert G. Brannan, Ph.D., David H. Holben, Ph.D., RD

You are being asked to participate in research. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

Explanation of Study

Those participating in this study will be asked to taste tropical fruits. The tasters will undergo a sensory description (e.g., smell, taste, texture) of the samples in addition to ranking samples in order to determine acceptance and preference.

Participants need no formal training. Participants will sample fruit and generate a list of terms that describe that sample. Participants will also sample a variety of fruits and rank the preference of these fruits as compared to one another.

Risks and Discomforts

Please inform your panel leader if you have allergies to fruits.

No known risks or discomforts have been identified with consuming tropical fruits.

Benefits

By participating in this study, individuals may gain a sense of achievement and importance in being a key factor of this study. Many will feel a sense of social responsibility donating their time and supporting a graduate student's research. Participation in this study could potentially enhance future job skills as some of the students participating in the study may be employed in the area of food and nutrition.

Confidentiality and Records

All efforts to protect confidential information will be taken. All documents with personal information will be kept in a locked filing cabinet. No personal information will be announced or exchanged in the group environment.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with:

* Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research;
* Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU;

Compensation

No Compensation will be received for participation in this study.
Contact Information
If you have any questions regarding this study, please contact:

Dane E. Salabak       ds11904@ohio.edu       330.416.5770 or
Robert G. Brannan, Ph.D   brannan@ohio.edu    740.593.2879

If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

By signing below, you are agreeing that:
• you have read this consent form (or it has been read to you) and have been given the opportunity to ask questions
• known risks to you have been explained to your satisfaction,
• you understand Ohio University has no policy or plan to pay for any injuries you might receive as a result of participating in this research protocol
• you are 18 years of age or older
• your participation in this research is given voluntarily
• you may change your mind and stop participation at any time without penalty or loss of any benefits to which you may otherwise be entitled.
• You do not have an allergy with fruit

Signature___________________________________________ Date________________________

Printed Name________________________________________
Participate in a Tropical Fruit Study

- Sample a variety of tropical fruits.
- Help create a vocabulary to describe a unique tropical fruit.
- Rank taste preference of several tropical fruits.

Study will only take a few minutes to participate in.

Interested subjects will have to meet the following initial criteria:
- At least 18 years old
- In good health
- No food allergies

Contact Dane Salabak at ds111904@ohio.edu or Robert Brannan Ph.D. at brannan@ohio.edu for more information
Head this way to participate in a Tropical Fruit Study!

- Sample a variety of tropical fruits.
- Help create a vocabulary to describe a unique tropical fruit.
- Rank taste preference of several tropical fruits.

Study will only take a few minutes to participate in.

Interested subjects will have to meet the following initial criteria:
- At least 18 years old
- In good health
- No food allergies
Tropical Fruit Study Ballots

Tropical Fruit Taste Test #1
Please taste the tropical fruit pulp. Identify as many tropical fruit flavors as you can and write them in the order of their intensity.
1. ______________________
2. ______________________
3. ______________________
4. ______________________
5. ______________________

Tropical Fruit Taste Test #2
Please taste the three tropical fruit samples on your tray. Rank them in the order of 1 (most liked), 2 (middle), and 3 (least liked). There are no ties. You must rank them 1, 2, 3. Then, guess what each of the tropical fruits are.
1. ______________________ What is it? ______________________
2. ______________________ What is it? ______________________
3. ______________________ What is it? ______________________
Federal regulations require IRB approval prior to implementing proposed changes to research projects. Such changes include any change to the originally approved proposal, including, but not limited to changes in number of participants, changes in recruitment/research procedures, and changes in supporting documents (consent form, debriefing form, questionnaires, advertisements, etc.).

Please complete this form, and attach all revised documents or supporting information.

Proposal #: 08E253 Date: 01.19.09

Proposal Title: Tropical Fruit Study

Principal Investigator Information

Name: Dane Salabak Department: College of Health and Human Services

Address: 11 Station Street Ap: A, Athens Ohio 45701

Email: dsl11904@ohio.edu Phone: 330.416.5770

Study Status

- [X] Project not yet started (no participants enrolled)
- [ ] Closed to new participant entry (data analysis/intervention occurring)

1. Describe the proposed changes and why they are being made.

We are adding a general information section which will be completed prior to participation in the study. This section will be used to generate background information on the participants in the study. All other components of the study, including the consent form and the ballots, will remain the same. This change is being made so more is known about the participants in the study.
2. Describe how, if at all, the proposed changes affect the risks of the study.

