EFFECT OF PROTEOLYTIC ENZYME AND FIBER OF PAPAYA FRUIT ON HUMAN DIGESTIVE HEALTH

BY

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THESIS

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ABSTRACT

The World Health Organization (WHO, 2005) recommends consumption of fruits and vegetables as part of a healthy diet with daily recommendation of 5 servings or at least 400 g per day. Fruits and vegetables are good sources of vitamins, minerals, antioxidants, and fiber. Papaya fruit is known for his high nutrient and fiber content, and with few exceptions, it is generally consumed ripe due to its characteristic flavor and aroma. Digestion improvement has been attributed to consumption of papaya; this we speculate is attributed to the fiber content and proteolytic enzymes associated with this highly nutritious fruit. However, research is lacking that evaluates the impact of papaya fruit on human digestion. Papain is a proteolytic enzyme generally extracted from the latex of unripe papaya. Previous research has focused on evaluating papain activity from the latex of different parts of the plant; however there are no reports about papain activity in papaya pulp through fruit maturation. The activity of papain through different stages of ripeness of papaya and its capacity of dislodging meat bolus in an *in vitro* model was addressed. The objective of this study was to investigate whether papain activity and fiber content are responsible for the digestive properties attributed to papaya and to find a processing method that preserves papaya health properties with minimal impact on flavor. Our results indicated that papain was active at all maturation stages of the fruit. Ripe papaya pulp displayed the highest enzyme activity and also presented the largest meat bolus displacement. The *in vitro* digestion study indicated that ripe papaya displayed the highest protein digestibility; this is associated with proteolytic enzymes still active at the acidity of the stomach. Results from the *in vitro* fermentation study indicated that ripe papaya produced the highest amount of Short Chain Fatty Acids SCFA of the three papaya substrates (unripe, ripe, and processed). SCFA are the most important product of fermentation and are used as indicators of the amount of substrate

fermented by microorganisms in the colon. The combination of proteolytic enzymes and fiber content found in papaya make of this fruit not only a potential digestive aid, but also a good source of SCFA and their associated potential health benefits. Irradiation processing had minimal impact on flavor compounds of papaya nectar. However, processed papaya experienced the lowest protein digestibility and SCFA production among the papaya substrates. Future research needs to explore new processing methods for papaya that minimize the detrimental impact on enzyme activity and SCFA production.

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CHAPTER 1

INTRODUCTION

Consumption of fruits and vegetables has been promoted because of their vitamins, minerals, antioxidants, and fiber content. Several studies evaluated the impact of fruit and vegetables on human health, concluding that consumption of fruits and vegetables promotes improvement in bowel function, increased satiety, and reduced risk of stroke and certain cancers (Kelsay et al., 1978; World Cancer Research Foundation, 1997; Rolls et al., 2004; He et al., 2006). The World Health Organization (WHO, 2005) attributed approximately 14 % of gastrointestinal cancer deaths, 11 % of heart disease death, and 9 % stroke deaths to insufficient consumption of fruit and vegetables.

Papaya is considered one of the most beneficial fruits as a good source of nutrients, fiber, and proteolytic enzymes. Its consumption has been attributed to aid digestion. Previous researchers focused their study on papain activity from the latex of the unripe fruit or other parts of the plant, and also in the quantification of papain present in the pulp (Mezhlumyan et al., 2003; Tripathi et al., 2011). However, papain content should not be related as a guide to predict papain activity. To our knowledge the protease activity in papaya fruit from mature green through fully ripe stages has not been previously investigated.

Proteolytic enzymes from different sources have been studied as alternatives to aid meat bolus displacement in the esophagus (Cavo et al., 1976, Thomas et al., 2004). My research began with evaluation of proteolytic enzyme activity in papaya pulp through maturation. To corroborate the protease action of papaya nectar we used an *in vitro* meat bolus model developed by Thomas et al. (2004). Our hypothesis was that there is proteolytic activity in papaya pulp through maturation and these enzymes will assist to dislodge meat bolus (Chapter 3).

Interesting findings from the protease activity and meat bolus displacement research prompted us to our next study, which was to understand whether proteolytic activity is partially responsible for the digestive properties attributed to papaya. Our goal was to determine whether proteolytic enzymes from papaya fruit are active in the gastrointestinal tract or if they are inactivated by the low pH of the stomach. To address this question I evaluated protein degradation by papaya proteases at the acidic pH of the stomach, using an *in vitro* digestion model. In addition, to evaluate the impact of fiber from papaya on human digestion, an *in vitro* fermentation study was conducted using human subjects as source of inoculum. This study was important to clarify the role of papaya proteolytic enzymes and fiber content in human digestion (Chapter 4).

The results of the *in vitro* experiments were expressed as protein digestibility (nitrogen disappearance) and Short Chain Fatty Acid SCFA production. SCFAs are the most important product of fermentation and also have been attributed to provide many health benefits (McBurney and Thompson, 1989; Adiotomre et al., 1990). Some of these health benefits are prevention and management of: coronary heart diseases, type 2 diabetes and obesity, with decrease of LDL cholesterol values and enhancement of colon health (He et al., 1995; Lairon et al., 2005; Roberfroid, 2007; Vos et al., 2007).

Papaya is a highly perishable fruit and during transportation may suffer from chill injury, bruising, wrinkling, and softening; all of these factors affect the acceptability of this fruit (Penteado and Leitao, 2004). Preservation of papayas is a needed field of study to add value, improve shelf life, and enhance accessibility of the fruit. To maintain the health benefits of this highly perishable fruit, it is important to develop processing methods that have minimal impact on its nutritional properties and flavor.

Papaya nectar using a combination of irradiation (5 kGy) and mild heat (80^{O}C, 5 min) as an alternative to traditional pasteurization was previously developed in our laboratory by Parker et al. (2010). Informal sensory testing revealed that irradiated samples taste sweeter than the control. To clarify the effect of irradiation on the papaya nectar flavor our project focused on studying the impact of processing on the flavor compounds of papaya nectar. Our goal was to identify flavor compounds present in irradiated and non-irradiated samples, with the aim of identifying specific compounds that could be responsible for the enhanced sweetness perception detected in the irradiated samples. The results from this experiment would help understand the cause of enhancement of sweetness in irradiated papaya nectar samples and resolve whether or not this was due to flavor components (Chapter 5).

Overall, this research will provide a better understanding of 1) the behavior of proteolytic activity in papaya pulp through its ripeness stages, 2) how the combination of papaya proteases and fiber provide health benefits in the human GI tract, and 3) the impact of irradiation on the flavor compounds of papaya nectar.

CHAPTER 2

LITERATURE REVIEW

Papaya cultivation, nutritional aspects, and applications

Papaya (*Carica papaya*) is a tropical fruit originally from tropical Central and South America. The fruit is grown in every tropical and subtropical country (Salunkhe and Desai, 1984), due to its rapid growth, easy cultivation, fast economic returns, and adaptation to diverse soils and climates (Seelig, 1970). This perennial plant, incorrectly referred as a tree, is a large herb with a soft wooded hollow stem of rapid growth that reaches heights up to 9 m (Samson, 1986). The fruit is oval to practically round, sometimes pear shaped or elongated club-shaped and weighs up to 9 kg (Morton, 1987). Papaya is a melon like fruit; the ripe fruit has a sweet taste and agreeable flavor. Ripe papaya typically is consumed fresh for dessert, in fruit salad or processed. Unripe fruit is consumed cooked similarly to a vegetable or used in salad, especially in South East Asia. Other parts of the plant, such as root and leaves, are used as curative remedies because of their many medicinal properties (Cherian, 2000; Runnie et al., 2004).

Demand for papaya is increasing due to its high content of vitamins (A and C) and minerals (K, P, Fe), and low content of sodium and calories; also, papaya contain basically no starch (Samson, 1986; Cano, et al., 1995; Wall, 2006). Papaya contains significant amount of papain and chymopapain, proteolytic enzymes. They have diverse uses as meat tenderizers, digestive medicine, in brewing applications, manufacture of chewing gums, and in pharmaceutical applications in the skin care and tanning industry (Nakasone and Paull, 1998).

Carica papaya belongs to the Caricaceae family. Recently, classification of the Caricaceae family has been revised to divide the family into the following: *Cylicomorpha,*

Horovitzia, Vasconcella, Jacarita, Jacarilla, and Carica. Papaya represents the only species within the genus *Carica*. Some of the species that before were comprised in the genus *Carica* are the mountain papayas, now classified into the genus *Vasconcella,* which has the largest number of species (21) (Badillo, 2001). They are also known as highland papayas, they are native to Andean regions with Ecuador as a center of origin, where they grow at altitudes between 1800 to 3000 m (Morton, 1987). Babaco (*Carica pentagona*, Heilborn) is the only commercially cultivated mountain papaya, normally cultivated in the mountain valleys of Ecuador (Morton, 1987). Unripe mountain papayas are a potential source of papain; some have papain activity reported 15 times higher than in papaya latex (Drew et al., 1997; Scheldeman, 2002). Highland papayas could be potentially used as genetic resources to improve common papaya, and they carry resistant genes to ringspot virus and cold weather.

Papaya fruit cultivation and development

Papayas are usually grown from seeds. Germination occurs 2-4 weeks after sowing, some orchards use seedlings which are planted 6-8 weeks after germination. Some papaya plants are dioeceous; they have male and female flowers in separated plants. Others produce hermaphrodite flowers, they have both female and male organs in the same flower. There are also some plants that are monoecious, which have both types of flowers, male and female, and according to the season they will produce male or female type of flowers (Iwu, 1993). The sex of the plant cannot be determined until 6 months after germination (Gonsalves, 1998). To maximize yield, a ratio of one male per 8-10 female plants is recommended in dioeceous varieties (Nakasone and Paull, 1998; Chay-Prove et al., 2000).

For optimal growth, papaya needs a well-drained, aerated, and rich in organic matter soil; with pH between 5.5-6.7 (Morton, 1987). Papaya is a frost-sensitive plant that can only be grown between latitude 32º North and South. For optimal growth the plant requires temperatures between 22-26^{O}C and also a well annually distributed rainfall from 100-150 cm (Litz et al., 1983). Papaya fruit are 7-50 cm long according to the variety, with a succulent flesh 2.5-5 cm thick; the skin is smooth and green when unripe and turns yellow or orange when ripe (Storey, 1969).

The leaves are soft, lobulated, long-petiolated, and can measure up to 80 cm long (Nakasone and Paull, 1998). They grow in clusters close to the top of the plant. The first fruit are expected 10-14 months after germination; they can take up to 5 months to develop, dependent upon the cultivar. Papaya contains a white milky juice, called latex, in all parts of the plant; latex is extracted from the unripe fruit (Iwu, 1993; El Moussaoui et al., 2001). The fruits, leaves, and latex are used for medicinal purposes. It is believed that papaya fruit contain immune-stimulating and antioxidant agents (Aruoma et al., 2006).

Nutritional aspects of papaya, uses, and medicinal applications

Recently the impact of natural antioxidants and dietary fiber on human health has received significant attention. Fruit and vegetables are critical components of the human diet due to their high antioxidant capacity, permitting prevention of cellular damage caused by free radicals, and also due to their fiber content (Liebman, 1992). Papaya, similarly, is a good source of vitamins, minerals, and fiber. The fiber content of papaya varies according to ripening stage, cultivar, and determination method. Researchers have reported Total Dietary Fiber (TDF) values for ripe papaya that vary from 11.7 to 35.2 % dry matter base (Chang et al., 1998; Ramulu and

Rao, 2003; Mahattanatawee et al., 2006; Roberts et al., 2008). Inclusion of fiber in the diet improves digestion and provides several health benefits such as reduced risk of cardiovascular disease, type 2 diabetes and colon cancer (Schulze et al., 2004; Schatzkin et al., 2007). Papaya often ranks high in fruit nutritional charts as a strong source of vitamin C, carotenoids, potassium, magnesium and folate (Vinci et al., 1995; Gebhardt and Thomas, 2002). One cup of papaya (140 g) provides 80 % to 96 % of the dietary reference intake (DRI) for vitamin C and 3 to 9 % of the DRI of vitamin A for an adult (USDA, 2010)

High concentrations of lycopene have been reported in red flesh papaya cultivars such as Sunrise and SunUp (Wall, 2006). Papaya contains higher ascorbic acid than oranges (108 mg/100g compared to 67 mg/100 g) (Lim et al., 2007). Compared to other exotic Mauritian fruits, papaya ranks second after guava in flavonoid content (Luximon-Ramma et al., 2003).

Papaya has several traditional medicinal applications in humans and animals in different parts of the world. In Mauritius smoking dried papaya leaves is done to alleviate asthma attacks. Papaya fruit and seeds were shown to have anthelmintic and antiamoebic activity (Okeniyi et al., 2007). Ripe papaya fruit is also consumed for different digestive conditions, as a diuretic, stomachic, and antiseptic (Iwu, 1993).

Papaya latex was used in folk medicine as an abortive agent, an antimicrobial agent, an antiseptic externally applied to burns and scalds, and as a cure for dyspepsia (Reed, 1976). In Ghana, dried leaf infusion is used to treat stomach problems and as a purgative (Akah et al., 1997). Several studies have been conducted to evaluate the medicinal potential of different parts of the papaya plant, such as leaves, fruits, shoots, seeds, roots, and latex (Canini et al., 2007; Adeneye and Olagunju, 2009; Otsuki et al., 2010). Unripe papaya may have antiulcer properties based on significant reduction of ulcer index in rats exposed to aqueous and methanol

extracts of whole unripe papaya (Ezike et al., 2009). Crude papaya latex extract can produce sustained uterine contractile activity at different stages of pregnancy in rats, which could cause abortion (Cherian, 2000). Unripe and semi-ripe papaya fruit are applied on the uterus to cause abortion in women in India. A study conducted on male mice suggested that aqueous extracts of papaya seed could be used as a helpful male contraceptive (Chinoy et al., 1994). Lohiya et al., (2005) demonstrated that methanol seed extracts could be used as a possible male anti-fertility drug

Researchers have reported that intake of two table spoons of pulverized papaya seeds mixed with hot water twice per day is used in the traditional management of diabetes and obesity (Adeneye and Olagunju, 2009). This study adapted traditional Nigerian dietary treatment into a study designed to evaluate the hypoglycemic, hypolipidemic, and cardio-protective potential of the seed extract in normal Wistar rats. After 30 days significant reductions in total cholesterol, triglycerides, fasting blood glucose, LDL, VLDL, and atherogenic and coronary artery indices were reported; the results were dose-dependent.

To explain some of the medicinal activity of papaya leaves, phenolic compounds were analyzed using papaya leaf extract (Canini et al., 2007). Quantitative analysis revealed high concentrations of phenolic acids. Papaya leaf extract, prepared from dried leaves, had antitumor effects, which were strengthened by application of higher doses of the extract (Otsuki et al., 2010). This group also observed an improvement in important antitumor molecules (cytokines) involved in the regulation of the immune system. However, it is uncertain which compounds in papaya leaves were responsible for this effect.

Chemical compounds

Plants produce several compounds to protect themselves against pest and herbivores. Papaya, for example, produces oxalates, hydrocyanic acid and benzyl isothiocyanate often referred as antinutrients (Umoh, 1995). Concentrations of these antinutrients decrease during ripening of the fruit (Tang, 1971). Another interesting compound that papaya contains is benzyl-glucosinolate, which is normally found in the Cruciferae family. Flath and Forrey (1977) detected among the volatiles of papaya two compounds, benzyl isothiocyanate and phenylacetonitrile, both products of the enzymatic degradation of benzyl glucosinolate (MacLeod and Pieris, 1983).

Papaya is a climacteric fruit; thus, chemical changes occur after the fruit is harvested. During ripening of the papaya fruit there is an incremental increase in soluble solids, total carbohydrates, carotenoids, and protein, with a decrease in moisture, fiber, and antinutrient compounds (Tang, 1971; Chan et al., 1979; Sankat and Maharaj, 1997; Bari et al., 2006).

Carica papaya is composed of many biologically active compounds, many of which are found concentrated in the latex, which is present in all parts of the plant (Madrigal et al., 1980). Within papaya plants the concentration of bio actives will vary with position on plant, age of plant and cultivar. Also, concentration of bioactives differs between male and female plants; female plants exude more latex than hermaphrodite and male plants.

Papaya latex is rich in cysteine proteinases, which are proteolytic enzymes (caricain, chymopapain, papain, and glycyl endopeptidase); these constitute 80 % of latex enzymes. Other enzymes also present in the latex include: glycosyl hydrolases (β-1,3- glucanases, chitinases, and lyzozymes), protease inhibitors (cystatin, and glutaminyl cyclotransferases), and lipases (El Moussaoui et al., 2001).

Additional papaya constituents include: the alkaloid carpaine, found in unripe fruit, leaves, and seeds; carbohydrates, fixed oil, and glycosides present in seeds; and saponins and xylitol, associated with bark (Iwu, 1993). The most abundant compound in the latex is chymopapain, but papain is twice as potent (Iwu, 1993). The enzyme activity of papain is higher in the latex extracted from fully developed fruit (mature green) than the one extracted in earlier stages of developed (Skeleton et al., 1969). Another group of researches study the papain content in mature green and ripe papaya. Papain content in mature green papaya was approximately 32 % higher than in ripe papaya (Tripathi et al., 2010), however this should not be considered as an indicator of enzyme activity (Madrigal et al., 1980).

Enzymes

Enzymes are defined as "proteins with catalytic activity"; they have the capacity to accelerate reactions by 10^3 to 10^{11} times compared to non-enzyme catalyzed reactions (Whitaker, 1994). Since enzymes are not consumed by the reaction they may be part of many other reactions. Another important characteristic of enzymes is their specificity, when they bind the substrate in the active site to convert it into product; enzymes that are highly specific catalyze only one particular reaction, while others catalyze reactions involving specific types of chemical bonds or functional groups (Whitaker, 1996). They are also responsible for several chemical reactions that are indispensable for living organisms; they have the same physical and chemical characteristics as all other proteins found in nature; thus enzymes are part of our daily protein consumption.

One of the most important groups of enzymes in the food industry is proteolytic enzymes. They have multiple applications in the food industry as meat tenderizers, for chill proofing of

beer, in the manufacture of cheeses, and in bread making where they modify gluten properties (Hewitt et al., 2000; Knight, 1980).

Proteolytic enzymes

Proteolytic enzymes are also referred to as peptide hydrolase, peptidase, or peptidase hydrolase. Based on mechanism of action, researchers suggested dividing them into four groups: a) serine proteases, b) sulfhydryl proteases, also called thiol proteases or cysteine proteases, c) metal containing proteases, and d) aspartic proteases, also called carboxyl proteases or acidic proteases (Wong, 1995).

Generally the quantity of proteolytic enzyme found in the majority of food products is small. Studies suggested that an important fraction of papaya proteases is stored in an inactive form in the fruit; thus active proteases in fresh papaya latex represent a small amount of the total enzymatic potential (Azarkan et al., 2004). The most important proteolytic enzymes found in fruits are papain (EC 3.4.22.2), ficin (EC 3.4.22.3), bromelain (EC 3.4.22.4), and actinidin (EC 3.4.22.14), found in papayas, figs, pineapples, and kiwis, respectively.

Papain

For centuries meat tenderizers have been used to reduce toughness of meat. Mexican Indians used to wrap meat in papaya leaves so the juices of the leaves would be absorbed and tenderize the meat before cooking. This is attributed to the presence of papain enzyme, which is extracted from the fully grown unripe fruit, by making superficial and longitudinal incisions in the fruit; the white liquid that drips from the fruit is called latex. Latex is collected and sun dried or dried in chambers until the moisture content is reduced to 5 % or 8 %; this is known as crude

papain (Buttle *et al*., 1989; Jacquet *et al.*, 1989). This enzyme is produced in many parts of the world and sold in form of powder. This powder contains, in addition to papain, other enzymes such as chymopapain, and papaya proteinase III and IV; chymopapain is the most abundant of them.