Proposed changes will not affect the risk of the study.

3. Describe how, if at all, the proposed changes affect the benefits of the study.

Proposed changes will help researchers to further quantify the research gathered into subgroups based on general information provided by participants.

4. Does the proposed revision affect the consent/assent document(s)? □ Yes □ No

If yes:
Will any participants need to be re-consented as a result of the changes? If so, please describe process to be used.

Include two copies of the revised consent/assent documents, one with changes highlighted, and one without highlighting.

Principal Investigator Signature

Date

Advisor Signature

Date

If new investigator is added, a revised page 1 of the project outline form, a signed signature page of the Project Outline Form, and proof of training is required.

Please note that approval of an amendment does not change the expiration date of the study.

Please return this form to: Office of Research Compliance, 117 Research & Technology Center, Ohio University, Athens, OH 45701-2979
The amendment, detailed below, and submitted for the following research study has been approved by the Institutional Review Board at Ohio University. Approval date of this amendment does not affect the expiration date of the original approval.

Amendment: General information questions to generate background information.

Project: Tropical Fruit Study

Project Director: Dane Salabak
Robert Brannan
David Holben

Advisor: Robert Brannan
(if applicable)

Department: Human and Consumer Sciences

Robin Stack, C.I.P., Human Subjects Research Coordinator
Office of Research Compliance

Date: 01/20/2009
Appendix B: Descriptive Sensory Analysis Ballot
Appendix C: IRB - Descriptive Sensory Analysis

Please Do Not Staple

OHIO UNIVERSITY
INSTITUTIONAL REVIEW BOARD (IRB)
PROJECT OUTLINE FORM

Title of Research Proposal: Optimization of the Pre-treatment to Reduce Oil Absorption in Frozen Pre-fried Breaded Products Using Whey Protein Isolate

Investigator(s) Information
Primary Investigator
Name Robert G. Brannan Department Human and Consumer Sciences

Address Grover Center W324, Ohio University
(If off-campus, include city, state and zip code)

Emailbrannan@ohio.edu Phone (740) 593-2879

Training Module Completed? X Yes □ No (Attach Certificate as Appendix H)
(http://csswww.cats.ohiou.edu/research/compliance/citiprogram.html)

Co-investigators
Name Eunice Mah Department Human and Consumer Sciences

Address W324 Grover Center, Ohio University
(If off-campus, include city, state and zip code)

Emailem272706@ohio.edu Phone (740) 590-1871

Training Module Completed? X Yes □ No (Attach Certificate as Appendix H)

Attach sheets for additional co-investigators if necessary, and check here □

Advisor Information (If applicable)

Name Robert G. Brannan Department Human and Consumer Sciences

Address W337 Grover Center, Ohio University Phone (740) 593-2879

Emailbrannan@ohio.edu

Training Module Completed? X Yes □ No (Attach Certificate as Appendix H)

Please refer to Guidelines for assistance in completing the form.

Office of Research Compliance 1 Rev. 08/2002
Anticipated Starting Date  February 8, 2007  Duration mos yrs
(Work, including recruitment, cannot begin prior to IRB approval. This date should never precede the submission date)

Funding Status
Is the researcher receiving or applying for external funding?  □ Yes  X No
(Note - This refers to funding from entities outside of Ohio University)

If yes, list source
(Note - If an application for funding has been submitted, a FULL copy of the funding application must accompany this form at APPENDIX C)

If yes, describe any consulting or other financial relationships with this sponsor.

Is there a payment of any kind connected with enrollment of participants on this study that will be paid to persons other than the research participants?
□ Yes  X No

(If yes, describe.)

Review Level
Based on the definition in the guidelines, do you believe your research qualifies for:
□ X Exempt Review  Category 6
□ Expedited Review  Category
□ Full Committee Review

Final determination of review level will be determined by Office of Research Compliance in accordance with the categories defined in the Code of Federal Regulations

Prior Approval
If this or a similar protocol been approved by OU IRB or any other, please attach copy of approval and label as Appendix E.

Recruitment/Selection of Subjects
Estimated Number of Human Participants 16

Characteristics of subjects (check as many boxes as appropriate).
□ Minors  □ Physically or Mentally Disabled  □ Elementary School Students
X Adults  □ Legal Incompetency  □ Secondary School Students
□ Prisoners  □ Pregnant Females  X University Students
□ Others (Specify)_____________________

Briefly describe the criteria for selection of subjects (inclusion/exclusion). Include such information as age range, health status, etc. Attach additional pages if necessary.

Please refer to Guidelines for assistance in completing the form.

Office of Research Compliance  2  Rev. 08/2002
How will you identify and recruit prospective participants? If subjects are chosen from records, indicate who gave approval for the use of the records. If records are "private" medical or student records, provide the protocol, consent forms, letters, etc., for securing consent of the subjects for the records. Written documentation for cooperation/permission from the holder or custodian of the records should be attached. (Initial contact of subjects identified through a records search must be made by the official holder of the record, i.e. primary physician, therapist, public school official.)

There will be several recruitment informational sessions where participants will be asked to fill in a questionnaire containing questions on food allergies, food preferences, and medical conditions related to diet. Participants will also be required to fill in their contact information and availability. They will then be screened based on their answers and a minimum of 10 participants and a maximum of 20 participants will be selected for future training.

Please describe your relationship to the potential participants, i.e. instructor of class, co-worker, etc. If no relationship, state no relationship.

No relationship, though some may be co-workers or students in classes taught by principle investigator

Attach copies of all recruitment tools (advertisements, posters, etc.) and label as APPENDIX B

Performance Sites

List all collaborating and performance sites, and provide copy of IRB approval from that site and/or letters of cooperation or support.

Research will be conducted in Grover Center, Ohio University

Project Description

Please provide a brief summary of this project, using non-technical terms that would be understood by a non-scientific reader. Attach an additional page, if needed, but please limit this description to no more than one typewritten page.

The goal of this project is to determine if battered and breaded, fully fried, boneless chicken patties can be produced in which the amount of frying oil that enters the patty is reduced, thus reducing the calories, grams of fat, and calories from fat in the cooked patty. This research will focus on the effects of whey protein concentration and pH on fat uptake in fried foods and determine an optimal pH and protein concentration for the development of a whey protein isolate coating. Samples will be prepared using the standard commercial breading procedure at varied pH and protein concentration. The samples (including control samples) will then be tested for lipid content, moisture content and texture attributes. Finally, descriptive sensory analysis may be conducted on the samples. The lipid and moisture content of the samples will determine the effectiveness of the whey protein isolate coating in reducing oil uptake. Texture analysis will be used in relation to the sensory analysis to determine the best formulation of whey protein isolate coating to apply with regards to sensory

Please refer to Guidelines for assistance in completing the form.
acceptance.

Please describe the specific scientific objectives (aims) of this research and any previous relevant research.

The specific objective of this study are to verify the 68% reduction in oil absorption observed in breaded, fully cooked chicken patties that were subjected to a post-breading dip of 10% whey protein isolate. The results of this research will be used to compliment and verify the results obtained by a similar study done by Brannan & Teyke (2006). Previous research has shown that treating battered and breaded, fully fried chicken patties with 10% whey protein isolate solution produced a 68% reduction in oil pickup from the frying oil, as shown in Figure 1 (Brannan & Teyke, 2006).

![Figure 1: Breadcr y-cooked chicken patties which were dipped in whey protein isolate solutions (10%) that were adjusted to different pH levels. Total fat content is shown below each label.](image-url)
Methodology: please describe the procedures (sequentially) that will be performed/followed with human participants.

Fresh chicken breast obtained from a local grocery will be ground using a meat grinder and formed into patties. They will be coated with a standard batter formulation is based on the work of Sahin, Sumnu, & Altunakar (2005) and breading consisting of Japanese bread crumbs and crackermeal obtained commercially. Control patties will be immediately fried in hot oil until an internal temperature of 170 F is obtained. Treated patties will be dipped in 2.5%, 5%, and 10% solution of whey protein isolate (see attached GRAS Notice No. GRN 000037) which has been adjusted to pH 2.3, and 8 using sodium bisulfate (see attached GRAS Notice No. GRN 000003), then immediately fried in hot oil until an internal temperature of 170 F is obtained. Once the patties are fully cooked, they will be frozen (0 F) until they are sampled. Patties will be rethermalized in an oven or a deep fat fryer until an internal temperature of 160 F is reached.