The Food and Drug Administration (FDA) does not allow the use of crude papain; thus, before being used by the food industry papain needs to be purified; this process is done by precipitation of papain with an organic solvent. Different factors affect papain activity and stability such as temperature, metal ions, and pH (Whitaker, 1994). Evaluation of thermal stability of papain and proteinase 3 was conducted at pH 4.0, for 6 minutes, and temperatures from 20-95^{O}C (Sumner et al., 1993). They determined that at temperatures over 55^{O}C papain activity was reduced, while proteinase 3 maintained its activity up to 90^{O}C. Loss of activity was attributed to changes occurring at the active site. Metal ions had an impact on papain activity; metal ions such as calcium and magnesium increased enzyme activity by 18 % and 24 %, respectively at $1x10^{-3}$ M concentration (Kaul et al., 2002).

Optimal pH for papain stability and activity is 6.0-7.5, a study reported maintenance of papain's native structure at pH 2.6, when combined with temperatures below 20^{O}C (Hernandez-Arana and Soriano-Garcia, 1988). Hoover and Kokes (1946) investigated the optimum pH for digestion of different proteins by papain. This optimal pH varied according to the type of protein being hydrolyzed. The effect of pH 2.5, 5, and 7 on casein proteolysis was determined; the trend of the reaction was similar at pH 2.5 and 7; however the digestion rate at pH 7 was five times greater than at pH 5.

Gomes et al., (1997) demonstrated the impact of high pressures plus temperature on papain activity. They found that pressure of 800 MPa for 10 minutes decreased enzymatic

activity (39 %) at ambient temperature (20^{O}C); this reduction in activity was higher (87 %) when the enzyme solution was exposed to higher temperature (60^{O}C). They suggested that decreased papain activity after pressure processing could be mainly due to oxidation of the thiolate group at the active site. They also observed that pressures equal or lower than 600 MPa had minimum impact on enzyme activity; independently of the temperature.

Papaya industry

Recent consumer focus has been on the health benefits provided by consumption of fruits and vegetables. This is reflected, in part, by an increasing demand of fruits worldwide. Papaya constitutes one of the 4 major tropical fruits in addition to mango, pineapple, and avocados. As a group the major tropical fruits comprise 75 % of tropical fresh fruit production. The US, Europe, China, Japan, and Canada are the biggest importers of tropical fruits (FAO, 2003). Papaya production constitutes nearly 20 % of major tropical fruit production. Major papaya producing countries are Brazil (~60 %), India (~20 %), and Nigeria (~10 %). Papaya is imported and air freighted to the US from Hawaii and various growing regions around the world.

US market

The main suppliers of papaya for the US market are Mexico, Belize, Brazil, and Hawaii. Mexico is the largest supplier of papaya to the US mainland followed by Hawaii (OTEA, 2002). The 2 most prominent types of papayas imported to the US mainland are Maradol and Solo. Maradol is a larger papaya (up to 1.8 Kg); it is sweet, with redish orange flesh, and is also known as Mexican papaya, supplied mainly by Mexico and Belize. Solo papayas are small fruit (up to

0.5 Kg); they are red or yellow, and are mainly shipped from Hawaii and Brazil to the US mainland.

The biggest crisis that the Hawaii papaya industry has faced was the papaya ringspot virus (PRSV) attack that resulted in dramatic losses to the Hawaii papaya industry (Gonsalves, 2004). To combat this, producers grow genetically modified (GMO) papayas, Rainbow with yellow flesh and SunUp with red flesh; both cultivars have resistance to PRSV.

Of the total papaya area planted in 2008, the Rainbow variety comprised 67 % SunUp represented 10 %, and the non-GMO Solo Kapoho variety 14 % (Stice, 2009). Even though the GMO varieties provide great resolution to the PRSV problem, countries such as Japan and Canada were previously not willing to purchase GMO papayas. Hawaii exports approximately 50% of its papaya production to the mainland and to foreign countries. Papaya production in Hawaii is expected to increase, since Canada and Japan are now opening up their markets to GMO papayas (Pesante, 2003).

Papaya is highly perishable and thus has a limited shelf life and distribution channel. During transportation of fresh fruit, papaya is susceptible to chill injury, bruising, wrinkling, and softening, all of which affect the acceptability of this fruit (Penteado and Leitao, 2004). Post-harvest losses of papaya vary in each country, with estimates of 20 % to 40 % (NAS, 1978; Liu and Ma, 1983); in some cases losses are reported up to 50 % (Paull et al., 1997). Hawaiian papaya production in 2006 was approximately 28 million pounds, from which approximately 10% was processed into puree. Additionally, 3.5 million pounds of culls were reported; if they were not processed into puree, they were discarded, or used to feed livestock. There is need for study of papaya preservation to add value, to improve shelf life, and expand availability of the fruit. According to Jago (2004), consumption of refrigerated juice in the US was approximately

4.4 billion liters. Orange juice was highest in consumption followed by apple, pineapple, and grapefruit.

Processing techniques, impact, and potential applications in fruit juices

Processing is designed to extend the shelf life of food products, providing consumers high quality, safe foods with minimum nutrient loss. Conventional technologies apply minimal processing to control microorganisms, such as mild heat treatment (pasteurization) and non-thermal treatments like high hydrostatic pressure, irradiation (cold pasteurization) or a combination of these processing techniques.

Pasteurization is a mild heat treatment (<100°C) applied to food for a specific period of time. The main goals of pasteurization are: to inactivate pathogenic and spoilage microorganisms and to inactivate deteriorative enzymes. However, pathogens present differential resistance to heat; this resistance changes according to diverse factors such as pH, water activity, and other characteristics of the food. For example, pathogenic and spoilage bacteria are more heat resistant when pH is close to neutrality (Fellows, 1994).

Irradiation involves the process of exposing food to ionizing energy, which passes through the food without leaving any radioactive residue (Elias and Cohen, 1977). When ionizing energy passes through food, molecular bonds are broken, and new stable products are formed. Food is not in direct contact with radioactive materials, it passes through a radiation chamber on a conveyor belt (Simic, 1983; Urbain, 1986).

The main sources of ionizing energy approved for food include gamma rays, produced from radioisotopes cobalt 60 and cesium 137, x-rays, and electron beams. Gamma rays and x-rays have the highest penetration of thick packaging materials and food products; the penetration

of electron beams is limited to 8 cm (Kilcast, 1994; McLaughlin, 1999). Irradiation can be used to inhibit sprouting in root crops, to slow ripening in fruits, for insect disinfestation, and to eliminate spoilage and food borne pathogens. In the latter, results obtained are similar to those of conventional pasteurization; this is why irradiation is sometimes referred to as "cold pasteurization". The main difference between irradiation and pasteurization is the source of energy; pasteurization is based on heat and irradiation on ionizing energy. Both methods are useful to extend the shelf life of fresh foods.

Another processing method that has caught the attention of scientists and the food industry is High Hydrostatic Pressure (HHP), a potential non-thermal alternative to pasteurization or irradiation of food products. High hydrostatic pressure processing it has been known since 1899 (Hite, 1899). However, there were limitations to the application of this technology, including lack of manufacturing units and lack of adequate packaging material resistant to high pressures. It was until 1990 when the first food products produced using high hydrostatic pressure were launched commercially in Japan (Fellows, 1994).

HHP consists of placing food packages inside a pressure chamber and applying pressures of 500-1000 MPa; water is the most common medium used to transmit pressure, it is pressurized using special pumps (Berk, 2008). Food is exposed to pressure for a determined residence time, the pressure is uniformly distributed. HHP only affects non-covalent bonds such as hydrogen, ionic, and hydrophobic bonds, allowing destruction of microorganisms without altering small molecules such as volatile compounds, pigments, vitamins, and other compounds related to product sensory and nutritional quality (Knorr, 1996).

Impact of pasteurization on papaya juice

Generally pasteurization results in minimal changes to the nutritional and sensory characteristics of the food. However, pasteurization of fruit juices which are heat sensitive can cause loss of volatile compounds, thus a reduction of sensory quality. Papaya is one example of fruit with a poor ability to withstand thermal processes. According to previous studies, traditional pasteurization methods develop cooked flavor in papaya juice; this is one of the reasons why papaya juice is often mixed with other fruits to mask the off flavors (Argaiz and Lopez-Malo, 1996; Tiwari, 2000). Heat treatment has been combined with non-thermal processing techniques, such as ultrasound treatment and irradiation, for potential microbial inactivation (Farkas, 1990; Athmaselvi, 2008; Parker et al., 2010).

The shelf life and the intensity of the heat treatment depend on the pH of the juice; the majority of fruit juices have a low pH<4.6 (Fellow, 1988). However, papaya juice has a higher pH >4.9 (Brekke et al., 1973; Penteado and Leitao, 2004). At pH greater than 4.6, pasteurization is not sufficient to inactivate the spores of microorganisms such as *Clostridium botulinum*, which is the most heat resistant spore forming pathogenic bacterium (Hauschild, 1989). Thus, to prevent spore growth, papaya juice should be keep refrigerated or acidified to pH<4.6. Acidification of papaya puree at pH 3.55 inhibited bacterial activity (Chan et al., 1973). Acidification is combined with heat treatment (pasteurization) for overall destruction of pathogenic microorganisms. Pasteurization is also used to inactivate enzymes, especially the ones that have similar ranges of D and z values to those of the microorganisms.

The enzyme pectin methylesterase (PE) is present in papaya and is responsible for gel formation in non-processed papaya juice (Chan et al., 1973); which can be observed within 1-2 days (Parker et al., 2010). Pasteurization reduces the activity of PE to an acceptable level.

Impact of irradiation on papaya

Impact on nutrients

Ascorbic acid and carotenoid concentrations in papaya were not affected by irradiation doses of 0.75 kGy, and there were no other nutritional and chemical changes observed compared to the control (Boylston et al., 2002). Similar results were reported for concentrations of ascorbic acid and carotenoids in two papaya cultivars irradiated at 2.0 kGy (Beyers et al., 1979). Irradiation doses of 0.8 kGy did not affect the free amino acid, total amino acid, and fatty acid composition of papaya (Blakesley et al., 1979).

The impact of irradiation on nutrients is not significant compared to other food processes; cooking for example has a larger detrimental impact on nutrients (Leskova et al., 2006). Irradiation doses up to 50 kGy had no significant impact on macronutrients and minerals in food (WHO, 1994). There are reports of vitamin loss during irradiation, which varies with other factors such as temperature, type of food, dose of irradiation, and presence of oxygen. Vitamin degradation can be minimized using irradiation at low temperatures and in absence of oxygen; prevention of further losses of vitamins could be accomplished by storage in sealed packages at low temperatures (Wilkinson and Gould, 1998).

Sensitivity to irradiation varies among vitamins. Vitamin C and B1 are the most sensitive water-soluble vitamins and vitamin D the least. The most sensitive fat soluble vitamins are A and E (WHO, 1994). Others have reported losses from 8-20 % of ascorbic acid when doses of 3.0 kGy were applied to papaya pulp (Wilkinson and Gould, 1998).

Different reductions in ascorbic acid were reported between two varieties of papaya Rainbow and SunUp, 18 % and 11 % respectively, when irradiation doses of 5 kGy were applied (Parker et al., 2010). When a combination of irradiation and mild heat were applied to both

varieties, ascorbic acid content in Rainbow was similar to the control, and that of SunUp was significantly different from the control. These results suggested that ascorbic acid in SunUp variety was more susceptible to heat than in Rainbow. Even-though there is degradation of vitamins during irradiation the changes to the food components in general are minimal; these changes are not exclusive to irradiation (Beyers and Thomas, 1979). Irradiation of papaya at 2.0 kGy did not affect carotenoids; however canning resulted in a 90 % loss.

Impact on papaya flavor

Papaya fruit processed at doses of 0.75 kGy and 1.0 kGy did not show significant changes in total soluble solids and titratable acidity, corresponding to no detectable differences in sweetness and tartness (Camargo et al., 2007). Flavor and aroma of irradiated papayas were less strong than the control (Boylston et al., 2002). High doses of irradiation could produce flavor changes; orange juice exposed to high doses of irradiation (10 kGy) developed unacceptable flavor and browning (Thakur and Arya, 1993).

Informal tasting of irradiated papaya nectar (5kGy and 7.5 kGy) revealed an increase in sweetness compared to the control; however analysis of the sugar profile and total soluble solids did not reveal any significant changes (Parker et al., 2010). The higher sweetness perception in irradiated samples could be due to changes in flavor compounds that could be masking (or altering) sweetness perception (Bakker, 1995; Parker et al., 2010). A study of papaya flavor volatiles detected 85 volatile compounds using capillary gas chromatography analysis; the GC volatile patterns were not significantly different between irradiated (0.75 kGy) and non-irradiated papaya (Blakesley et al., 1979). The lack of difference could be due to the low doses of irradiation used.

Researchers reported increased glucose and decreased galacturonic acid in strawberry fruit after irradiation at 4 kGy (D'Amour et al., 1993). The increase in glucose could be attributed to cellulose hydrolysis; however the neutral sugars were not affected. According to the authors this could be an indicator of depolymerization of the polygalacturonic acid chains but not of the neutral sugar side chains of pectin

Papayas exposed to irradiation remain firmer longer than the non-irradiated; this retention in firmness could be associated with changes in cell wall pectin (Akamine and Wong, 1966; Akamine and Moy, 1983). Firmness of papaya fruit is an important factor to take in account for shelf life prediction, estimation of handling and transportation issues, and prediction of consumer acceptance.

Effect of irradiation on enzymatic activity

Each enzyme has different resistance to ionizing radiation. Catalase, for example, is 60 times more resistant than carboxypepetidase. When sulfhydryl compounds are present they tend to protect enzymes from ionizing radiation (Whitaker, 1994). The ionizing radiation needed to inactivate enzymes is higher than that needed to destroy microbial spores (D'Innocenzo and Lajolo, 2001). Irradiation at 0.5-1.0 kGy did not affect PE activity in papaya fruit (Zhao et al., 1996). On the contrary, another study measured activity patterns after 6 days of exposing papaya to irradiation doses of 0.5 kGy; polygalacturonase (PG), PE, and β-galactosidase activity was lower in irradiated samples than the non-irradiated (D'Innocenzo and Lajolo, 2001).

Furthermore, PE was active at irradiation doses up to 7.5 kGy, causing gel formation in papaya nectar (Parker et al., 2010). Irradiation was not effective for inactivating PE, even at doses up to 48 kGy (Wilkinson and Gould, 1996). To destroy microorganisms and inactivate

enzymes without using high doses of irradiation, heat treatment is typically combined with irradiation.

High Hydrostatic Pressure (HHP)

Impact on microorganisms

Bacterial inactivation by HHP depends on the species, strain of the microorganism, and storage conditions. Gram positive bacteria are more resistant to pressure changes than gram negative bacteria. Bacteria in the log phase of growth are more sensitive to high pressures than those in stationary or dormant phase. *Escherichia coli* cells in stationary phase are more resistant to high pressures than those in exponential phase. When *E. coli* cells in both phases were exposed to pressures of 200 MPa, the cells in the exponential phase were inactivated by 99.9% and the cells in stationary phase were only inactivated by 30% (Manas and Mackey, 2004). Moreover, *E. coli* present in cashew apple juice was exposed to pressure treatments of 400 MPa for 3 minutes at 25^{O}C, resulting in an 8 log reduction. The D values for *E. coli* ranged from 16.43 min at 250 MPa to 1.21 min at 400 MPa, the z value was 123 MPa (Lavinas et al., 2008).

Hoover et al. (1989) found that pressures of 350 MPa (30 min) or 400 MPa (5 min) caused a 10-fold reduction in vegetative cells of bacteria, yeasts or molds. Furthermore, to eliminate spores a combination of mild heat (60^{O}C) and pressures of 400 MPa at different times according to the bacterial resistance are needed (Seyderhelm and Knorr, 1992).

Researchers achieved 5-log reduction of *Clostridium Botulinum* type E spores of strains Alaska and Beluga when combining pressures of 827 MPa and mild heat (40-50^{O}C) for 5 minutes; pressures of 827 MPa alone were not enough to inactivate the *Clostridium botulinum* type E spores (Reddy et al., 1999). Spores of *Bacillus anthracis*, which are notoriously resistant

to heat treatments, irradiation, desiccation, and disinfection, have been inactivated with a combination of heat treatment (70^{O}C) and pressure of 500 MPa for 4 min (Clery-Barraud et al., 2004).

Bacyssochlamys nivea ascospores are resistant to heat and to pH variation; normally ascospores survive commercial pasteurization of fruit juices, causing spoilage, which can visually be observed due to inflation of the package (Ferreira et al., 2009). Application of pressure cycling was more effective than constant high pressure for inactivation of *Byssochlamys nivea* ascospores in pineapple juice. Pressure of 600 MPa and 80^{O}C in three 5-minute cycles or five 3-minute cycles inactivated 10^5-10^6 CFU/mL of ascospores.

High hydrostatic pressure (HHP) can inactivate microorganisms and enzymes (Hendrickx et al., 1998). However the mechanism of degradation, denaturation, activation, and deactivation of the different components such as enzymes, microorganisms, and nutrients could be affected not only by HHP but also by other factors, including pH and temperature.

HHP effect on enzymatic activity

It is unclear as to how HHP affects enzymatic activity; however, it is known that high pressure is capable of destroying intracellular vacuoles, and damaging cell walls and cytoplasmic membranes (Cano et al., 1997). Enzymes demonstrated different sensitivity to pressure, some are inactivated at low pressures (100 MPa) and others can tolerate high pressure up 1200 MPa without being affected. Others are activated by high pressure (refs). Other factors such as pH, substrate composition, and temperature could have an effect on the activation or inactivation of enzymes under high pressures (Hendrickx et al., 1998).

There are important food enzymes that have responded differently upon exposure to high pressure, such as polyphenol oxidase (PPO), which is thought to be one of the most important enzymes causing deterioration in fruits during postharvest handling and storage (Martinez and Whitaker, 1995). PPO catalyzes browning in fruits and vegetables, causing color deterioration and slight changes in flavor and softening of the tissue. HHP can inactivate or activate PPO, the grade of activation and inactivation depends on the pressure, enzyme origin, duration of pressure processing, pH of the medium, and additives present (Anese et al., 1994). Pressures up to 400 MPa caused activation of PPO in raspberry (Garcia-Palazon et al., 2004).

Pectin could be responsible for gel formation in some fruit juices under certain conditions. Pectin methyl esterase (PE), catalyzes deesterification of pectin, allowing potential bonding interactions that would change the texture of fruit juices (Giovane et al., 1990). Pressures up to 650 MPa did not inactivate PE in apple juice, PE showed great stability to high pressures. After exposure to pressures of 750 MPa for 90 minutes at 50^{O}C, there was an 80% reduction of PE activity (Valdramidis et al., 2009). Other important enzymes, such as lipase, exposed to pressures up to 350 MPa, had increased activity; however, pressures higher than 350 MPa, maintained or decreased lipase activity (Eisenmenger and Reyes-De-Courcuera, 2010).

In vitro digestion

Recently people have become more health conscious; thus, consumption of high dietary fiber products has become more popular due to their attributed impact on digestive health. To evaluate the impact of dietary fiber on the human digestive tract, it is necessary to study food digestion and fermentation; *in vivo* studies are expensive and time-limiting. On the other hand, animal studies are difficult to extrapolate due to differences in the hindgut function among

different mono-gastric species (Moughan, 1999). This makes the *in vitro* analysis an alternative to simulate an *in vivo* situation. However, it is not easy to simulate the gastrointestinal tract function, particularly the large intestine due to its microbial environment. Bacteria produce enzymes that digest the substrate that enters in the large intestine; this is an anaerobic process. The product of this fermentation is used by the bacteria for their maintenance and growth (Ewing and Cole, 1994).

The most commonly applied techniques for *in vitro* digestion/fermentation methods are continuous, semi-continuous, and batch. They either remove the product of fermentation constantly, alternating, or not at all (Barry et al., 1995; Karppinen, 2003). Of all the above mentioned methods the batch method is the easiest and the most common method.

The amount of substrate fermented is frequently measured by monitoring either metabolite production or substrate disappearance (Coles et al., 2005). The most important indicator of fermentability is short chain fatty acid (SCFA) production, which fits in the category of metabolite production. Another widely used indicator of fermentation is organic matter (OM) and dry matter (DM) disappearance, which belongs to the category that measures substrate disappearance such as non-starch polysaccharide (NSP), resistant starch (RS), protein and other fermentable substrates.

Fiber impact on digestion and health

The definition of dietary fiber has been debated for many years; there are more than 20 definitions. The term "dietary fiber" was used to refer to the non-digestible part of the plant cell wall, such as hemicellulose, cellulose, and lignin (Hipsley, 1953). Finally in 1995 the dietary fiber definition rose to a consensus. Dietary fiber was defined as "remnants of edible plant cells,

polysaccharides, lignin, and associated substances resistant to hydrolysis and digestion by the alimentary enzymes of humans" (AOAC, 1995). Lee and Prosky (1995) proposed to enlarge the definition of dietary fiber, including also resistant oligosaccharides in addition to non-starch polysaccharides, resistant starch, and lignin.

The American Association of Cereals Chemist (AACC) in 2000 defined dietary fiber as "the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine, that promotes physiological effects including laxation and (or) blood cholesterol attenuation, and (or) blood glucose attenuation." This definition included polysaccharides, oligosaccharides, lignin, and associated plant substances.

The Institute of Medicine (IOM) (2002) agreed with the definition proposed by Lee and Prosky (1995) and defined total dietary fiber as the "sum of functional and dietary fiber", where "functional" fibers are isolated, non-digestible carbohydrates that have beneficial physiological effects on humans, and dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants".

Dietary fiber is comprised of insoluble (IDF) and soluble dietary fibers (SDF), differentiated by their solubility in water. Non-digested food components that resist hydrolysis and digestion in the stomach and small intestine, eventually enter the colon for fermentation. Insoluble fiber is partially fermented by the microflora in the colon; it plays an important role in fecal bulking and decreasing fecal transit time through the bowel. Soluble fibers are entirely fermented by colonic microflora, they do not impact bulk matter of feces. Different factors influence resistance to digestion of starches in the small intestine, including: the granular

structure of the starch, the amylose to amylopectin ratio, and the starch source (Cummings, 1991).

Several potential health benefits of consumption of dietary fiber have been demonstrated. Some have been submitted and approved by the U.S. Food and Drug Administration (FDA) as health claims those that have been approved include those associated with risk reduction of coronary heart disease (CHD) and cancer. However, these claims are approved for foods and do not apply to dietary supplements. Other potential claims associated with high consumption of dietary fiber are related to satiety and weight control, because foods with high fiber content, specifically whole grains, "help you to feel full with less calories" (USDA, 2000 and 2005).

The IOM (2002) reported that functional and dietary fiber augment fecal weight and the number of fecal movements per day; they also facilitate stool transit. Other studies suggested that consumption of certain fibers could delay glucose uptake and soothe insulin responses (Lindstrom et al., 2006). Another strong health claim is the one that suggested negative correlation between fiber intake and risk reduction of type 2 diabetes (IOM, 2002).

He et al. (2007) demonstrated that increasing intake of fruit and vegetables from less than 3servings/day to more than 5servings/day is associated with a 17% reduction in CHD risk. However, they did not study the individual impact of each source of fiber, fruits or vegetables, on reduction of CHD risk. Moreover, a pooled analysis from 10 cohort studies compared three sources of fiber: cereals, fruits, and vegetables (Pereira et al., 2004). Incidence of CHD decreased 10 % and 16 % for each 10g/d increase of dietary cereal and fruit fiber, respectively. Strong inverse association was also found in reduction of CHD deaths, with risk reduction of 25% and 30 % for each 10g/d increase of dietary cereal and fruit fiber, respectively. The results of this study suggested that fruits are a better source of dietary fiber than cereals.

In addition to the effect on CHD, fruit consumption may have other health benefits, including: improving bowel function, increasing satiety, and reducing the risk of stroke and some cancers (Kelsay, et al., 1979; World Cancer Research Fundation, 1997; Rolls et al., 2004; He et al., 2006). Papaya fruit is a good source of total dietary fiber (35 % dry matter); from which soluble dietary fiber SDF and insoluble dietary fiber IDF constitute about 50 % each of the total dietary fiber TDF (Chang et al., 1998; Ramulu and Rao, 2003). SDF are completely fermented and IDF are either partially or not fermented by bacteria in the colon. Among the different products of fermentation, the most important are the short chain fatty acids SCFA.

Short Chain Fatty Acids (SCFA)

Short chain fatty acids (SCFA) are organic fatty acids with 1 to 6 carbon atoms and the principal anions occur from bacterial fermentation of polysaccharide, oligosaccharide, protein, peptide, and glycoprotein precursors in the colon (Miller and Wolin, 1979; Cummings, 1984; Cummings and Macfarlane, 1991).

The main SCFA absorbed by different regions of the colon are acetate, butyrate, and propionate (Ruppin et al., 1980; Cummings et al., 1995). Acetate is the most important SCFA in the colon and in the blood; it is directly absorbed in the colon and transported to the liver (Cook and Sellin, 1998). Propionate is better absorbed in the human colon than acetate (Saunders, 1991). In the case of propionate many assumptions are made based on animal studies, there is much inconsistency in human studies. Studies have suggested that high production of propionate by bacteria could inhibit hepatic cholesterol synthesis (Chen et al., 1984; Hara et al., 1998). Butyrate is important in regulation of cell proliferation and differentiation and is the main source

of energy of colonic epithelial cells. It is the most important SCFA for colonocyte metabolism (Topping and Clifton, 2001; Roberfroid, 2005).

Short chain fatty acids are synthesized by colonic micro flora mostly from undigested carbohydrates; a small amount also originates from non-absorbed protein. The type of SCFA produced is influenced by individual substrate specificity, affinity of different gut species and their ability to compete for these substrates, and also host factors such as transit time, mucus secretion, and drugs can influence the type of SCFA produced (Macfarlane et al., 1992). In addition, researchers have found that bacterial numbers, fermentation, and proliferation are highest in the proximal colon where substrate availability is larger (Macfarlane and Gibson, 1995).

Carbohydrates are fermented primarily in the proximal colon by saccarolytic bacteria; quantitatively carbohydrates occupy the most important role in the production of SCFA (Macfarlane and Macfarlane, 2003). The major carbohydrate source is the resistant starch. The products of carbohydrate fermentation are mainly linear SCFA, CO_2, and H_2. Furthermore, proteolytic bacteria in the colon ferment proteins and amino acids yielding branched SCFA, H_2, CO_2, CH_4, phenols, and amines (Macfarlane and Macfarlane, 2003; Roberfroid, 2005).

Several population surveys concluded that SCFA production is in the order of acetate>propionate>butyrate, in a molar ratio of approximately 60:20:20, respectively (Cummings et al., 1979; Topping and Clifton, 2001). SCFA are metabolized in 3 main sites of the body: 1) cells of the ceco-colonic epithelium, which use butyrate as a main source of energy, 2) liver cells, which metabolize residual butyrate and propionate used for gluconeogenesis, also acetate is use by the liver, 3) muscle cells which use residual acetate to generate energy (Roberfroid, 2005).

Recently, colonic health has been associated with maintenance of overall health and reducing the risk of various diseases such as inflammatory bowel disease, irritable bowel disease, cardiovascular disease, and cancer (Floch and Curtiss, 2002).There is increasing evidence that SCFA are important in colon health and they may also be important in the prevention and management of certain diseases.

REFERENCES

Adeneye, A.; Olagunju, J. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of Carica papaya Linn. in Wistar rats. *Biology and Medicine.* **2009**, 1-10.

Akamine, E. K.; Moy, J. H. Delay in postharvest ripening and senescence of fruits. *Preservation of food by ionizing radiation.* **1983**, *3*, 129–158.

Akamine, E. K.; Wong, R. T. F. *Extending the shelf life of papayas with gamma irradiation.* **1966**, 35-40.

Akah, P. A.; Oli, A. N.; Enwerem, N. M.; Gamaniel, K. Preliminary studies on purgative effect of Carica papaya root extract. *Fitoterapia.* **1997**, *68*, 327-331.

Anese, M.; Nicoli, M. C.; Dall'Aglio, G.; Lerici, C. R. Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *J. Food Biochem.* **1994**, *18*, 285-293.

Argaiz, A.; Lopez-Malo, A. Kinetics of First Change on Flavour, Cooked Flavour Development and Pectinesterase Inactivation. *Fruit processing.* **1996**, *6*, 145-150.

Aruoma, O. I.; Colognato, R.; Fontana, I.; Gartlon, J.; Migliore, L.; Koike, K.; Coecke, S.; Lamy, E.; Mersch-Sundermann, V.; Laurenza, I. Molecular effects of fermented papaya preparation on oxidative damage, MAP Kinase activation and modulation of the benzo [a] pyrene mediated genotoxicity. *Biofactors.* **2006**, *26*, 147-159.

Athmaselvi, K. A. In *In Effect of Ultrasound Treatments in Papaya Juice Processing;* II International Symposium on Papaya. **2008**, pp 621-624.

Azarkan, M.; Wintjens, R.; Looze, Y.; Baeyens-Volant, D. Detection of three wound-induced proteins in papaya latex. *Phytochemistry.* **2004**, *65*, 525-534.

Badillo, V. M. Nota correctiva Vasconcellea St. Hill. y no Vasconcella (Caricaceae). *Ernstia.* **2001**, *11*, 75-76.

Bakker, J. Flavor interactions with the food matrix and their effects on perception. *Food Science and Technology.* New York, Marcel Dekker. **1995**, 411-411.

Bari, L.; Hassan, P.; Absar, N.; Haque, M. E.; Khuda, M.; Pervin, M. M.; Khatun, S.; Hossain, M. I. Nutritional analysis of two local varieties of papaya (Carica papaya L.) at different maturation stages. *Pak J Biol Sci.* **2006**, *9*, 137-140.

Barry, J. L.; Hoebler, C.; Macfarlane, G. T.; Macfarlane, S.; Mathers, J. C.; Reed, K. A.; Mortensen, P. B.; Nordgaard, I.; Rowland, I. R.; Rumney, C. J. Estimation of the fermentability of dietary fibre *in vitro*: a European interlaboratory study. *Br. J. Nutr.* **1995**, *74*, 303-322.

Beyers, M.; Thomas, A. C.; Van Tonder, A. J. Gamma Irradiation of subtropical fruits. 1. Compostional tables of mango, papaya, strawberry, and litchi fruits at the edible-ripe stage. *J. Agric. Food Chem.* **1979**, *27*, 37-42.

Blakesley, C. N.; Loots, J. G.; Du Plessis, L.; De Bruyn, G. Gamma irradiation of subtropical fruits. 2. Volatile components, lipids and amino acids of mango, papaya, and strawberry pulp. *J. Agric. Food Chem.* **1979**, *27*, 42-48.

Brekke, J. E.; Chan, H. T.; Cavaletto, C. G. In *Papaya puree and nectar;* Hawaii Agricultural Experiment Station, University of Hawaii. **1973**, 1-8.

Boylston, T. D.; Reitmeier, C. A.; Moy, J. H.; Mosher, G. A.; Taladriz, L. Sensory Quality and Nutrient Composition of Three Hawaiian Fruits Treated by S-irradiation. *J. Food Qual.* **2002**, *25*, 419-433.

Buttle, D. J.; Kembhavi, A. A.; Sharp, S. L.; Shute, R. E.; Rich, D. H.; Barrett, A. J. Affinity purification of the novel cysteine proteinase papaya proteinase IV, and papain from papaya latex. *Biochem. J.* **1989**, *261*, 469.

Camargo, R. J.; Tadini, C. C.; Sabato, S. F. Physical-chemical analyses of irradiated papayas (Carica papaya L.). *Radiat. Phys. Chem.* **2007**, *76*, 1866-1868.

Cano, M. P.; Ancos, B.; Lobo, G. Peroxidase and polyphenoloxidase activities in papaya during postharvest ripening and after freezing/thawing. *J. Food Sci.* **1995**, *60*, 815-817.

Cano, M. P.; Hernandez, A.; Ancos, B. High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *J. Food Sci.* **1997**, *62*, 85-88.

Canini, A.; Alesiani, D.; D'Arcangelo, G.; Tagliatesta, P. Gas chromatography-mass spectrometry analysis of phenolic compounds from Carica papaya L. leaf. *Journal of Food Composition and Analysis.* **2007**, *20*, 584-590.

Chang, S. C.; Lee, M. S.; Lin, C. J.; Chen, M. L. Dietary fiber content and composition of fruits in Taiwan. *Asia Pac. J. Clin. Nutr.* **1998**, *7*, 206-210.

Chan, H. T.; Hibbard, K. L.; Goo, T.; Akamine, E. K. Sugar composition of papayas during fruit development. *HortScience.* **1979**, *14*, 140-141.

Chan Jr, H. T.; Flath, R. A.; Forrey, R. R.; Cavaletto, C. G.; Nakayama, T.; Brekke, J. E. Development of off-odors and off-flavors in papaya puree. *J. Agric. Food Chem.* **1973**, *21*, 566-570.

Chay-Prove, P.; Ross, P.; O'Hare, P.; Macleod, N.; Kernot, I.; Evans, D.; Grice, K.; Vawdrey, L.; Richards, N.; Blair, A. Agrilink Series: Your Growing Guide to Better Farming. *Papaw Information Kit. Queensland Horticulture Institute and Department of Primary Industries, Qld, Nambour*. **2000**.

Chen, W. J.; Anderson, J. W.; Jennings, D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *Proc. Soc. Exp. Biol. Med.* **1984**, *175*, 215-218.

Cherian, T. Effect of papaya latex extract on gravid and non-gravid rat uterine preparations in vitro. *J. Ethnopharmacol.* **2000**, *70*, 205-212.

Chinoy, N. J.; D'Souza, J. M.; Padman, P. Effects of crude aqueous extract of Carica papaya seeds in male albino mice. *Reproductive Toxicology.* **1994**, *8*, 75-79.

Clery-Barraud, C.; Gaubert, A.; Masson, P.; Vidal, D. Combined effects of high hydrostatic pressure and temperature for inactivation of Bacillus anthracis spores. *Appl. Environ. Microbiol.* **2004**, *70*, 635.

Coles, L.; Moughan, P.; Darragh, A. In vitro digestion and fermentation methods, including gas production techniques, as applied to nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. *Anim. Feed Sci. Technol.* **2005**, *123*, 421-444.

Cook, S. I.; Sellin, J. H. Review article: short chain fatty acids in health and disease. *Aliment. Pharmacol. Ther.* **1998**, *12*, 499-507.

Cummings, J. H.; Hill, M. J.; Bone, E. S.; Branch, W. J.; Jenkins, D. J. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. *Am. J. Clin. Nutr.* **1979**, *32*, 2094.

Cummings J. H. Microbial digestion of complex carbohydrates in man. Proc. Nutr. Soc. **1984**, 43, 35-44.

Cummings, J.; Macfarlane, G. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Microbiol.* **1991**, *70*, 443-459.

Cummings, J. H.; Rombeau, J. L.; Sakata, T. In Physiological and clinical aspects of short-chain fatty acids. *Cambridge University Press*. **1995**.

D'Amour, J.; Gosselin, C.; Arul, J.; Castaigne, F.; Willemot, C. Gamma-Radiation Affects Cell Wall Composition of Strawberries. *J. Food Sci.* **1993**, *58*, 182-185.

D'Innocenzo, M.; Lajolo, F. Effect of gamma irradiation on softening changes and enzyme activities during ripening of papaya fruit. *J. Food Biochem.* **2001**, *25*, 425-438.

Drew, R. A.; Magdalita, P. M.; O'Brien, C. M. *In Development of Carica interspecific hybrids;* International Symposium on Biotechnology of Tropical and Subtropical Species Part 2. 461, **1997**, pp 285-292.

Eisenmenger, M. J.; Reyes-De-Corcuera, J. I. Kinetics of lipase catalyzed isoamyl acetate synthesis at high hydrostatic pressure in hexane. *Biotechnol. Lett.* **2010**, 1-5.

Elias, P. S.; Cohen, A. J. In *Radiation chemistry of major food components.* Elsevier. **1977**.

El Moussaoui, A.; Nijs, M.; Paul, C.; Wintjens, R.; Vincentelli, J.; Azarkan, M.; Looze, Y. Revisiting the enzymes stored in the laticifers of Carica papaya in the context of their possible participation in the plant defense mechanism. *Cellular and Molecular Life Sciences.* **2001**, *58*, 556-570.

Ewing, W. N.; Cole, D. J. A. In *The living gut: an introduction to micro-organisms in nutrition. Context Publications*. **1994** .

Ezike, A.; Akah, P.; Okoli, C.; Ezeuchenne, N.; Ezeugwu, S. Carica papaya (paw-paw) unripe fruit may be beneficial in ulcer. *Journal of Medicinal Food.* **2009**, *12*, 1268-1273.

Farkas, J. Combination of irradiation with mild heat treatment. *Food control.* **1990**, 223-229.

Ferreira, E. H. R.; Rosenthal, A.; Calado, V.; Saraiva, J.; Mendo, S. Byssochlamys nivea inactivation in pineapple juice and nectar using high pressure cycles. *J. Food Eng.* **2009**, *95*, 664-669.

Flath, R. A.; Forrey, R. R. Volatile components of papaya (Carica papaya L., Solo variety). *J. Agric. Food Chem.* **1977**, *25*, 103-109.

Floch, M. H.; Curtiss, J. A. H. Probiotics and functional foods in gastrointestinal disorders. *Curr. Gastroenterol. Rep.* **2001**, *3*, 343-350.

Garcia-Palazon, A.; Suthanthangjai, W.; Kajda, P.; Zabetakis, I. The effects of high hydrostatic pressure on [beta]-glucosidase, peroxidase and polyphenoloxidase in red raspberry (Rubus idaeus) and strawberry (Fragaria× ananassa). *Food Chem.* **2004**, *88*, 7-10.

Gebhardt, S.E.; Thomas, R.G. Nutritive value of foods. US Department of Agriculture, Agricultural Research Service, *Home and Garden Bulletin*. **2002**, 72.

Gomes, M. R. A.; Sumner, I. G.; Ledward, D. A. Effects of high pressure on papain activity and structure. *J. Sci. Food Agric.* **1997**, *75*, 67-72.

Gonsalves, D. Transgenic papaya in Hawaii and beyond. **2004**, 1-5.

Gonsalves, D. Control of papaya ringspot virus in papaya: a case study. *Annu. Rev. Phytopathol.* **1998**, *36*, 415-437.

Giovane, A.; Quagliuolo, L.; Castaldo, D.; Servillo, L.; Balestrieri, C. Pectin methyl esterase from Actinidia chinensis fruits. *Phytochemistry.* **1990**, *29*, 2821-2823.

Hauschild, A. H. W. Clostridium botulinum. In M. P. Doyle (Ed.), Foodborne bacterial pathogens. New York, NY: Marcel Dekker Inc. **1989**, 111-189.

Hara, H.; Haga, S.; Kasai, T.; Kiriyama, S. Fermentation products of sugar-beet fiber by cecal bacteria lower plasma cholesterol concentration in rats. *J. Nutr.* **1998**, *128*, 688.