Two types of sensory analysis will be performed, descriptive analysis using trained panel, then consumer testing using untrained but screened consumers of the product. For all sensory analysis, the patties will be rethermalized from frozen by either frying in hot oil (191°C) or baking at 191°C until an internal temperature of 77°C is obtained. Potential participants for the descriptive sensory analysis will fill out a questionnaire during the recruitment sessions asking for known food allergies, medical conditions pertaining to food and diet, food preferences, contact information, and availability. They will then be screened based on all these criteria and will be contacted to confirm participation via email or phone. Participants who are willing to be part of the research will be asked to sign a consent form with an introductory paragraph describing the purpose of the study as well as the voluntary nature of participation, a confidentiality statement requesting participants to withhold disclosing details of the research to others apart from those directly involved in the research. The panelists will be trained on basic sensory qualities using foods obtained at the supermarket. Once the panel is trained, they will be asked to describe the chicken patties as prepared above. For the consumer panel, untrained panelists will be recruited. After informed consent has been obtained, panelists will be presented with a series (up to six) of pairs of fried patties. Each pair will consist of a control and a treated patty. Panelists will be asked to taste each patty and rate the patties on a hedonic scale in three categories: taste, texture (crunchiness), and appearance. The most that any panelists will taste in a session is twelve patties.

Please refer to Guidelines for assistance in completing the form.
Describe any potential risks or discomforts of participation and the steps that will be taken to minimize them.

Risks associated with participating in this study will be minimal to none. Participants on the descriptive sensory panel will be screened according to known food allergies or medical conditions. Allergen information will be stated in the Informed Consent form.

Describe the anticipated benefits to the individual participants. If none, state that. (Note that compensation is not a benefit, but should be listed in the compensation section on the next page.)

None

Describe the anticipated benefits to society and/or the scientific community. There must be some benefit to justify the use of human subjects.

The usage of whey protein as food coatings has long been investigated and applied (Gennadios et al., 1997; Mellema, 2003; Dairy Management Inc., 2003). However, the extent of commercial application of whey protein coatings as oil barriers is limited to separating oil-rich products such as nuts from other components of heterogeneous foods such as cereal (Dairy Management Inc., 2003). Furthermore, application of other types of coatings to reduce oil uptake are not commercially feasible due to physical, chemical or economical constraints. A cursory search for available patents on oil-barriers in foods reveal that the technology either involves multiple steps that are time consuming (long post-dipping drying time), employment of non-natural chemical additives or involve treatments that compromise the quality of the finished product (U.S. Patent No. 4,917,908, 1990; U.S. Patent No. 5,126,152, 1992). The application of this particular whey protein coating would only involve a post-breading dip in a mixture of whey protein and water without any synthetic ingredients without any additional time consumption. To facilitate the integration of the usage of coatings made from whey protein in fried foods, optimization of this pre-treatment must first be done. The results from this research will hopefully be able to contribute to existing studies on the oil-barrier capabilities of whey protein films and become the stepping stone to the commercial application of such coatings on frozen pre-fried foods. Since whey has many disposal issues, increase utilization of whey would not only be a financial benefit for the dairy industry but will also help decrease the amount of whey that is being disposed. Fried food manufacturers may also benefit by being able to promote their foods as being low- or reduced-fat products. Ultimately, the commercial application of reduced-fat frozen pre-fried foods may contribute to the reduction of fat intake in the American diet.

Please refer to Guidelines for assistance in completing the form.
Describe procedures in place to protect confidentiality. Who will have access to raw data? Will raw data be made available to anyone other than the Principal Investigator and immediate study personnel (e.g., school officials, medical personnel)? If yes, who, how, and why? Describe the procedure for sharing data. Describe how the subject will be informed that the data may be shared.

Informed consent forms will be signed and filed independently of the ballots used for sensory analysis. Ballots will not have any markers of individual identification. Informed consent forms and ballots will be stored in Grover Center and no one will have access to the raw data.

Will participants be: Audiotaped? ☑ Yes
☑ No

Videotaped? ☑ Yes
☑ No

If so, describe how/where the tapes will be stored (i.e. locked file cabinet in investigator office), who will have access to them, and at what point they will be destroyed.

Provide details of any compensation (money, course credit, gifts) being offered to participants, including how the compensation will be prorated for participants who discontinue participation prior to completion.

Compensation will be provided to panelists in the form of gift certificates to the Atrium Café in Grover Center.