He, F. J.; Nowson, C. A.; MacGregor, G. A. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *The Lancet.* **2006**, *367*, 320-326.

He, F.; Nowson, C.; Lucas, M.; MacGregor, G. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *J. Hum. Hypertens.* **2007**, *21*, 717-728.

Hendrickx, M.; Ludikhuyze, L.; Van den Broeck, I.; Weemaes, C. Effects of high pressure on enzymes related to food quality. *Trends Food Sci. Technol.* **1998**, *9*, 197-203.

Hernandez-Arana, A.; Soriano-Garcia, M. Detection and characterization by circular dichroism of a stable intermediate state formed in the thermal unfolding of papain. *Biochimica Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology.* **1988**, *954*, 170-175.

Hewitt, H. H.; Whittle, S.; Lopez, S.A.; Bailey, E.Y.; Weaver, S.R. Topical uses of papaya in chronic skin ulcer therapy in Jamaica. *West Indian Medical Journal.* **2000**, 32-33.

Hipsley, E. H. Dietary "fibre" and pregnancy toxaemia. Brit. Med. J. **1953**, *2*, 420.

Hite, B. H. In: The effect of pressure in the preservation of milk: a preliminary report*; West Virginia Agricultural Experiment Station*. **1899**, 152.

Hoover, D. G.; Metrick, C.; Papineau, A. M.; Farkas, D. F.; Knorr, D. Biological effects of high hydrostatic pressure on food microorganisms. *Food technology.* **1989**.

Hoover, S. R.; Kokes, E.L.C. Effect of pH upon proteolysis by papain. *J. Biological Chemistry*. **1946**, 199-207.

Iwu, M. M. In *Handbook of African medicinal plants;* CRC. **1993**, 1-10.

Jacquet, A.; Kleinschmidt, T.; Schenk, A. G.; Looze, Y.; Braunitzer, G. The thiol proteinases from the latex of Carica papaya L. III. The primary structure of chymopapain. *Biol. Chem. Hoppe-Seyler.* **1989**, *370*, 425-434.

Jago, D. New food products from around the world. *Food & Beverage International.* **2006**, *5*.

Karppinen, S. Dietary fibre components of rye bran and their fermentation in vitro. **2003**.

Kaul, P.; Sathish, H.; Prakash, V. Effect of metal ions on structure and activity of papain from Carica papaya. *Food/Nahrung.* **2002**, *46*, 2-6.

Kelsay, J. L.; Behall, K. M.; Prather, E. S. Effect of fiber from fruits and vegetables on metabolic responses of human subjects, II. Calcium, magnesium, iron, and silicon balances. *Am. J. Clin. Nutr.* **1979**, *32*, 1876.

Kilcast, D. Effect of irradiation on vitamins. *Food Chem.* **1994**, *49*, 157-164.

Knight, R. J. Origin and world importance of tropical and subtropical fruit crops. In: Nazy S, Shaun SE (Eds) *Tropical and Subtropical Fruits: Composition, Properties and Uses*. AVI Publishing, Connecticut, USA. **1980**, 1-120.

Knorr, D. Advantages, opportunities and challenges of high hydrostatic pressure application to food systems. *Progress in Biotechnology.* **1996**, *13*, 279-287.

Macfarlane, G. T.; Gibson, G. R.; Cummings, J. H. Comparison of fermentation reactions in different regions of the human colon. *J. Appl. Microbiol.* **1992**, *72*, 57-64.

Macfarlane, G. T.; Gibson, G. R. Microbiological aspects of the production of short-chain fatty acids in the large bowel. *Physiological and Clinical Aspects of Short-Chain Fatty Acids.* **1995**, *1995*, 87-105.

Macfarlane, S.; Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **2003**, *62*, 67-72.

MacLeod, A. J.; Pieris, N. M. Volatile components of papaya (Carica papaya L.) with particular reference to glucosinolate products. *J. Agric. Food Chem.* **1983**, *31*, 1005-1008.

Madrigal, L. S.; Ortiz, A. N.; Cooke, R. D.; Fernandez, R. H. The dependence of crude papain yields on different collection (‘tapping’) procedures for papaya latex. *J. Sci. Food Agric.* **1980**, *31*, 279-285.

Manas, P.; Mackey, B. M. Morphological and physiological changes induced by high hydrostatic pressure in exponential-and stationary-phase cells of Escherichia coli: relationship with cell death. *Appl. Environ. Microbiol.* **2004**, *70*, 1545.

Martinez, M.; Whitaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195-200.

Mahattanatawee, K.; Manthey, J. A.; Luzio, G.; Talcott, S. T.; Goodner, K.; Baldwin, E. A. Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *J. Agric. Food Chem.* **2006**, *54*, 7355-7363.

Mclaughlin, W. L. In *In Gamma-ray sources vs. X-ray or electron beams for quarantine treatment;* Proc. Workshop The Use of Irradiation as Quarantine Treatment of Food and Agricultural Commodities. Honolulu, HI, USA. **1999**, 78-89.

Miller, T. L.; Wolin, M. J. Fermentations by saccharolytic intestinal bacteria. *Am. J. Clin. Nutr.* **1979**, *32*, 164.

Morton, J. F.; Dowling, C. F. Fruits of warm climates. **1987**, 1-18.

Moughan, P. J. In vitro techniques for the assessment of the nutritive value of feed grains for pigs: a review. *Aust. J. Agric. Res.* **1999**, *50*, 871-880.

Murcia, M. A.; Jimenez, A. M.; Martinez-Tome, M. Evaluation of the antioxidant properties of Mediterranean and tropical fruits compared with common food additives. *Journal of Food Protection.* **2001**, *64*, 2037-2046.

Nakasone, H. Y.; Paull, R. E. In *Tropical fruits.* CAB International. **1998.**

Lavinas, F. C.; Miguel, M. A. L.; Lopes, M. L. M.; Valente Mesquita, V. L. Effect of high hydrostatic pressure on cashew apple (*Anacardium occidentale L.*) juice preservation. *J. Food Sci.* **2008**, *73*, M273-M277.

Leskova, E.; Kubíková, J.; KOváciková, E.; Kosicka, M.; Porubská, J.; Holcíková, K. Vitamin losses: retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis.* **2006**, *19*, 252-276.

Liebman, B. Fresh fruit: A papaya a day? Nutrition Action Healthletter, **1992**, 1–11.

Lim, Y. Y.; Lim, T. T.; Tee, J. J. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* **2007**, *103*, 1003-1008.

Lindström, J.; Peltonen, M.; Eriksson, J. G.; Louheranta, A.; Fogelholm, M.; Uusitupa, M.; Tuomilehto, J. High-fibre, low-fat diet predicts long-term weight loss and decreased type 2 diabetes risk: the Finnish Diabetes Prevention Study. *Diabetologia.* **2006**, *49*, 912-920.

Litz, R. E.; O'Hair, S. K.; Conover, R. A. In vitro growth of Carica papaya L. cotyledons. *Scientia Horticulturae.* **1983**, *19*, 287-293.

Lohiya, N. K.; Mishra, P. K.; Pathak, N.; Manivannan, B.; Bhande, S. S.; Panneerdoss, S.; Sriram, S. Efficacy trial on the purified compounds of the seeds of Carica papaya for male contraception in albino rat. *Reproductive Toxicology.* **2005**, *20*, 135-148.

Luximon-Ramma, A.; Bahorun, T.; Crozier, A. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *J. Sci. Food Agric.* **2003**, *83*, 496-502.

Okeniyi, J. A. O.; Ogunlesi, T. A.; Oyelami, O. A.; Adeyemi, L. A. Effectiveness of dried Carica papaya seeds against human intestinal parasitosis: a pilot study. *Journal of Medicinal Food.* **2007**, *10*, 194-196.

Otsuki, N.; Dang, N. H.; Kumagai, E.; Kondo, A.; Iwata, S.; Morimoto, C. Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects. *J. Ethnopharmacol.* **2010**, *127*, 760-767.

Parker, T. L.; Esgro, S. T.; Miller, S. A.; Myers, L. E.; Meister, R. A.; Toshkov, S. A.; Engeseth, N. J. Development of an optimised papaya pulp nectar using a combination of irradiation and mild heat. *Food Chem.* **2010**, 118, 861-869.

Paull, R. E.; Nishijima, W.; Reyes, M.; Cavaletto, C. Postharvest handling and losses during marketing of papaya (Carica papaya L.). *Postharvest Biol. Technol.* **1997**, 11, 165-179.

Penteado, A. L.; Leitao, M. F. F. Growth of Salmonella Enteritidis in melon, watermelon and papaya pulp stored at different times and temperatures. *Food control.* **2004**, 15, 369-373.

Pereira, M. A.; O'Reilly, E.; Augustsson, K.; Fraser, G. E.; Goldbourt, U.; Heitmann, B. L.; Hallmans, G.; Knekt, P.; Liu, S.; Pietinen, P. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch. Intern. Med.* **2004**, 164, 370.

Pesante, A. Fresh Papayas. Market Outlook Report. (accessed May/15, 2009).

Ramulu, P.; Udayasekhara Rao, P. Total, insoluble and soluble dietary fiber contents of Indian fruits. *Journal of food composition and analysis.* **2003**, 16, 677-685.

Reddy, N. R.; Solomon, H. M.; Fingerhut, G. A.; Rhodehamel, E. J.; Balasubramaniam, V. M.; Palaniappan, S. Inactivation of Clostridium botulinum type E spores by high pressure processing. *J. Food Saf.* **1999**, 19, 277-288.

Reed, C. F. Information summaries on 1000 economic plants. *Typescripts submitted to the USDA.* **1976**, 102–103.

Roberts, M.; Minott, D. A.; Tennant, P. F.; Jackson, J. C. Assessment of compositional changes during ripening of transgenic papaya modified for protection against papaya ringspot virus. *J. Sci. Food Agric.* **2008**, 88, 1911-1920.

Roberfroid, M. B. Introducing inulin-type fructans. *Br. J. Nutr.* **2005**, 93, S13-S25.

Rolls, B. J.; Ello-Martin, J. A.; Tohill, B. C. What can intervention studies tell us about the relationship between fruit and vegetable consumption and weight management? *Nutr. Rev.* **2004**, 62, 1-17.

Runnie, I.; Salleh, M. N.; Mohamed, S.; Head, R. J.; Abeywardena, M. Y. Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. *J. Ethnopharmacol.* **2004**, 92, 311-316.

Ruppin, H.; Bar-Meir, S.; Soergel, K. H.; Wood, C. M.; Schmitt Jr, M. G. Absorption of short-chain fatty acids by the colon. *Gastroenterology.* **1980**, 78, 1500-1507.

Samson, J. A. Mango, avocado and papaya. *Tropical Fruits.* **1986**, 185-221.

Sankat, C.K.; Maharaj R. Papaya, in Postharvest Physiology and Storage of Tropical and Subtropical Fruits, ed. By Mita S. CAB International, Wallingford. **1997**, 167–189.

Salunkhe, D. K.; Desai, B. B. Postharvest biotechnology of fruits. *Boca Raton,* Florida.

Saunders, D. R. Absorption of short-chain fatty acids in human stomach and rectum. *Nutr. Res.* **1991**, 11, 841-847.

Schatzkin, A.; Mouw, T.; Park, Y.; Subar, A. F.; Kipnis, V.; Hollenbeck, A.; Leitzmann, M. F.; Thompson, F. E. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. *Am. J. Clin. Nutr.* **2007**, 85, 1353.

Scheldeman, X. Distribution and potential of cherimoya (Annona cherimola Mill.) and highland papayas (Vasconcellea spp.) in Ecuador. **2002**.

Schulze, M. B.; Liu, S.; Rimm, E. B.; Manson, J. A. E.; Willett, W. C.; Hu, F. B. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *Am. J. Clin. Nutr.* **2004**, 80, 348.

Seelig, R. A. Papaya Fruit and vegetable facts and pointers. *United Fresh Fruit and Vegetable Association,* Washington, DC. **1970**, 7.

Seyderhelm, I.; Knorr, D. Reduction of Bacillus stearothermophilus spores by combined high pressure and temperature treatments. *ZFL.Internationale Zeitschrift fuer Lebensmitteltechnologie und Verfahrenstechnik (Germany, FR).* **1992**.

Simic, M. G. In: Josephson ES, Peterson MS (eds) Preservation of food by ionizing radiation, vol 2. **1983**.

Stice, K. United States Market Analysis- Fiji and Pacific Island Papaya. (accessed January/23, 2010).

Storey, W. B. Papaya. *Outlines of perennial crop breeding in the tropics. The Netherlands: Wageningen.* **1969**, 389–408.

Sumner, I. G.; Harris, G. W.; Taylor, M. A. J.; Pickersgill, R. W.; Owen, A. J.; Goodenough, P. W. Factors effecting the thermostability of cysteine proteinases from Carica papaya. *European Journal of Biochemistry.* **1993**, 214, 129-134.

Thakur, B. R. A. M.; Arya, S. S. Effect of sorbic acid on irradiation-induced sensory and chemical changes in sweetened orange juice and mango pulp. *Int. J. Food Sci. Tech.* **1993**, 28, 371-376.

Tang, C. S. Benzyl isothiocyanate of papaya fruit. *Phytochemistry.* **1971**, 10, 117-121.

Topping, D. L.; Clifton, P. M. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **2001**, 81, 1031.

Umoh, I. B. Chemical composition of very ripe, just ripe and unripe pawpaw fruits carica papaya. University of Calabar, Nigeria. *Unpublished results.* **1995**.

Urbain, W. M. Food irradiation. Food Science and Technology. **1986**, 170-216.

USDA. National Nutrient Data Base for Standard Reference. http://www.nal.usda.gov/fnic/-foodcomp/search (accessed August 28, 2010).

Valdramidis, V.; Graham, W.; Beattie, A.; Linton, M.; McKay, A.; Fearon, A.; Patterson, M. Defining the stability interfaces of apple juice: Implications on the optimisation and design of High Hydrostatic Pressure treatment. *Innovative Food Science & Emerging Technologies.* **2009**, 10, 396-404.

Vinci, G.; Botre, F.; Mele, G.; Ruggieri, G. Ascorbic acid in exotic fruits: a liquid chromatographic investigation. *Food Chem.* **1995**, 53, 211-214.

Wall, M. M. Ascorbic acid, vitamin A, and mineral composition of banana (Musa sp.) and papaya (Carica papaya) cultivars grown in Hawaii. *Journal of Food Composition and Analysis.* **2006**, 19, 434-445.

Whitaker, J. R. Principles of enzymology for the food sciences. **1994**, 120-123.

Whitaker, J. R. Enzymes. In: Food Chemistry by Fenema**. 1996**, 489-492.

WHO. Safety and nutritional adequacy of irradiated food. World Health Organization, Geneva. 1994. http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=4-&codcol=15-&codcch=410 (accessed October 25, 2010).

Wilkinson, V. M.; Gould, G. In *Food Irradiation: a reference guide.* Woodhead Pub Ltd. **1998**, 224-226.

Wong, D.W.S. Proteolytic Enzymes. In: Food Enzymes Structure and Mechanism. **1995**, 124.

World Cancer Research Fundation Panel. Food, Nutrition and the Prevention of Cancer: A Global Perspective. American Institute for Cancer Research: Washington, DC. **1997**, 1-9.

Zhao, M.; Moy, J.; Paull, R. E. Effect of gamma-irradiation on ripening papaya pectin. *Postharvest Biol. Technol.* **1996**, 8, 209-222.

CHAPTER 3

Determination of proteolytic enzyme activity in papaya fruit and processed nectar

ABSTRACT

Papaya often ranks highest among fruit for its levels of ascorbic acid, carotenoids, potassium, and fiber. Additionally, papaya fruit is often noted for a beneficial digestive impact. Proteolytic enzymatic action is often cited as the main reason for this. However, also commonly stated is that papain is only active in unripe fruit. The literature is lacking in evidence of full characterization of the activity of papain during maturation of papaya fruit. Clarification of the role of papain in potential beneficial digestive effects of consumption of papaya fruit is needed. The objective of this study was to evaluate proteolytic activity in papaya and assess its potential to serve as a digestive aid. Papain activity in fresh papaya pulp was evaluated at three stages of ripeness. Stability of papain activity was also determined at regular intervals of storage at -20 C for several weeks. Digestibility of papaya, pineapple and kiwi pulp was also assessed using *in vitro* digestibility assays of meat bolus, indicating proteolytic capability. One key finding of our research was that there is significant measurable papain proteolytic activity in the pulp of fresh papaya fruit. Enzymatic protease activity stability studies also indicated a 20-35% reduction in enzymatic activity after 6 weeks storage at -20C. Papain digestive action was effective in meat bolus digestion, intermediate between the action of pineapple and kiwi in 4 hours of assay, at 23 hours of assay pineapple and kiwi fruit proteolytic action exceeded that of papaya. Additionally, ripe papaya showed the greater proteolytic action than unripe and half ripe fruit after 6 hours.

This research represents one of the first reports of active papain protease activity in ripe papaya, which could be one of the factors that make papaya a powerful digestive aid.

INTRODUCTION

Papain is an enzyme extracted from full grown unripe papaya fruit by making superficial and longitudinal incisions in the fruit. The white liquid that drips from the fruit is called latex. Papaya latex is rich in cysteine proteinases. Latex is collected and either sun dried or dried in chambers until the moisture content is reduced to 5-8% this is known as crude papain (Liener, 1974). Crude papain is produced in many parts of the world and sold in granular form. This dry product contains other proteases besides papain (Caygill, 1979). Commercially available purified papain is a combination of chymopapain and papain plus a trace of other enzymes. Protease enzymes constitute approximately 80% of all enzymes present in the latex (Buttle et al., 1989; Jacquet et al., 1989; El Moussaoui et al., 2001).

The most commercially important proteolytic enzymes found in fruits are papain (EC 3.4.22.2), ficin (EC 3.4.22.3), bromelain (EC 3.4.22.4), and actinidin (EC 3.4.22.14) present in papaya, fig, pineapple, and kiwi respectively. Proteases are minor components of the human diet. A fraction of papaya proteases are stored in the inactive form in the fruit; thus active proteases in fresh papaya latex represent a small amount of the total enzymatic potential (Azarkan et al., 2006). Bioactive components dispersed through the papaya plant in latex vary depending on location in the plant, age, and sex of the plant (Madrigal et al., 1980).

Previous studies reported protease activity in latex obtained from unripe fruit and other parts of the plant such as leaves and stems (Mezhlumyan et al., 2003; Thomas et al., 2004). Skeleton et al. (1969) investigated papain yield and proteolytic activity from the latex of the

developing papaya fruit, demonstrating an increase until the papaya fruit was fully developed (mature green); however, fully ripe fruit was not analyzed in this study. Tripathi et al. (2010) monitored papain content in mature green and ripe papaya (transgenic vs. non-transgenic). Papain content in mature green papaya was approximately 32 % higher than in ripe papaya; the same trend was observed in both cultivars. Madrigal et al. (1980) suggested that papain content should not be used as a guide to predict proteolytic activity in papaya, since the same quantity of latex with higher papain content produced lower protease activity than the one with lower papain yield. To our knowledge, protease activity from mature green papaya fruit through fully ripe stages has not been investigated.

Papain activity and stability are affected by various factors, including temperature and pH. Sumner et al. (1993) evaluated the thermal stability of papain and proteinase III (pH 4.0, for 6 min, from 20-95^{O}C). At temperatures over 55^{O}C papain activity declined while proteinase III maintained its activity up to 90^{O}C. Papain activity loss was attributed to changes that occurred at the active site.