**Instruments**

List all questionnaires, instruments, standardized tests below, with a brief description, and provide copies of each, labeled as APPENDIX C.

A simple hedonic ballot will be developed.

How will the data be analyzed? State the hypothesis and describe how the analysis of the data will test that hypothesis.

ANOVA will be used to test for significance and a suitable means separation test (Duncan’s, LSD, etc) will be used to separate means where significance is obtained.

The analysis will test the hypothesis by showing if significant differences exist with respect to oil and moisture concentration, color, texture, and flavor.

*Please refer to Guidelines for assistance in completing the form.*
**Informed Consent Process**

Attach copies of all consent documents or text and label as APPENDIX A.

Informed consent is a process, not just a form. Potential participants/representatives must be given the information they need to make an informed decision to participate in this research. How will you provide information/obtain permission?

*Informed consent form will be presented and explained to panelists prior to any testing. Panelists' signatures on the informed consent forms constitute permission.*

How and where will the consent process occur? How will it be structured to enhance independent and thoughtful decision-making? What steps will be taken to avoid coercion or undue influence?

*Informed consent form will be presented and explained to panelists prior to training of descriptive panelists and prior to any tasting by participants in the consumer tests. The informed consent form will include an introduction to the study including the purpose and estimated length of the study. Participants will then either choose to participate or not to participate in the survey. Panelists' signatures on the informed consent forms constitute permission.*

Will the investigator(s) be obtaining all of the informed consents? X Yes  □ No

If not, identify by name and training who will be describing the research to subjects/representatives and inviting their participation?

Will all adult participants have the capacity to give informed consent? If not, explain procedures to be followed.

Yes

If any participants will be minors, include procedures/form for parental consent and for the assent from the minor.

No minors will be participating

Are you requesting a waiver or alteration of informed Consent? □ Yes  X No

*Please refer to Guidelines for assistance in completing the form.*
An IRB may approve a consent that does not include, or alters, some or all of the elements of informed consent. Provide justifications below for the waiver.

a. Describe how the proposed research presents no more than minimal risk to participants.

The products tested are all common food items or Generally Recognized as Safe

b. Why will a waiver of informed consent not adversely affect the rights and welfare of participants?

NA

c. Why is it impracticable to carry out the research without a waiver or alteration of informed consent?

NA

d. How will pertinent information be provided to participants, if appropriate, at a later date?

NA

Even if waiver of written informed consent is granted, you will likely be required to obtain verbal permission that reflects the elements of informed consent (if appropriate). Please specify below information to be read/given to participants.

NA

Will participants be deceived or incompletely informed regarding any aspect of the study?

☐ Yes    ☒ No

If so, provide rationale for use of deception.

Attach copies of post-study debriefing information and label as APPENDIX D.

Please refer to Guidelines for assistance in completing the form.
Investigator Assurance

I certify that the information provided in this outline form is complete and correct.

I understand that as Principal Investigator, I have ultimate responsibility for the protection of the rights and welfare of human subjects, conduct of the study and the ethical performance of the project.

I agree to comply with Ohio University policies on research and investigation involving human subjects (O.U. Policy # 19.052), as well as with all applicable federal, state and local laws regarding the protection of human subjects in research, including, but not limited to the following:

- The project will be performed by qualified personnel, according to the OU approved protocol.
- No changes will be made in the protocol or consent form until approved by the OU IRB.
- Legally effective informed consent will be obtained from human subjects if applicable, and documentation of informed consent will be retained, in a secure environment, for three years after termination of the project.
- Adverse events will be reported to the OU IRB promptly, and no later than within 5 working days of the occurrence.
- All protocols are approved for a maximum period of one year. Research must stop at the end of that approval period unless the protocol is re-approved for another term.

I further certify that the proposed research is not currently underway and will not begin until approval has been obtained. A signed approval form, on Office of Research Compliance letterhead, communicates IRB approval.

Principal Investigator Signature____________________________________ Date _____________

Co-Investigator Signature___________________________________________ Date _____________

Please refer to Guidelines for assistance in completing the form.

Office of Research Compliance 10  Rev. 08/2002
Faculty Advisor/Sponsor Assurance

By my signature as sponsor on this research application, I certify that the student(s) or guest investigator is knowledgeable about the regulations and policies governing research with human subjects and has sufficient training and experience to conduct this particular study in accord with the approved protocol. In addition:

- I agree to meet with the investigator(s) on a regular basis to monitor study progress.
- Should problems arise during the course of the study, I agree to be available, personally, to supervise the investigator in solving them.
- I assure that the investigator will report significant or untoward adverse events to the IRB in writing promptly, and within 5 working days of the occurrence.
- If I will be unavailable, as when on sabbatical or vacation, I will arrange for an alternate faculty sponsor to assume responsibility during my absence.

I further certify that the proposed research is not currently underway and will not begin until approval has been obtained. A signed approval form, on Office of Research Compliance letterhead, communicates IRB approval.

Advisor/Faculty Sponsor Signature ___________________________ Date ____________

*The faculty advisor/sponsor must be a member of the OU faculty. The faculty member is considered the responsible party for legal and ethical performance of the project.*
Checklist:
- Completed and Signed IRB-1 (this form)
- Appendix A – copies of all consent documents (in 12 pt. Font) including
  - Informed Consent to Participate in Research (adult subjects)
  - Parental Permission/Informed Consent (parents of subjects who are minors or children)
  - Assent to Participate in Research (used when subjects are minors or children)
- Appendix B – copies of any recruitment tools (advertisements, posters, etc.)
- Appendix C – copies of all instruments (surveys, standardized tests, questionnaires, interview topics, etc.).
- Appendix D – Copies of debriefing text
- Appendix E – Approval from other IRB, School District, Corporation, etc.
- Appendix F – Any additional materials that will assist the Board in completing its review
- Appendix G – Copies of any IRB approvals
- Appendix H – Copies of Human Subjects Research Training Certificates
  (for all key personnel involved in non-exempt research)

All fields on the form must be completed, regardless of review level. If a field is not applicable, indicate by inserting n/a. Incomplete forms will result in delayed processing. Forward this completed form and all attachments to:

Human Subjects Research  
Office of Research Compliance  
RTEC 117

Questions? Visit the website at [www ohio.edu/research/compliance/](http://www ohio.edu/research/compliance/) or email compliance@ohio.edu

Please refer to Guidelines for assistance in completing the form.

Office of Research Compliance   12   Rev. 08/2002
Ohio University Consent Form Template (must be in 12 point font)

Title of Research: Optimization of the Pre-treatment to Reduce Oil Absorption in Frozen Pre-fried Breaded Products Using Whey Protein Isolate
Principal Investigator: Robert G. Brannan
Co-Investigator: Eunice Mah
Department: Human and Consumer Sciences

Federal and university regulations require signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing this form.

Explanation of Study
The purpose of this research is to determine if battered and breaded, fully fried, boneless chicken patties can be produced in which the amount of frying oil that enters the patty is reduced, thus reducing the calories, grams of fat, and calories from fat in the cooked patty. Samples will consist or battered, breaded, and deep-fried chicken patties. Treated patties will be dipped in a whey protein isolate solution (an approved, Generally Regarded as Safe food product) before being fried. Participants who are willing to participate will undergo training before being asked to taste these samples. You will be asked to taste the food and give requested feedback. The duration of your participation is anticipated to be around ...... including training and tasting.

Risks and Discomforts
There are no foreseeable risks. It is important that you provide us all known food allergies of medical complications that may be aggravated by participation in this research.

Benefits
The level of fat intake for the average American is much higher than the recommended dietary fat intake leading to various health complications. The high level of fat intake may be closely related to the increase of convenient fried food products in the market. A strategy that utilizes safe, readily available and relatively inexpensive whey protein that reduces fat in fried foods by two-thirds while maintaining their desirable textural and flavor attributes may promote health unforeseen ways

Confidentiality and Records

Compensation

Contact Information
If you have any questions regarding this study, please contact Eunice Mah at em272706@ohio.edu or (740) 590-1871.

Please refer to Guidelines for assistance in completing the form.

Office of Research Compliance 13 Rev. 08/2002
If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

I certify that I have read and understand this consent form and agree to participate as a subject in the research described. I agree that known risks to me have been explained to my satisfaction and I understand that no compensation is available from Ohio University and its employees for any injury resulting from my participation in this research. I certify that I am 18 years of age or older. My participation in this research is given voluntarily. I understand that I may discontinue participation at any time without penalty or loss of any benefits to which I may otherwise be entitled. I certify that I have been given a copy of this consent form to take with me.

Signature __________________________________________  Date ______________
Printed Name ________________________________________