Impact of irradiation processing on protease action is not clear; based upon previous reports; one may expect irradiation processing to have a significant impact on protease activity. The enzyme resistance to irradiation varies not only due to the irradiation doses but also due to other factors involved such as pH, temperature, moisture content, and purity of the enzyme (Whitaker, 1996). The ionizing radiation dose needed to inactivate enzymes is higher than that needed to destroy microbial spores. Irradiation doses of 0.5-1.0 kGy did not affect pectinesterase (PE) activity in papaya fruit (Zhao et al., 1996). It has been suggested that the presence of sulfhydryl compounds in papaya could protect enzymes from ionizing radiation (Whitaker, 1969).

Other studies demonstrated that PE was active at irradiation doses up to 7.5 kGy, which caused gel formation in papaya nectar (Parker et al., 2010). Irradiation by itself was not effective inactivating PE enzyme in papaya, even at doses up to 48 kGy (Wilkinson and Gould, 1998). To inactivate PE without using high doses of irradiation, heat treatment has been typically combined with irradiation. Our laboratory previously developed used irradiation doses (5 kGy) combined with heat treatment (80^{O}C, 5 min) to develop papaya nectar of similar consistency to tomato juice that maintains its natural flavor (Parker et al., 2010). However, the impact of irradiation on the protease activity of papaya nectar was not studied. One of the attributed health benefits of papaya is as a digestive aid; this is speculated due to its proteolytic enzyme activity.

The objective of this study was to evaluate protease activity at three significant stages of papaya development and to determine the impact of processing on the enzymatic activity of papaya nectar. In addition, to corroborate the activity of the enzyme, a meat bolus *in vitro* assay was performed with papaya nectar at these three ripeness stages. We hypothesized that papaya's digestive aid properties could be partially attributed to the protease activity of the pulp.

MATERIALS AND METHODS

Materials

Carica papaya L. Rainbow variety was graciously donated by the Hawaii Papaya Industry Association and the Hawaii Department of Agriculture. To eliminate fruit fly infestation they were heat treated with vapor (47^{O}C) for 4 hr at 90% humidity and cooled 1 hr prior to shipment by 2 day air. Papayas were harvested when they were fully developed, yet green (0% yellow), 25% yellow, and 60% yellow. The 0% yellow (unripe) fruit was pulped immediately

after arrival; the 25% and 60% yellow fruit were stored at 23 C until they were 50% (half ripe) and 100% yellow (ripe) before pulping, respectively.

Sample preparation

Initial sample preparation was similar for all papayas sets, they were halved, their seeds were removed and their pulp was obtained using a Kitchen Aid K5SS with a pulper attachment (Hobart Corporation, Troy, OH). Brix was measured using a Westover Scientific RHB-32ATC hand-held refractometer (Mill Creek, WA). Samples analyzed were: 0% yellow (unripe), 50% yellow (half ripe), 100% yellow (ripe), 100% yellow (processed using Ultra High Temperature) (UHT), and 100% yellow (processed by irradiation; 5 kGy + 80^{O}C, 5 min) (irradiated). Samples were frozen (-20^{O}C) prior to irradiation processing in a Gammacell 220 Excel with a cobalt-60 source, MDS Nordion, Ottawa, ON, Canada, in the irradiation facility at the University of Illinois. After irradiation, samples were heated 5 min at 80 C. Samples, ripe papaya unprocessed and processed, were lyophilized to prevent enzyme inactivation, ground, and sieved using a 0.5 mm screen. The other samples (unripe, half ripe, and ripe) were stored at -20^{O}C until enzyme activity was determined.

Determination of proteolytic activity

Protease activity was measured according to Arnon (1970) using casein as a substrate. The reaction mix (5.0 mL) contained aliquots (0.1-0.8 mL) of diluted papaya pulp (0.6 mg/mL); 0.2 mL of activating solution (0.02 M sodium EDTA, 0.005 M cysteine, pH 8.0); 0.1-0.8 mL 0.05 M Tris-HCl buffer, pH 8.0; and 1mL of 1% casein in the same Tris buffer solution. Following incubation (37^{O}C, 10 min), 3 mL 5% v/v trichloroacetic acid (TCA) was added to stop

the reaction. Tubes were stored at room temperature 40 min to precipitate non-hydrolyzed casein, which was concentrated by centrifugation (10000 g, 10 min). Absorbance of clear supernatant was measured at 280 nm. The reading was corrected for a blank, containing the same reactants except that the sample was added after addition of TCA.

The standard curve was prepared under the same conditions as the sample using a reaction mix (6.2 mL) where commercial papain (0.1- 2.0 mL, 0.2mg/mL in 0.05M Tris-HCl buffer, pH 8.0) was utilized. The same procedure, previously detailed for the sample, was followed to measure the absorbance of the commercial papain. Enzymatic activity in samples was calculated by interpolation of the standard curve. The commercial papain utilized had an activity of 0.9 units/mg solid. One unit of enzyme activity is defined as "the activity which gives rise to an increase of one unit of absorbance at 280 nm per minute digestion" (Arnon, 1970).

Protein content of the samples was determined using bovine serum albumin as standard by the Bradford method (1976). Cysteine, casein, and papain were purchased from Sigma Chemical Company (St. Louis, MO). The EDTA was obtained from Fisher Scientific, Inc. (Pittsburgh, PA), and Tris buffer from AMRESCO, Inc. (Solon, OH). Proteolytic enzymatic activity was also determined in the latex of unripe papaya, in the seeds and skin + flesh of ripe papaya. Since preliminary results showed no significant differences in the activity of the skin + flesh compared to the pulp, further research was not performed on the skin + flesh.

In vitro bolus experiment

This is study was based on methodology previously described by Thomas et al. (2004) with slight modifications. Proteolytic enzymes from different sources were studied as alternatives to aid meat bolus displacement in the esophagus; they have been successful used to

dislodge esophageal meat bolus (Thomas et al., 2004). However, the purpose of using this approach in our study is to confirm the enzymatic action present in papaya pulp at different stages of maturation. This method will serve as a good indicator of protease action in a model system.

Fruit juices evaluated in our esophageal model were kiwi, pineapple, and developmentally staged papaya. Kiwi and pineapple have been demonstrated previously to hydrolyze meat proteins through enzymatic action, thereby dislodging meat bolus in this model system (Thomas et al., 2004). Water was used as a blank and papain (Sigma Aldrich, St. Louis, MO) solutions (0.1 mg/mL and 0.2 mg/mL) were utilized as a positive control. Juices were prepared and stored at -20^{O}C until thawed for analysis.

Precooked chicken meat (Tyson) was purchased at a local store. Pieces were finely chopped into smaller pieces. Plastic syringes (10 mL) were modified by cutting the end at the 0 mL mark, the inner stopper was removed. The syringe was tightly packed with (~ 2.3 g) chicken between 8 mL and 10 mL marker. In each syringe 2 mL of solution was added on top of the chicken after being tightly packed. Syringes were arranged in racks and incubated at 37°C. The movement of the meat in response to proteolytic activity was recorded over a period of 20 hours. Additional test solution was added over the test period as needed.

Statistical analysis

Data was analyzed as a completely randomized design using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC). Least square means were reported for all response criteria. The statistical model was $Y= \mu+S+e$, where Y denotes the observed variable, u the overall mean, S represents the effect of substrate at different maturity stages, and e the experimental error.

When significant ($P<0.05$) differences were detected, individual means were compared using the least significant difference (LSD) and the mean separation was conducted (SAS Institute, Inc., Cary, NC). Differences among means with a *P* value of less than 0.05 were considered significant.

RESULTS AND DISSCUSION

The objective of this study was to evaluate protease activity through developmental stages of papaya ripeness and determine the impact of processing on the enzymatic activity of papaya nectar. In response to suggestions that papaya provides an additional health benefit of serving as a digestive aid, we speculated that papaya's digestive aid properties could be partially attributed to protease activity in the pulp. One of the overall goals of our laboratory is to identify a strategy for processing papaya that will produce microbiologically safe nectar with minimal impact on flavor and nutrient content. It was also of importance to evaluate the impact of our selected processing methods on protease activity.

Protease activity during development

Enzymatic activity progressed from lowest (unripe, 5.7 U/g pulp) to highest (ripe, 11.9 U/g pulp) over the course of development (Figure 3.1). Ripe fruit displayed 50% higher enzyme activity than unripe fruit; these activities are lower compared to the activity found in the seeds (29.2 U/g seed) and seem insignificant compared to activities obtained from the latex (6110 U/g latex). Increases in proteolytic activity in ripe papaya are associated with an increase in protein content of the fruit as it ripens. This trend of increasing protein content during ripening was reported previously (Abu-Goukh et al., 2010; Bari et al., 2006). Our finding is supported by

previous research that reported that protein in papaya decreased up to physiological maturity (mature green fruit) then increased up to coincided with the climacteric peak of respiration (fully ripe fruit) then drastically decreased in the over ripe stage (Abu-Goukh et al., 2010). The roll of protein in fruits is more functional, such as enzymes, than for storage; therefore they are associated with changes in metabolic activity during fruit development (Wills et al., 1998). Thus the increase in protein during ripening of papaya coincide with the increase of activity of papain and other enzymes reported before such as cellulase, xylanase, and polygacturonase (Abu-Goukh and Bashir, 2003; Chen and Paull, 2005).

Specific activity measurements (U/mg protein) are presented in Figure 3.2. Unripe papaya fruit had the highest protease action (11.33 U/mg protein). This value was similar to that reported by Galindo-Estrella et al. (2009) for unripe papaya (Maradol cultivar; 10 U/mg protein). An inverse trend was observed between specific activity and ripeness stage. A decrease of 25% in specific activity of protease was observed in ripe papaya compared to unripe papaya. A study analyzed papain levels (mg papain/g pulp) in mature green fruit and fully ripe fruit; papain levels were higher in mature green fruit and progressively decreased by 32%, during ripening (Tripathi et al., 2010). However, our study focused on the papain activity not quantitation of papain present in the fruit.

Processing impact on enzyme activity

Irradiated papaya nectar had the lowest activity (0.85 U/g pulp), representing 65% less activity than the ripe fruit. Likely this was due to the application of heat treatment (80^{O}C, 5 min) utilized for inactivation of PE, utilized to prevent gel formation in nectar. Previous studies reported decreased enzyme activity (polygalacturonase PG, PE, and β-galactosidase) in papaya

fruit after irradiation doses of 0.5 kGy (D'Innocenzo and Lajolo, 2001). However, other researchers reported that irradiation doses up to 48 kGy were not enough to inactivate PE (Wilkinson and Gould, 1998; Parker et al., 2010). Since our laboratory is also investigating the use of ultra-high temperature processing (UHT) for papaya nectar, the impact of this treatment (121 C, 3 sec) was also assessed for comparison. Protease activity was 55% lower than that of ripe papaya fruit. Processed samples (both irradiated and UHT) were the lowest in specific activity as was expected.

Enzyme stability during storage

Impact of storage (-20C) on enzyme activity of ripe papaya pulp, over a 6 week period was evaluated (Table 3.1). After 2 weeks storage there was an approximately 20% loss of enzyme activity in the pulp. Activity continued to decrease by approximately 35% after 6 weeks storage, as compared to fresh. Similar trends were reported by Baeza et al. (1990). They reported 40% activity loss in lyophilized latex of *Carica candamarsensis* after 12 weeks storage at -20 C.

Indicator of digestive action

Meat bolus displacement was used as indicator of protease digestive action. The objective of our study was to corroborate the protease enzyme activity present at the different maturation stages of papaya fruit measuring the impact on bolus displacement. After 4 hours incubation in the meat bolus study kiwi juice showed the fastest displacement of the bolus among the fruit juices compared to water (control) and to the different papain solutions (Table 3.2). Similar results were reported in a previous study under same conditions after 4.5 hours of incubation (Thomas et al., 2004). After 8 and 20hrs of incubation pineapple, kiwi, and papaya juice

produced the greatest displacement of the meat bolus in the syringes (Table 3.2) which could be associated to the enzymatic activity present in these fruits (pineapple 40 U/g pulp, kiwi 25 U/g pulp, and papaya 12 U/g pulp).

Meat bolus displacement was also evaluated using papaya as it progressed from mature green to fully ripe (Figure 3.3, Table 3.3). After 5, 18, and 20 hours of incubation, ripe papaya showed the largest bolus displacement among the three papaya ripeness stages, indicating that proteolytic enzymes are active through the different stages of ripeness of papaya fruit, sufficient to dislodge meat bolus in 5 hours.

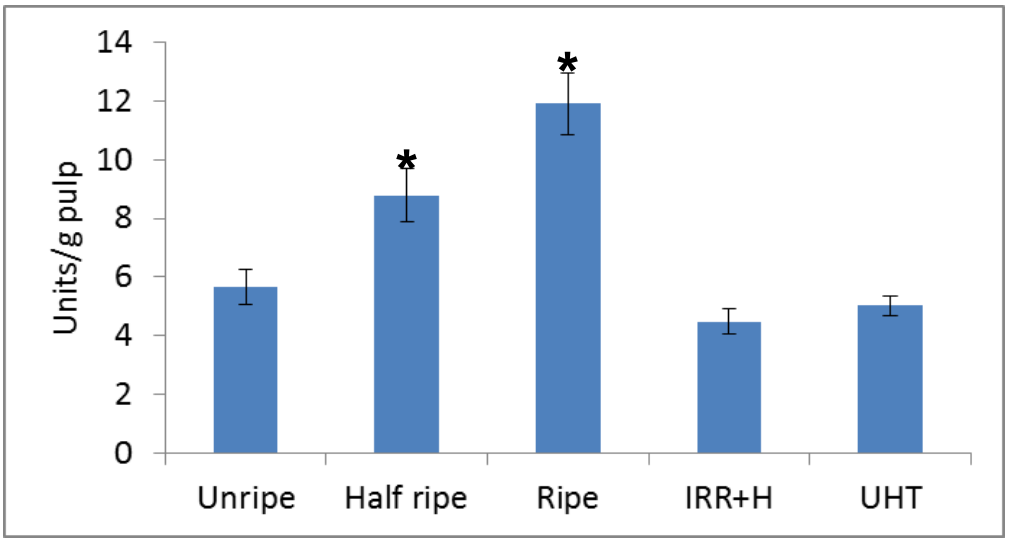
CONCLUSIONS

This study demonstrates the presence of active proteolytic enzymes throughout the ripeness stages of papaya. Of the three stages, ripe papaya had the greatest enzyme activity (U/g pulp). The specific activity (U/mg protein) was higher in unripe papaya, due to the low protein content.

Both processing methods (irradiation and UHT) showed a detrimental impact on the proteolytic activity (loss of 60 %, compared to non-processed). The meat bolus study confirms the proteolytic activity presence through the different ripeness stages of papaya fruit. However, it is still unclear whether this activity is partially responsible for the digestive properties attribute to papaya. Also it is not clear if the amount of activity present in the fruit would be enough to have an impact on the human GI tract, especially at the acidic pH of the stomach. To solve these inquires our next study was focused on evaluation of protein degradation by papaya proteases at the acidic pH of the stomach, using an *in vitro* monogastric model.

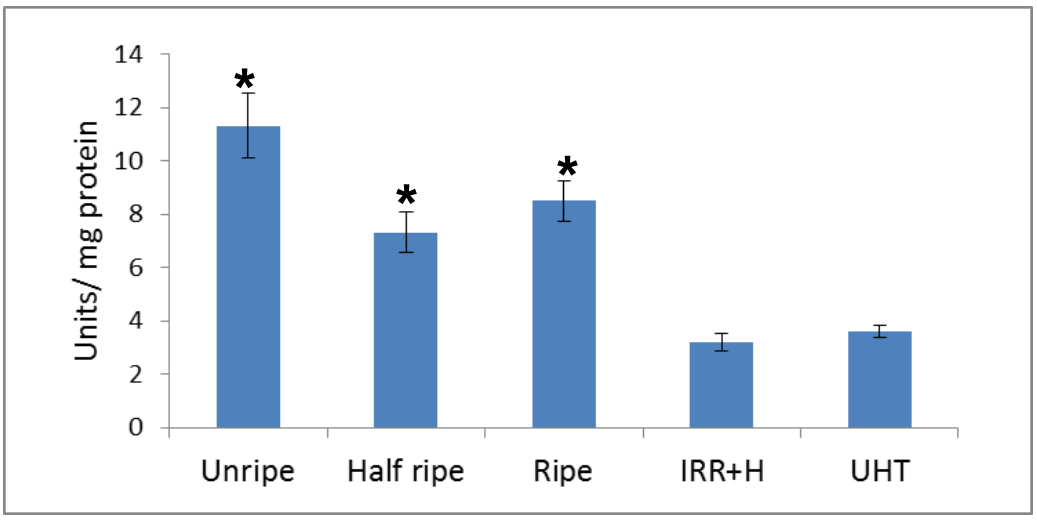
FIGURES

Figure 3.1. Evaluation of proteolytic activity of papaya fruit at three ripeness stages and processed papaya nectar[a].



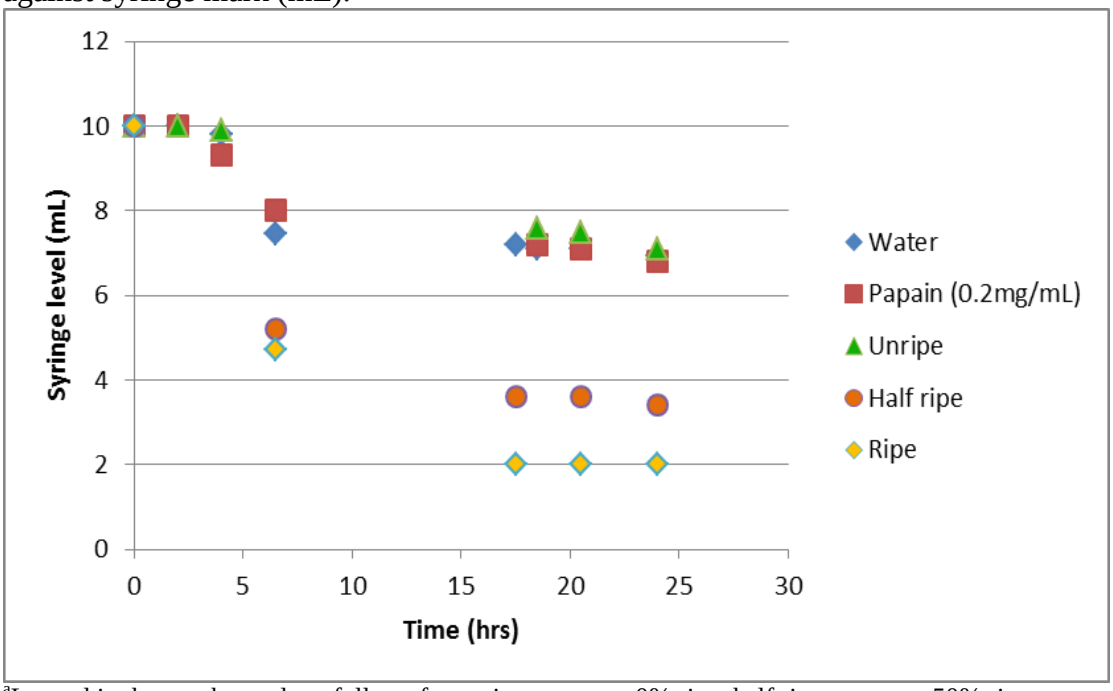
[a]IRR+H = irradiated (5 kGy) + heat (80C, 5min); half ripe = 50 % ripe, UHT = ultra-high temperature processing (121 C, 3 sec). Groups marked with stars were significantly different from all other conditions at α=0.05 level.

Figure 3.2. Specific activity of papaya fruit at three ripeness stages and processed papaya nectar[b].



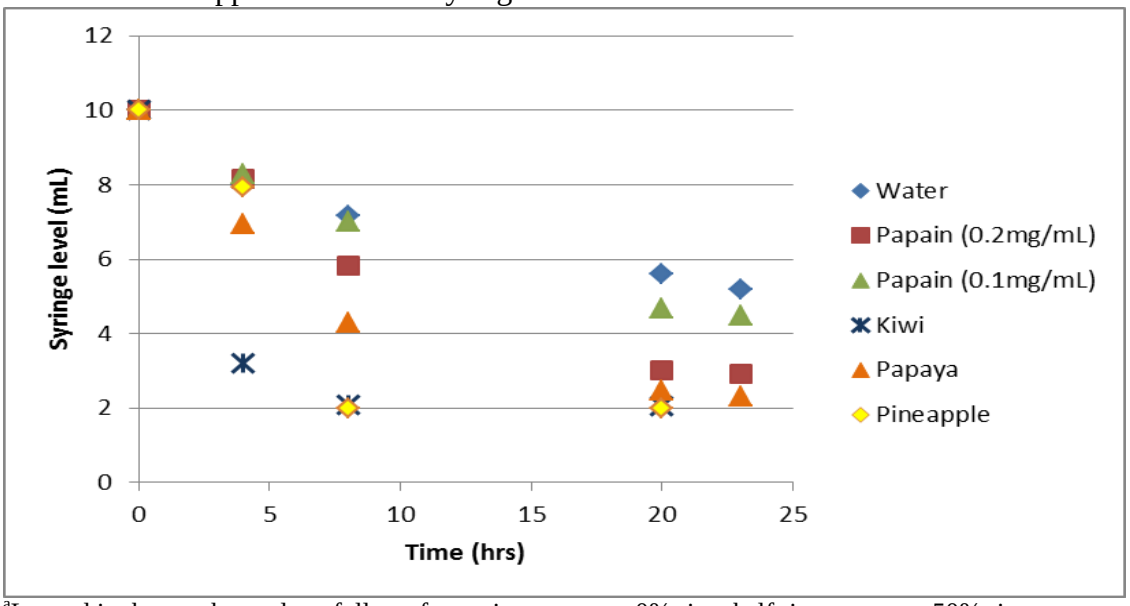
[b]IRR+H = irradiated (5 kGy) + heat (80C, 5min); half ripe = 50 % ripe, UHT = ultra-high temperature processing (121 C, 3 sec). Groups marked with stars were significantly different from all other conditions at α=0.05 level.

Figure 3.3. Average displacement of meat bolus using papaya juice at three ripeness stage against syringe mark (mL).



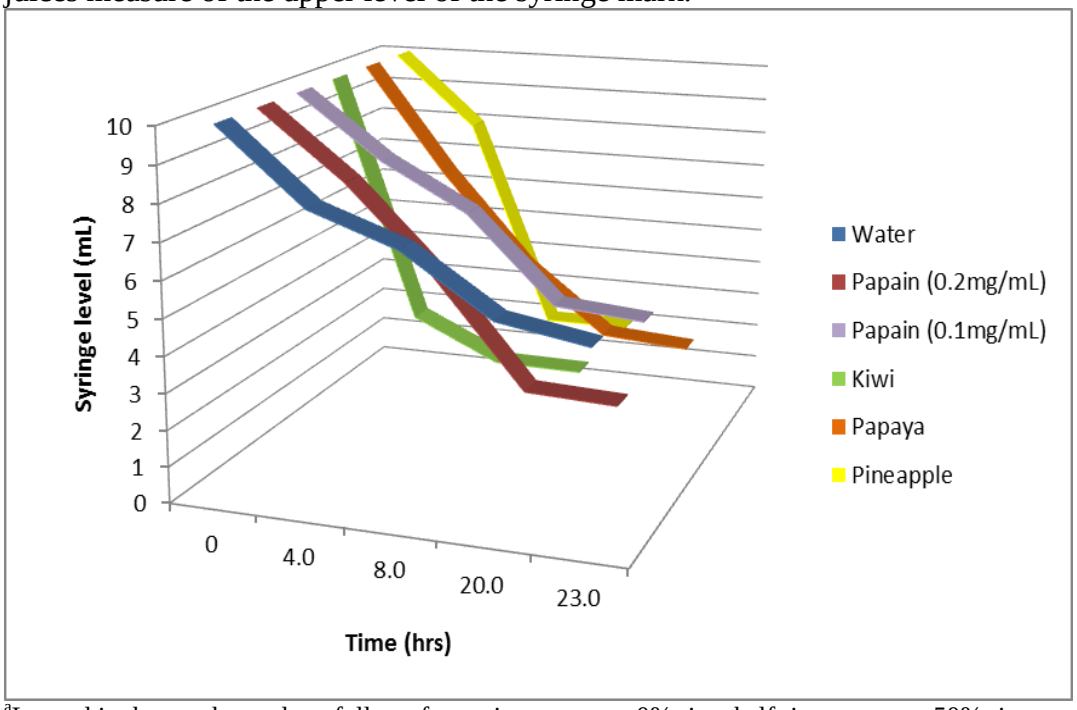
[a]Legend in the graph stands as follows for unripe papaya = 0% ripe, half ripe papaya = 50% ripe, ripe= 100% ripe.

Figure 3.4. Average downward movement (mL) of meat bolus using different fruit juices measure of the upper level of the syringe mark.



[a]Legend in the graph stands as follows for unripe papaya = 0% ripe, half ripe papaya = 50% ripe, ripe= 100% ripe.

Figure 3.5. Average downward movement (mL) of meat bolus using different fruit juices measure of the upper level of the syringe mark.



[a]Legend in the graph stands as follows for unripe papaya = 0% ripe, half ripe papaya = 50% ripe, ripe= 100% ripe.

TABLES

Table 3.1. Effect of storage on enzyme activity (U/g pulp) of ripe papaya during 6 weeks at -20C.

Fruit part	Week 0	Week 2	Week 4	Week 6
Flesh	13.7± 1.0	11.4± 0.6	9.4± 0.7	9.3± 1.0
Skin + Flesh	12.2± 1.4	12.9± 0.8	12.7± 0.5	8.1± 0.9

Table 3.2. Average downward movement (mL) of meat bolus using different fruit juices measure of the upper level against the syringe mark.

Time (Hrs.)	Water	Papain (0.2mg/mL)	Papain (0.1mg/mL)	Kiwi	Papaya	Pineapple
0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
4	8.0 ± 1.0	8.2 ± 0.1	8.3 ± 0.5	3.2 ± 1.5	6.9 ± 0.9	7.9 ± 1.5
8	7.2 ± 0.9	5.8 ± 1.3	7.0 ± 0.8	2.1 ± 0.2	4.3 ± 0.8	2.0 ± 0.0
20	5.6 ± 1.2	3.0 ± 0.6	4.7 ± 2.3	2.1 ± 0.8	2.5 ± 0.1	2.0 ± 0.0
23	5.2 ± 1.4	2.9 ± 0.5	4.5 ± 2.2	-	2.3 ± 0.6	-

[&]Maximum value recorded from the syringe (2.0 mL). [b]Values are expressed as mean ± standard deviations.

Table 3.3. Average downward movement (mL) of meat bolus using papaya juice at three ripeness stages, measure of the upper level against the syringe mark.

Time (hrs.)	Water	Papain (0.2mg/mL)	Unripe	Half ripe	Ripe
0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
2	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	-	-
4	9.8 ± 0.0	9.3 ± 0.1	9.9 ± 0.2	-	-
6.5	7.47 ± 0.8	8.0 ± 0.5	-	5.2 ± 2.0	4.7 ± 2.4
17.5	7.2 ± 0.6	-	-	3.6 ± 2.0	2.0 ± 0.0
18.5	7.2 ± 0.8	7.2 ± 1.0	7.6 ± 0.7	-	-
20.5	7.1 ± 0.7	7.1 ± 1.0	7.5 ± 0.9	3.5 ± 2.6	2.0 ± 0.0
24	6.9 ± 0.7	6.8 ± 1.2	7.1 ± 1.0	3.4 ± 2.4	2.0 ± 0.0

[&]Minimum value recorded from the syringe (2.0 mL). [b]Values are expressed as mean ± standard deviations.

REFERENCES

Abu-Goukh, A. B. A.; Shattir, A. E. T.; Mahdi, E. F. M. Physico-chemical changes during growth and development of papaya fruit. II: Physical changes. Agric. Biol. J. N. Am. **2010**, 1(5), 871-877.

Medicinal Plants for Livestock: Beneficial or Toxic? Animal Sci. Dept., Cornell Univ. (accessed April 14, 2011) http://www.ansci.cornell.edu/plants/medicinal/index.html

Arnon, R. Papain. *Meth. Enzymol.* **1970**, 19, 226-244.

Azarkan, M.; Dibiani, R.; Baulard, C.; Baeyens-Volant, D. Effects of mechanical wounding on Carica papaya cysteine endopeptidases accumulation and activity. *Int. J. Biol. Macromol.* **2006**, 38, 216-224.

Baeza, G.; Correa, D.; Salas, C. Proteolytic Enzymes in *Carica candamarcensis*. *J. Sci. Food Agric*. **1990**, 51, 1-9.

Bari, L.; Hassan, P.; Absar, N.; Haque, M. E.; Khuda, M.; Pervin, M. M.; Khatun, S.; Hossain, M. I. Nutritional analysis of two local varieties of papaya (Carica papaya L.) at different maturation stages. *Pak. J. Biol Sci.* **2006**, 9, 137-140.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, 72, 248-254.

Buttle, D. J.; Kembhavi, A. A.; Sharp, S. L.; Shute, R. E.; Rich, D. H.; Barrett, A. J. Affinity purification of the novel cysteine proteinase papaya proteinase IV, and papain from papaya latex. *Biochem. J.* **1989**, 261, 469.

Caygill, J.C. Sulphydryl plant proteases. Enzyme Microb. Technol. **1979**, 1, 233-242.

D'Innocenzo, M.; Lajolo, F. Effect of gamma irradiation on softening changes and enzyme activities during ripening of papaya fruit. *J. Food Biochem.* **2001**, *25*, 425-438.

El Moussaoui, A.; Nijs, M.; Paul, C.; Wintjens, R.; Vincentelli, J.; Azarkan, M.; Looze, Y. Revisiting the enzymes stored in the laticifers of Carica papaya in the context of their possible participation in the plant defence mechanism. *Cellular and Molecular Life Sciences* **2001**, 58, 556-570.

Galindo-Estrella T., H., G.E.; R., M., J.; Sandoval-Fabian, G., Chel-Guerrero, L.; Rodriguez-Buenfil, I.; Gallegos-Tintore, S. Proteolytic activity in enzymatic extracts from Carica papaya L. cv. Maradol harvest by-products. *Process Biochemistry* **2009**, 44, 77-82.

Jacquet, A.; Kleinschmidt, T.; Schnek, A. G.; Looze, Y.; Braunitzer, G. The thiol proteinases from the latex of Carica papaya L. III. The primary structure of chymopapain. *Biol. Chem. Hoppe-Seyler* **1989**, 370, 425-434.

Madrigal, L. S.; Ortiz, A. N.; Cooke, R. D.; Fernandez, R. H. The dependence of crude papain yields on different collection ('tapping') procedures for papaya latex. *J. Sci. Food Agric.* **1980**, 31, 279-285.

Mezhlumyan, L. G.; Kasymova, T. D.; Yuldashev, P. K. Proteinases from Carica papaya latex. *Chemistry of natural compounds.* **2003**, 39, 223-228.

Liener, I. E. The Sulfhydryl Proteases. Food Related Enzymes. **1974**, Vol 136, 202-219.

Parker, T. L.; Esgro, S. T.; Miller, S. A.; Myers, L. E.; Meister, R. A.; Toshkov, S. A.; Engeseth, N. J. Development of an optimised papaya pulp nectar using a combination of irradiation and mild heat. *Food Chem.* **2010**, 118, 861-869.

Roberts, M.; Minott, D. A.; Tennant, P. F.; Jackson, J. C. Assessment of compositional changes during ripening of transgenic papaya modified for protection against papaya ringspot virus. *J. Sci. Food Agric.* **2008**, 88, 1911-1920.

Skelton, G. Development of proteolytic enzymes in growing papaya fruit. *Phytochemistry* **1969**, 8, 57-60.

Sumner, I. G.; Harris, G. W.; Taylor, M. A. J.; Pickersgill, R. W.; Owen, A. J.; Goodenough, P. W. Factors effecting the thermostability of cysteine proteinases from Carica papaya. *European Journal of Biochemistry* **1993**, 214, 129-134.

Thomas, L.; Low, C.; Webb, C.; Ramos, E.; Panarese, A.; Clarke, R. Naturally occurring fruit juices dislodge meat bolus obstruction in vitro. *Clinical Otolaryngology & Allied Sciences* **2004**, 29, 694-697.

Tripathi, S.; Suzuki, J. Y.; Carr, J. B.; McQuate, G. T.; Ferreira, S. A.; Manshardt, R. M.; Pitz, K. Y.; Wall, M. M.; Gonsalves, D. Nutritional composition of Rainbow papaya; the first commercialized transgenic fruit crop. *Journal of Food Composition and Analysis.* **2010.**

Whitaker, J. R. Papain-and ficin-catalyzed reactions. Effect of pH on activity and conformation of ficin. *Biochemistry.* **1969**, 8, 1896-1901.

Wilkinson, V. M.; Gould, G. In *Food irradiation: a reference guide.* Woodhead Pub Ltd. **1998**, 93-95.

Zhao, M.; Moy, J.; Paull, R. E. Effect of gamma-irradiation on ripening papaya pectin. *Postharvest Biol. Technol.* **1996**, 8, 209-222.

CHAPTER 4

Impact of papaya nectar on human digestion using an *in vitro* model

ABSTRACT

Papaya fruit consumption has become popular due to his high nutritional value and digestive properties. However, little research has been conducted to evaluate the impact of papaya fruit on human digestion. The objective of this study was to determine whether the digestive properties attributed to ripe papaya fruit are due to the activity of proteolytic enzymes present in the fruit, due to its fiber content, or to the combination of both. To accomplish this objective *in vitro* digestion and fermentation assays were performed to determine protein digestibility, organic matter disappearance (OMD), and short chain fatty acid (SCFA) production. Unripe papaya, ripe papaya, and processed ripe papaya were utilized as substrates. Processed papaya fruit was pulped, diluted, and exposed to irradiation (5 kGy), followed by mild heat treatment (80°C, 5 min). To evaluate proteolytic activity nitrogen disappearance from casein and casein plus substrate were used in the *in vitro* digestion stage. In the *in vitro* fermentation stage, samples were inoculated with feces from three human donors; OMD and SCFA were measured as indicators of digestibility. Short chain fatty acid concentrations were determined using gas chromatography. Nitrogen disappearance was significantly higher in ripe fruit (ripe= 74.6 %, unripe= 65.84 %, processed= 65.60 %, casein= 65.4 %); this correlates with the highest enzymatic activity detected in the ripe fruit from our previous study. Short chain fatty acid analysis of the different substrates indicated that pectin (483.08 mg/g), positive control, had the highest SCFA production, ripe papaya (424.15 mg/g) and unripe papaya (414.62 mg/g) had higher production than processed (367.18 mg/g). Pectin and unripe papaya had similar OMD

(32.42 and 32.43 %) respectively, significantly higher than the ripe fruit unprocessed (20.86 %) and processed (22.24 %) which were not significantly different from each other. This research suggests that in addition to the fiber content of papaya, proteolytic enzymes present in the fruit are partially responsible for the digestive properties attributed to papaya fruit.

INTRODUCTION

Recently, consumer awareness of issues surrounding the intake of antioxidants and dietary fiber has heightened due to increasing reports of their potential impact on human health. Many reports support the concept that dietary fiber improves digestion and provides several health benefits, such as reduced risk of coronary heart disease CHD, type 2 diabetes, and colon and rectal cancer (Wolk et al., 1999; Schulze et al., 2004; Schatzkin et al., 2007).

Fruit and vegetable consumption has been promoted for vitamin, mineral, antioxidant, and fiber content. Several studies have evaluated the impact of fruit and vegetables on human health, concluding that consumption of fruits and vegetables promotes improvement in bowel function, increasing satiety, and reducing the risk of stroke and certain cancers (Kelsay, et al., 1978; World Cancer Research Foundation, 1997; Rolls et al., 2004; He et al., 2006).

Papaya fruit is not only a good source of vitamins and minerals but also of fiber and proteolytic enzymes (Wurtz and Bouchut, 1879; Liebman, 1992; USDA National Nutrient Database for Standard Reference, 2010). Papaya has been suggested for many years to play a role in digestion (Roy, 1873; Wittmack, 1878); this we speculate is attributed to the fiber content and proteolytic enzymes associated with this highly nutritious fruit. Papaya fruit contain approximately 35 % total dietary fiber, from which soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) each constitute about 50 % of the total dietary fiber TDF (Chang et al., 1998;

Ramulu and Rao, 2003). SDF is completely fermented by the bacteria in the colon and IDF is partially or non-fermentable. Among the different products of fermentation the most important are the short chain fatty acids SCFA, which are indicators of digestibility (McBurney and Thompson, 1989; Adiotomre et al., 1990). There is increasing evidence that SCFA are important in maintenance of colon health (Roberfroid, 2007; Vos et al., 2007) and may also be important in the prevention and management of certain diseases, such as coronary heart diseases, type 2 diabetes, and obesity, and in decreasing LDL cholesterol (He et al., 1995; Lairon et al., 2005).

Crude papain represents the group of proteolytic enzymes found in papaya. Crude papain is extracted from unripe papaya fruit by making superficial incisions to the surface of unripe, green fruit, which will yield a milky juice called latex that is collected and dried to reduce its moisture content to approximately 5 % (Buttle et al., 1989; Jacquet et al., 1989). Papain has also been extracted from leaves, stalks, petioles, and stems of the papaya plant (Balls et al., 1939; Galindo-Estrella et al., 2009). Proteolytic activity was analyzed in papaya by-products; unripe fruit and stems were the most promising source of papain. Crude papain contains other proteolytic enzymes besides papain such as: proteinase III or caricain, and proteinase IV or glycyl endopeptidase, and chymopapain, which is most abundant, but not the most active (Galindo-Estrella et al., 2009).

Researchers studied the stability of these four papaya proteinase enzymes at low pH and their degradation by pepsin to see their suitability for oral preparations to aid digestion (Huet et al., 2006). At low pH the four enzymes adopted a partly structured state (molten globule state) different from the native and unfolded states. They suggested that enzyme activity could not be regenerated from any molten state of papaya proteinases after 30 minutes incubation at 37°C under acidic conditions. They also noted that papaya proteinases are highly susceptible to

proteolysis by pepsin at pH 1.2 and 37°C, suggesting that only a small portion will survive gastric conditions. They suggested that oral preparations of the enzyme should be protected to be more effective in the gut.

Papain and bromelain both belong to the same group of proteinases; at low pH they form intermediate states known as molten globule and A-states, which are partly structured (Fink et al., 1994; Huet et al., 2006). Bromelain is extracted from pineapple (*Ananas comosus*) stem. It has been marketed as a digestive aid due to his high enzyme activity (Haq et al., 2002). However, is well known that enzyme stability is affected by factors such us pH and temperature. An *in vivo* study investigated whether non-encapsulated bromelain maintains its proteolytic activity throughout the gastrointestinal tract in murine mice (Hale, 2004). Oral bromelain remained sufficiently active to proteolytically remove the cell surface receptors throughout the whole gastrointestinal tract of the mice. However, when bromelain was formulated either with Maalox or sodium bicarbonate (antacids) the retention of proteolytic activity increased.

Little research has been published on evaluating the efficacy of papaya fruit in human digestion. The objective of this study was to determine whether the digestive properties typically attributed to papaya fruit are due to proteolytic enzymes present in the fruit or due to its fiber content, or to a combination of both. One of the goals was to determine whether the proteolytic enzymes of papaya fruit would remain active in the GI tract, or if they would be inactivated by the low pH in the stomach. The goal of this research was to determine whether proteolytic enzymes from papaya fruit are active in the gastrointestinal tract or if they are inactivated. To accomplish this objective an *in vitro* digestion assay for protein and organic matter disappearance (OMD) were performed. To understand how papaya fiber is helping digestion, an *in vitro* fermentation experiment was conducted to evaluate OMD and SCFA production.

MATERIALS AND METHODS

Substrates

Carica papaya L. Rainbow variety was used as a substrate. Papayas were graciously donated by the Hawaii Papaya Industry Association and the Hawaii Department of Agriculture. Papayas were heat treated (47^{O}C) with vapor for 4 hr at 90 % humidity to eliminate fruit fly infestation and cooled for 1 hr prior to shipment by 2-d air. Unripe papaya (0 % yellow) was pulped immediately after arrived and the 50 % yellow fruit were stored at room temperature (23^{O}C) until they were fully ripe (100 % yellow) before pulping. Fruit were harvested when they were 0% and approximately 50 % yellow.

Substrate preparation

Initial sample preparation was similar for all papayas sets, they were halved, their seeds manually removed and their pulp was processed using a Kitchen Aid K5SS with a pulper attachment (Hobart Corporation, Troy, OH). Brix was measured in the collected juice using a Westover Scientific RHB-32ATC hand held refractometer (Mill Creek, WA). The collected pulp was diluted to 8.0^{O}Brix using double deionized water. The samples used as substrates were: unripe papaya (0 % yellow), fully ripe papaya (100 % yellow) and processed (100 % yellow: irradiated at 5 kGy and heated at 80^{O}C, 5 min). Ripe samples were placed in 250 mL glass bottles and labeled as follows: control and 5kGy + H. Samples were frozen at -20^{O}C until processed. Frozen samples were irradiated in a Gammacell 220 Excel with a cobalt-60 source (MDS Nordion, Ottawa, ON, Canada) in the irradiation facility at the University of Illinois. After irradiation the samples were exposed to heat treatment.

Samples, unripe papaya, ripe papaya, and processed were lyophilized to prevent enzyme inactivation, ground, and sieved using a 0.5 mm screen. These substrates were used for fiber determination and for *in vitro* studies.

Proteolytic activity determination

An *in vitro* digestion experiment was conducted to determine whether the proteolytic enzyme remained active after been exposed to the stomach acidity. To evaluate nitrogen disappearance (protein digestibility) due to enzymatic activity the following substrates were used: casein (blank), casein + unripe papaya, casein + ripe papaya, and casein + processed papaya. Casein (250 mg) plus sample (250 mg) was weighed in triplicate into 50 mL Falcon centrifuge tubes. Also, three blanks (casein only) were prepared. Residues were ashed for organic matter disappearance OMD or put through the Leco instrument (FP2000, Leco Corp., St. Joseph, MI) to determine nitrogen disappearance.

Chemical analysis

Substrates were analyzed for dry matter (DM) and organic matter (OM) by AOAC (1984) methods. Crude protein (CP) content was determined from Leco nitrogen values (AOAC, 2000) using a conversion factor of (N x 6.25). Insoluble (IDF) and soluble dietary fiber (SDF) concentrations were determined and they were summed to give the total dietary fiber (TDF) content of each substrate (Prosky et al., 1992). Substrates were analyzed in duplicate. Characterization of relevant chemical composition of three substrates is presented in Table 1.

Donors and collection method

Human fecal samples from 3 individual male volunteers were pooled to serve as source inocula for *in vitro* fermentation. All donors were older than 18 years, consumed their normal diet, were free of gastrointestinal disease, and had not received antibiotics at least 3 months before or during the study. The Institutional Review Board of the University of Illinois approved the experimental protocol, and all subjects signed an informed consent prior to initiation of the experiment. On the morning of the experiment, each donor provided a fresh fecal sample, collected using a Commode Specimen Collection System (Sage Products, Crystal Lake, IL). To ensure viability of microbial population, samples collected were brought to the laboratory within 15 min of defecation.

Medium composition and substrate fermentation

For *in vitro* fermentation, substrates were de-sugared using 80 % ethanol prior to freeze drying. The substrates for fermentation were: unripe papaya, ripe papaya, processed papaya, cellulose (negative control), pectin (positive control), and blanks. Cellulose was obtained from Solka Floc (International Fiber Corp., Urbana, OH) and pectin from Pectin HM Rapid (TIC Gums, Belcamp, MD).

Substrates (~ 250 mg) were weighed in triplicate for each pull time (0 and 12 hr fermentation) into 16 mL Balch tubes that were used in a model that simulated large bowel fermentation. The composition of the *in vitro* medium is listed in Table 2. All media components, except vitamins and short chain fatty acids (SCFA), were added before autoclave sterilization of the medium. Vitamin solutions were added just before dispensing the medium, which was maintained under anaerobic conditions at all times after preparation. An aliquot of the

medium (26 mL) was aseptically transferred to appropriate Balch tubes, capped with butyl rubber stoppers, and sealed with aluminum caps. All tubes were stored at 4^{O}C for 12 hr to allow hydration of substrates before initiating fermentations. Tubes were placed in a 37^{O}C water bath approximately 30 min before inoculation.

Fecal samples from three donors were maintained at 37^{O}C until inocula were prepared (within 10 min). Equal amounts of each fecal sample were mixed together and diluted 1:10 (w/v) in anaerobic diluting solution by blending for 15 sec in a Waring blender under a stream of CO_2. Blended diluted feces were filtered through four layers of cheesecloth into 125 mL serum bottles under CO_2.

Samples and blank tubes were aseptically inoculated with 4 mL of diluted feces. Tubes were incubated at 39^{O}C with periodic mixing for 0 and 12 h, and processed immediately for analyses. The pH of tube contents was measured with a standard pH meter (Denver Instrument Co., Arvada, CO) at 0 and 12 h. A subsample (2 mL) was taken from each tube for SCFAs analyses. The remaining 28 mL was combined with 4 vols 95 % ethanol to precipitate the soluble polysaccharide fractions. Samples were filtered through Whatman 541 filter paper and washed sequentially with 78 % ethanol, 95 % ethanol, and acetone. The 78 % rinse was used to rinse out remaining soluble components in the residue after initial filtration. The 95 % ethanol solution was used to dilute the water in the residue after washing with 78 % ethanol. Acetone was used to remove any residual pigments and aid in drying the residue after washing with 95 % ethanol. Samples were dried at 105^{O}C overnight and weighed to determine DMD. They were ashed (500^{O}C, overnight) and weighed again to determine organic matter disappearance OMD.

Short-Chain Fatty Acid analysis

Samples to be analyzed for SCFAs (2 mL) were mixed with 0.5 mL 25 % metaphosphoric acid, precipitated 30 min, and centrifuged 25 min at 20000 G. The supernatant was decanted and frozen (-20^{O}C) in microfuge tubes. After freezing the supernatant was thawed and centrifuged at 10000 G for 10 min. Short chain fatty acid (acetate, propionate, butyrate) concentrations were determined using a Hewllett-Packard 5890A series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm x 4 mm i.d.) packed with 10 % SP-1200/1 % H3PO4 on 80/100 + mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). The temperatures for the oven, injector, and detector were 125, 180, and 175^{O}C, respectively. SCFA concentrations were corrected with the quantities of SCFA produced in blank tubes. All samples were run in triplicate.

Statistical analysis

Data was analyzed as a completely randomized design using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC). Least square means were reported for all response criteria. The statistical model was $Y = \mu+S+e$, where Y denotes the observed variable, μ is the overall mean, S represent the effect of substrate, and e is the experimental error. When significant ($P<0.05$) differences were detected, individual means were compared using the least significant difference (LSD) among means with a P value of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Chemical composition

Dry matter (DM) values for papaya substrates ranged from 72.39 to 76.82 %. The highest DM concentration was observed in unripe papaya (76.82 %), followed by processed papaya (74.59 %), and ripe papaya (72.39 %). Organic matter (OM) concentrations were similar among the substrates, differing by 4 %; the highest OM was observed in processed (97.13 %), followed by ripe (96.27 %), and the lowest in unripe fruit (93.57 %) (Table 4.1). A slight increase in DM (3 %) and OM (0.9 %) was observed after the sample was irradiated and heat treated.

Unripe papaya had the highest crude protein (CP) content (6.06 % DMB), followed by ripe (3.47 % DMB), and processed (2.62 % DMB) papaya. Crude protein of unripe papaya samples (6.1 % DMB.) was higher than that (4.7 % DMB) reported by Roberts et al. (2008). However, the CP values (3.5 % DMB) for ripe papaya in our sample was lower than the one reported (5 %) for the Sunrise Solo variety (Roberts et al., 2008). They did not find significant differences in CP across ripening stages. Tripathi et al. (2010) reported CP values for mature green papaya (5.75 % DMB), fruit ripened off the tree (6.06 % DMB), and fruit ripened on the tree (5.19 % DMB), no significant differences were detected between the ripe fruit on the tree and the mature green fruit.

Chemical changes in papaya fruit during growth and development were studied by Abu-Goukh et al. (2010) and Bari et al. (2006). Total protein decreased until the fruit reached physiological maturity, then increased until reaching maximum protein content at the climacteric peak of respiration, and decreased at the over ripe stage. Researchers found (Frenkel et al., 1968; Abu-Goukh and Bashir, 2003) that in the climacteric phase of respiration the number of free amino acids decreased, indicating an augment in protein synthesis; the opposite happened after

fruit was fully ripe (progressing towards becoming over ripe). The role of proteins in fruit is mainly functional, since proteins in fruit are mainly enzymes, required for the ripening process (Wills et al., 2007).

Total dietary fiber TDF, insoluble dietary fiber IDF, and soluble dietary fiber SDF comparisons amongst substrates are reported in Table 1. The highest TDF content was observed in unripe (35.77 % DMB) papaya. TDF decreased by 48 % when papaya reached the full ripe stage (18.65 % DMB), and after processing TDF declined by 18 % (15.23 % DMB). Experimental TDF values were similar to the values reported by others for green papaya (30.4 %, Mahattanatawee et al., 2006) and ripe papaya (14.2 %, Mahattanatawee et al., 2006; Roberts et al., 2008). There is high literature variability among TDF values depending on ripening stage, cultivars, and determination methods; TDF values for ripe papaya vary from 11.7 % (Ramulu and Rao, 2003) to 35.2 % (Chang et al., 1998).

The greatest IDF content was found in unripe (24.5 %) papaya; this decreased by 53 % (11.48 %) upon ripening and decreased by 36 % after processing (7.38 %). These values represent 68 %, 62 %, and 48 % of their respective TDF. The IDF concentration decreased by 53 % when the fruit ripened and by 36 % after processing. The SDF content decreased by 36 % when the fruit ripened and increased by 9 % after processing. This research demonstrated that irradiation processing had an effect on SDF and IDF concentrations. However, the processed samples showed the most balanced percentages of IDF and SDF. The proportion of IDF and SDF from this experiment is in agreement with those reported by other researchers (Chang et al., 1998; Ramulu and Rao, 2003).

Organic matter disappearance OMD in the *in vitro* digestion assay was significantly greater in the processed substrate (82.8 %) compared to unripe and ripe (57. 9 % and 80.6 %,

respectively). An increase in OMD was observed when fruit ripened (by 39 %) and after processing (by 3 %). Similar trends were observed for dry matter disappearance DMD after 24 hours of incubation (Table 4.3). The higher OMD observed in the processed substrate could be explained by the homogeneous proportion of IDF: SDF. Swanson et al. (2001) suggested that OMD improved as the ratio of insoluble: soluble fiber decreased which is in agreement with our results, where unripe had higher proportion of IDF: SDF (68:32 % of TDF) than ripe (62:38 % of TDF) and processed (48:52 % of TDF), which was most uniform. Since SDF are more fermentable than IDF this could explain why when the proportion IDF: SDF is lower, the values for OMD are higher (Cummings, 1984). However, not only the IDF: SDF ratio is important but also TDF content in the sample which is associated with OMD. The TDF content in the ripe and processed substrate was approximately half of TDF in the unripe; thus, lower OMD was expected in ripe papaya.

Nitrogen disappearance in ripe papaya (74.6 %) substrate was significantly higher (by 12 %) than in the other substrates after 24 hours of incubation (Table 4.3). When unripe (65.84 %) papaya and processed papaya (65.60 %) were used as substrates no significant differences in nitrogen disappearance were observed between them. Also when they were compared against casein (65.4 %), both substrates were slightly higher than the control (Table 4.3). The values from this experiment for ripe papaya are similar to protein digestibility values reported by Roberts et al. (2008). These results suggest that addition of ripe papaya could contribute to high nitrogen disappearance (protein digestibility) which suggests that papain remains active in ripe papaya after being exposed to the acidity of the stomach (pH 2.0).

Third stage *in vitro* fermentation

The unripe papaya substrate without sugar had the highest OMD (32.43 %) which was significantly different from the other papaya substrates and cellulose but was very similar to pectin (32.42 %) (Table 4.4). There was no significant impact of processing on ripe papaya OMD and DMD measurements. The highest OMD observed in unripe papaya could be attributed to its high content of TDF, which was higher than the values observed in the ripe samples. High values of OMD of sugar-containing samples (Table 4.3) as compared to those without sugar (Table 4.4) could be attributed to the amount of soluble carbohydrates present. The soluble sugars are speculated to have been lost during filtration, explaining the high OMD values from the *in vitro* digestion stage 2 samples (Table 4.3). The quantity of soluble carbohydrates used by the bacteria as an energy source is not well known. OMD by itself is not a precise measurement of bacterial fermentation, other indicators, such as total SCFA, are considered more accurate indicators of fermentability.

Changes in pH, total SCFA, and individual SCFA production after 12 hours of *in vitro* fermentation are reported in Table 4.5. The highly fermentable control (pectin) had the highest decrease in pH (-1.65). Amongst the substrates, the greatest pH reduction was observed in unripe papaya (-0.63), and the lowest in ripe papaya (-0.39). Generally, a change in pH is an indicator of total SCFA production. One might expect that those with the greatest decrease in pH would have the highest the total SCFA production. However, in this experiment no significant differences were observed in total SCFA production between unripe and ripe papaya. This indicates that pH changes alone cannot be used to predict SCFA production, since changes in pH values are expressed on a log scale which differs from the one that SCFA production are expressed.

Pectin fermentation generated the highest total SCFA production and cellulose the lowest (483.08 and 3.48mg/g DMB respectively). Among the substrates the highest production of total SCFA, acetate, and butyrate was observed in ripe papaya (424.15, 306.76, 55.73 mg/g DMB) but it was not significantly different from the amount produced by unripe (414.62, 295.41, 54.20 mg/g DMB). Processing had a detrimental impact on the total SCFAs, acetate, propionate, and butyrate production which decreased significantly (by 13 %, 6.6 %, 36.7 %, and 25 % respectively) after processing compared to ripe substrate (table 5). Total SCFA is an accurate indicator of fermentation; however the individual proportion of SCFA can provide us with additional information about the quality of the substrates.

The proportions of SCFA have small variation among substrates, the ratios of percentage of total SCFA for acetate: propionate: butyrate was 71:16:13 (unripe), 72:15:13 (ripe), and 78:11:11 (processed). Even though total SCFA significantly decreased after processing, the proportion of acetate increased compared to that in the other substrates. The acetate increase is in accordance with SDF increase after processing, which composed 52 % of the total SCFA (Table 4.1). For comparison to other reports, molar proportions were calculated for unripe (77:14:9), ripe (78:13:9), and processed (83:9:8) samples. Molar ratios of acetate: propionate: butyrate from our experiment were comparable to those reported for inulin (72:19:8) and oligofructose (78:14:8) after 24 h of *in vitro* fermentation (Wang and Gibson, 1993).

The greatest molar proportion of acetate (83 % of total SCFA) was produced by ripe processed papaya and the lowest by unripe papaya (77 % of the total SCFA). Acetate production by sample substrates was higher than that of pectin (71 %). The high acetate production found in all papaya substrates could be explained by the high content of pectin in papaya. Previously high levels of uronic acids (78.5 %) were reported in papaya fruit, indicating that pectin is the main

component of SDF (Chang et al., 1998). Our finding is in agreement with another study demonstrating that the type of SCFAs produced depends partially on the composition of the polysaccharide substrate (Cummings and Macfarlane, 1991). The higher the pectin content the higher the proportion of acetate is formed (Englyst et al., 1987).

The highest proportion of propionate among papaya substrates was produced by unripe fruit (14 % of total SCFA) and the lowest by ripe processed papaya (9 % of total SCFA). The main propionate function in humans is thought to be regulation of cholesterol synthesis. An increase in high-density lipoprotein (HDL) cholesterol was observed when a dose (7.5 g) of sodium propionate was consumed daily for 7 weeks by human subjects (Venter et al., 1990).

Butyrate plays an important role in prevention of colon cancer (Smith et al., 1998). In our experiment the butyrate proportion for unripe fruit was similar to ripe (9 % of total SCFA) and decreased after processing (to 8 % of total SCFA). Pectin had the highest propionate and butyrate proportion (16 % and 13 % of total SCFA). The molar proportion of butyrate from all samples in our experiment were higher than those reported for apple (68:26:6) and pear (70:25:5) (Casterline et al., 1997). Furthermore, production of propionate and butyrate has been associated with high concentrations of resistant starch (Bird et al., 2000).

Branched chain fatty acids BCFA (isovalerate, isobutyrate) and valerate (no branched but indicated with these due to similar origin) are produced by proteolytic bacteria which ferment protein residues in the gut. In our experiment ripe papaya showed the greatest production of BCFA (Table 4.6). This could be explained by high protein digestibility observed in ripe papaya which could be attributed to higher enzyme activity reported in ripe papaya (Table 4.3). These enzymes which are not digested constitute the substrate for the proteolytic bacteria in the gut.

CONCLUSIONS

The *in vitro* digestion experiment suggests that (after 6 hours incubation at 39 ^{O}C) ripe papaya has active proteolytic enzymes that might be only partially affected by the stomach acidity (pH 1.8) and pepsin degradation. This experiment indicated that ripe papaya showed the greatest nitrogen disappearance among the substrates, indicating that ripe papaya aids to digest protein in the GI tract. In addition to proteolytic enzymes, papaya also contains significant amounts of fiber. This study demonstrated that ripe papaya produced the highest amount of SCFA of the three papaya substrates. SCFA are used as indicator of the amount of substrate fermented by the microorganisms in the colon. The combination of proteolytic enzymes and fiber content found in papaya make of this fruit not only a potential digestive aid but also a good source of SCFA and all the health benefits that they provide. This research demonstrated that processed papaya experienced the lowest nitrogen disappearance and SCFA production among the papaya substrates. Future research studies should focus on the processing of papaya minimizing the detrimental impact on enzyme activity and SCFA production.

TABLES

Table 4.1. Chemical composition of three papaya substrates used in the *in vitro* experiment.

Substrate w sugar	% DMB					
	% DM	OM	CP	IDF	SDF	TDF
Unripe	76.82 ± 0.34	93.57 ± 0.01	6.061 ± 0.12	24.50 ± 0.02	11.27 ± 0.32	35.77 ± 0.31
Fully ripe	72.39 ± 0.10	96.27 ± 0.07	3.470 ± 0.09	11.48 ± 0.04	7.17 ± 0.43	18.65 ± 0.40
Processed	74.59 ± 0.37	97.13 ± 0.06	2.628 ± 0.01	7.38 ± 0.04	7.85 ± 0.12	15.23 ± 0.12

[a]Values are expressed as mean ± standard deviations. [b]IDF= insoluble dietary fiber, SDF= soluble dietary fiber, TDF= total dietary fiber (IDF+SDF), CP= crude protein, OM= organic matter, DM= dry matter, DMB= dry matter basis.

Table 4.2. Media composition used for the *in vitro* experiment stage 2.

Component	Concentration in medium
	mL/L
Solution A	330.0
Solution B	330.0
Trace mineral solution	10.0
Water- soluble vitamin solution	20.0
Folate: biotin solution	5.0
Riboflavin solution	5.0
Hemin solution	2.5
Short-chain fatty acid mix	0.4
Resazurin	1.0
Distilled Water	296.1
	g/L
Na_2CO_3	4.0
Cysteine HCl-H_2O	0.5
Trypticase	0.5
Yeast extract	0.5

Table 4.3. Organic matter (OMD), dry matter (DMD) and nitrogen disappearance (NitD) of three substrates and casein used in the *in vitro* experiment stage 1.

Substrate with sugar	%OMD (DMB)	%DMD (DMB)	%NitD (NitB)
Unripe	57.90[c]	50.30[b]	65.84[b]
Fully ripe	80.62[b]	74.81[a]	74.60[a]
Processed	82.88[a]	77.23[a]	65.60[b]
Casein	-	-	65.40[b]

[z]Values within the same column followed by different letter were significantly different at α=0.05 level.

Table 4.4. Organic matter and dry matter disappearance of three papayas substrates and 2 standards used in the *in vitro* experiment stage 2.

Substrate w/o sugar	%OMD (DMB)	%DMD (DMB)
Unripe	32.43[a]	32.27[a]
Fully ripe	20.86[b]	8.90[b]
Processed	22.24[b]	9.97[b]
Cellulose	0.00 [c]	0.00[b]
Pectin	32.42[a]	35.38[a]

[z]Values within the same column followed by different letter were significantly different at α=0.05 level.

Table 4.5. Acetate, Propionate, Butyrate, and Total Short- Chain Fatty Acid (SCFAs) production and pH change after 12 h of *in vitro* fermentation of three papaya substrates and 2 standards.

		mg/g DMB			
Substrate	pH change	Acetate	Propionate	Butyrate	Total SCFAs
Unripe	-0.63[d]	295.41[ab]	65.01[b]	54.20[b]	414.62[b]
Fully ripe	-0.39[b]	306.76[a]	61.66[b]	55.73[b]	424.15[b]
Processed	-0.47[c]	286.48[b]	39.01[c]	41.69[c]	367.18[c]
Cellulose	-0.02[a]	1.86[c]	1.45[d]	0.18[d]	3.48[d]
Pectin	-1.65[e]	313.07[a]	84.96[a]	85.05[a]	483.08[a]

[z]Values within the same column followed by different letter were significantly different at α=0.05 level.

Table 4.6. Total Brand Chain Fatty Acids (BCFA) production after 12 h of *in vitro* fermentation of three papaya substrates and two standards.

	mg/g DMB			
Substrate	Isobutyrate	Isovalerate	Valerate	Total BCFA
Unripe	0.20[b]	0.00[b]	2.10[b]	2.30[b]
Fully ripe	3.39[a]	0.72[a]	3.40[a]	7.51[a]
Processed	0.04[b]	0.00[b]	0.59[c]	0.63[c]
Cellulose	0.00[b]	0.00[b]	0.00[d]	0.00[c]
Pectin	0.00[b]	0.00[b]	0.00[d]	0.00[c]

[z]Values within the same column followed by different letter were significantly different at α=0.05 level.

REFERENCES

Abu-Goukh, A. B. A.; Bashir, H. A. Changes in pectic enzymes and cellulase activity during guava fruit ripening. *Food Chem.* **2003**, *83*, 213-218.

Abu-Goukh, A. B. A.; Shattir, A. E. T.; Mahdi, E. F. M. Physico-chemical changes during growth and development of papaya fruit. I: Physical changes. **2010**.

Adiotomre, J.; Eastwood, M. A.; Edwards, C. A.; Brydon, W. G. Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. *Am. J. Clin. Nutr.* **1990**, *52*, 128.

AOAC. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, DC. **1992**.

AOAC. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA. **2000**.

Balls, A. K.; Lineweaver, H. Isolation and properties of crystalline papain. *J. Biol. Chem.* **1939**, *130*, 669.

Bari, L.; Hassan, P.; Absar, N.; Haque, M.; Khuda, M.; Pervin, M.; Khatun, S.; Hossain, M. Nutritional analysis of two local varieties of papaya (Carica papaya L.) at different maturation stages. *Pak J Biol Sci.* **2006**, *9*, 137-140.

Bird, A. R.; Hayakawa, T.; Marsono, Y.; Gooden, J. M.; Record, I. R.; Correll, R. L.; Topping, D. L. Coarse brown rice increases fecal and large bowel short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *J. Nutr.* **2000**, *130*, 1780.

Buttle, D. J.; Kembhavi, A. A.; Sharp, S. L.; Shute, R. E.; Rich, D. H.; Barrett, A. J. Affinity purification of the novel cysteine proteinase papaya proteinase IV, and papain from papaya latex. *Biochem. J.* **1989**, *261*, 469.

Casterline Jr, J. L.; Oles, C. J.; Ku, Y. In vitro fermentation of various food fiber fractions. *J. Agric. Food Chem.* **1997**, *45*, 2463-2467.

Chang, S. C.; Lee, M. S.; Lin, C. J.; Chen, M. L. Dietary fiber content and composition of fruits in Taiwan. *Asia Pac. J. Clin. Nutr.* **1998**, *7*, 206-210.

Cummings, J.; Macfarlane, G. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Microbiol.* **1991**, *70*, 443-459.

Cummings J. H. (1984). Microbial digestion of complex carbohydrates in man. *Proc. Nutr. Soc*. **1984**, *43*, 35-44.

Edwin, F.; Jagannadham, M. Sequential Unfolding of Papain in Molten Globule State. *Biochem. Biophys. Res. Commun.* **1998**, *252*, 654-660.

Englyst H. N., Hay S., Macfarlane G. T. Polysaccharide breakdown by mixed populations of human fecal bacteria. *FEMS Microbiol Ecol*. **1987**, *95*, 163-171.

Fink, A. L., Calciano, L. J., Goto, Y., Kurostu, T., Pallerose, D. R. *Biochemistry.***1994**, *33*, 12504–12511.

Frenkel, C.; Klein, I.; Dilley, D. R. Protein synthesis in relation to ripening of pome fruits. *Plant Physiol.* **1968**, *43*, 1146.

Galindo-Estrella T., H., G.E.; R., M., J.; Sandoval-Fabian, G., Chel-Guerrero, L.; Rodriguez-Buenfil, I.; Gallegos-Tintore, S. Proteolytic activity in enzymatic extracts from Carica papaya L. cv. Maradol harvest by-products. *Process Biochemistry* **2009**, *44*, 77-82.

Hale, L. P. Proteolytic activity and immunogenicity of oral bromelain within the gastrointestinal tract of mice. *Int. Immunopharmacol.* **2004**, *4*, 255-264.

Haq, S. K.; Rasheedi, S.; Khan, R. H. Characterization of a partially folded intermediate of stem bromelain at low pH. *European Journal of Biochemistry* **2002**, *269*, 47-52.

He, J.; Klag, M. J.; Whelton, P. K.; Mo, J. P.; Chen, J. Y.; Qian, M. C.; Mo, P. S.; He, G. Q. Oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority of China. *Am. J. Clin. Nutr.* **1995**, *61*, 366.

He, F. J.; Nowson, C. A.; MacGregor, G. A. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *The Lancet* **2006**, *367*, 320-326.

He, F.; Nowson, C.; Lucas, M.; MacGregor, G. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *J. Hum. Hypertens.* **2007**, *21*, 717-728.

Huet, J.; Looze, Y.; Bartik, K.; Raussens, V.; Wintjens, R.; Boussard, P. Structural characterization of the papaya cysteine proteinases at low pH. *Biochem. Biophys. Res. Commun.* **2006**, *341*, 620-626.

Jaquet, A., Kleinschmidt, T., Schenk, A. G., Looze, Y., & Braunitzer, G. (1989). The thiol proteinases from the latex of carica papaya L. III. The primary structure of chymopapain. *Biol. Chem. Hoppe-Seyler.***1989**, *370*, 425-434.

Kelsay, J. L.; Behall, K. M.; Prather, E. S. Effect of fiber from fruits and vegetables on metabolic responses of human subjects, II. Calcium, magnesium, iron, and silicon balances. *Am. J. Clin. Nutr.* **1979**, *32*, 1876.

Lairon, D.; Arnault, N.; Bertrais, S.; Planells, R.; Clero, E.; Hercberg, S.; Boutron-Ruault, M. C. Dietary fiber intake and risk factors for cardiovascular disease in French adults. *Am. J. Clin. Nutr.* **2005**, *82*, 1185.

Liebman, B. Fresh fruit: A papaya a day? Nutrition Action Healthletter. **1992**, 10–11.
Mahattanatawee, K.; Manthey, J. A.; Luzio, G.; Talcott, S. T.; Goodner, K.; Baldwin, E. A. Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *J. Agric. Food Chem.* **2006**, *54*, 7355-7363.

McBurney, M. I.; Thompson, L. U. Effect of human faecal donor on in vitro fermentation variables. *Scand. J. Gastroenterol.* **1989**, *24*, 359-367.

Prosky, L., Asp, N. G., Schweizer, T. F., De Vries, J. W., & Furda, I. Determination of insoluble and soluble dietary fiber in foods and food products: Collaborative study. *AOAC Annual International Meeting.* **1992**, *75*, 360-367.

Ramulu, P.; Udayasekhara Rao, P. Total, insoluble and soluble dietary fiber contents of Indian fruits. *Journal of food composition and analysis.* **2003**, *16*, 677-685.

Roberts, M.; Minott, D. A.; Tennant, P. F.; Jackson, J. C. Assessment of compositional changes during ripening of transgenic papaya modified for protection against papaya ringspot virus. *J. Sci. Food Agric.* **2008**, *88*, 1911-1920.

Roberfroid, M. B. Inulin-type fructans: functional food ingredients. *J. Nutr.* **2007**, *137*, 2493-2502.

Rolls, B. J.; Ello-Martin, J. A.; Tohill, B. C. What can intervention studies tell us about the relationship between fruit and vegetable consumption and weight management? *Nutr. Rev.* **2004**, *62*, 1-17.

Roy,G. C. The solvent action of papaya juice on the nitrogenous articles of food. *Calcutta Med. J.* **1873**.

Schatzkin, A.; Mouw, T.; Park, Y.; Subar, A. F.; Kipnis, V.; Hollenbeck, A.; Leitzmann, M. F.; Thompson, F. E. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. *Am. J. Clin. Nutr.* **2007**, *85*, 1353.

Schulze, M. B.; Liu, S.; Rimm, E. B.; Manson, J. A. E.; Willett, W. C.; Hu, F. B. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *Am. J. Clin. Nutr.* **2004**, *80*, 348.

Smith, J. G.; Yokoyama, W. H.; German, J. B. Butyric acid from the diet: actions at the level of gene expression. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 259-297.

Swanson, K.; Grieshop, C.; Clapper, G.; Shields Jr, R.; Belay, T.; Merchen, N.; Fahey Jr, G. Fruit and vegetable fiber fermentation by gut microflora from canines. *J. Anim. Sci.* **2001**, *79*, 919.

Tripathi, S.; Suzuki, J. Y.; Carr, J. B.; McQuate, G. T.; Ferreira, S. A.; Manshardt, R. M.; Pitz, K. Y.; Wall, M. M.; Gonsalves, D. Nutritional composition of Rainbow papaya; the first commercialized transgenic fruit crop. *Journal of Food Composition and Analysis.* **2010**.

USDA. National Nutrient Data Base For Standard Reference. (accessed August/28, 2010). http://www.nal.usda.gov/fnic/foodcomp/search

Venter C. S., Vorster H. H., Cummings J. H. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am. J. Gastroenterol*. **1990**, *85*, 549-553.

Vos, A. P.; M'Rabet, L.; Stahl, B.; Boehm, G.; Garssen, J. Immune-modulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. *Crit. Rev. Immunol.* **2007**, *27*, 97-140.

Wang, X.; Gibson, G. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Microbiol.* **1993**, *75*, 373-380.

Wills, R. B. H.; McGLasson, W. B.; Graham, D.; Joyce, D. C. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals. *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals.* **2007**, vi-ix 227.

Wittmack, C. The fermentative action of the juice of the fruit of *Caricu pupuyu. J. Pharm.*, **1878**, *9*, 449.

Wolk, A.; Manson, J. A. E.; Stampfer, M. J.; Colditz, G. A.; Hu, F. B.; Speizer, F. E.; Hennekens, C. H.; Willett, W. C. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. *JAMA: the journal of the American Medical Association* **1999**, *281*, 1998.

Wurtz, A., Bouchut, E. Sur le ferment digestif du *Carica papaya.* Compt. rend. **1879**, *89*, 425

World Cancer Research Fundation Panel. Food, Nutrition and the Prevention of Cancer: A Global Perspective. American Institute for Cancer Research: Washington, DC, **1997**.

CHAPTER 5

Impact of irradiation processing on papaya (*Carica papaya var.* Rainbow) nectar flavor

ABSTRACT

Papaya fruit is a rich source of carotenoids, potassium, fiber, and ascorbic acid. However, dramatic limitations exist to global consumption of papaya, due to relatively high perishability of fresh fruit. There is a need to develop a high quality, acceptable processed papaya product with minimal nutrient losses and minimal flavor changes due to processing. A pulp-based papaya nectar was developed using irradiation to diminish negative impacts of thermal processing on flavor and nutrients. The objective of this study was to investigate flavor changes of papaya nectar upon exposure to irradiation plus mild heat treatment. Fully ripened papaya fruit (*Carica papaya* L., Rainbow variety) was pulped, diluted, and processed with irradiation (5 kGy) or a combination of irradiation and mild heat (80°C, 5 min). Volatile components were profiled using combined Solvent-Assisted Flavor Evaporation, Gas Chromatography–Mass Spectrometry, and Gas Chromatography–Olfactometry. Informal testing upon product development indicated irradiation increased sweetness perception of nectars. Flavor analysis of neutral/basic and acidic fractions revealed approximately 50 compounds. Among the components identified, alcohols, esters, aldehydes, and ketones, were investigated further through literature research for their potential role in sweetness perception of the nectars. Irradiation has been shown to serve as an acceptable alternative to thermal processing for papaya nectar. Enhancement of sweetness

perception by irradiation was not linked to specific flavor components, yet likely linked to structural changes in pulp initiated by irradiation.

INTRODUCTION

Papaya (*Carica papaya*) is a tropical fruit of rapid growth and short life span. It is a perennial plant originally from tropical America and is grown in every tropical and subtropical country. Papaya is popular due to its high content of vitamin (A and C), minerals (K, P, Fe), and low calorie content (Samson, 1986; Lobo and Cano, 1998). In the US, papaya is grown predominantly in Hawaii, where it constitutes a commercially important fruit crop.

Papaya constitutes the fifth largest crop in Hawaii; it has always been an important crop for the Hawaiian economy. The Solo papaya, introduced to Hawaii in 1910, was the most dominant variety for commercial production until the ringspot virus almost destroyed the entire Hawiian papaya crop (Gonsalves, 1998). The first genetically engineered papaya, Rainbow, was commercialized in 1998. This papaya included a gene that made the fruit resistant to papaya ringspot virus. The Rainbow papaya initially was an F-1 hybrid created by crossing the Kapoho Solo yellow-flesh variety with the SunUp red-flesh variety (Manshardt, 1998). Currently, approximately 77% of the total papaya acreage in Hawaii is planted with Rainbow variety (NASS, 2009).

Papaya is a highly perishable fruit, a characteristic that limits its large scale distribution. During transportation of fresh fruit, papaya may suffer from chill injury, bruising, wrinkling, and softening; all of these factors affect the acceptability of this fruit (Penteado and Leitao, 2004). These issues highlight the importance of the preservation of papayas as a needed field of study to add value, improve shelf life, and enhance accessibility of the fruit. Traditional pasteurization

methods result in development of cooked flavor in papaya juice; this is one of the reasons why papaya juice is often mixed with other fruits to mask the off flavors (Tiwari, 2000).

Our laboratory previously developed papaya nectar using a combination of irradiation (5 kGy or 7.5 kGy) and mild heat (80^{O}C, 5 min) as an alternative to traditional pasteurization (Parker et al., 2010). Informal sensory testing indicated that irradiated samples were sweeter than the control, in addition to the improvement of texture/mouthfeel, noticed through an apparent softening of the pulp. Preliminary carbohydrate analysis revealed no significant changes in sugar composition. Total soluble solids (Brix) and titratable acidity of the two varieties of papaya nectar (Rainbow and SunUp) were not significantly impacted by irradiation processing. Enhanced sweetness perception in irradiated samples could be due to changes in flavor compounds; that could be masking/altering sweetness perception (Bakker, 1995; Parker *et al.*, 2010).

The characteristic papaya fresh fruit aroma is sweet, cantaloupe like, with some muskiness (Morton, 1987). The volatile composition of papaya fruit was studied (McLeod and Pieris, 1983; Idstein and Schreier, 1985; Flath et al., 1990; and Pino et al., 2003). Papaya has a characteristic aroma due to many volatile compounds such as alcohols, esters, aldehydes, and sulfur compounds (Almora et al., 2004). Volatile recovery from papaya, Solo variety, was evaluated using four methods: vacuum trapping train, co-distillation extraction (vacuum), vacuum distillation, and co-distillation extraction at 1 atm (Flath and Forrey, 1977).

Regardless of the variability of recovery methods, linalool and benzyl isothiocyanate were the major compounds detected by all four methods. Linalool is a terpene alcohol, identified as one of the most important odorants present in papaya fruit (Chan et al., 1973; Flath and Forrey, 1977; Schreier and Winterhalter, 1986). Both linalool and benzyl isothiocyanate, are

produced by enzymatic degradation of benzyl glucosinolate by the thioglucosidase (myrosinase) enzyme after cellular disruption (Tang, 1971; Heidlas et al., 1984). Papaya fruit is one of the few known crops that contain glucosinolates outside of the Cruciferae family.

Low concentrations of linalool were reported in the solvent extraction of volatiles from fresh papaya from Sri Lanka (MacLeod and Pieris, 1983). These researchers found phenylacetonitrile in high amounts (17.7 %) and also suggested that methyl butanoate could be responsible for the sweaty note of papaya. Others reported that oxygenated terpenoids derived from linalool could play an important role in Brazilian papaya aroma (Winterhalter et al., 1986). Studies in Papaya Maradol roja variety showed an increase in linalool and decrease in benzyl isothocyanate production during ripening of the fruit (Almora et al., 2004).

Volatile components from Hawaii papaya, Solo, at four ripeness stages, from green to fully ripe were recovered by trapping with Tenax (Flath et al., 1990). As anticipated, the majority of volatile components were detected in fully ripe fruit, including the following major components: linalool, linalool oxide A, linalool oxide B, and ethyl acetate. Linalool and certain aldehydes (acetaldehyde, hexanal, phenylacetaldehyde, benzaldehyde) were also detected in all four ripeness stages of the fruit. Other studies reported esters as the predominant volatile components (41 %) in papaya Maradol variety (Pino et al., 2003). Methyl butanoate and ethyl butanoate were reported as the major compounds after simultaneous steam distillation-solvent extraction. Studies from Sri Lanka and Colombia also reported esters as the major volatile compounds in papaya, representing 50 % of total volatiles (Heidlas et al., 1984; MacLeod and Pieris, 1983).

The most widely studied cultivar of papaya is Solo. The composition of volatile components in fruits can vary between cultivars and over different regions of production. Few

reports exist on the identification of the flavor papaya volatiles of the Rainbow cultivar, which was utilized in this study.

Heat processing, such as pasteurization, can cause reduction of sensory quality in some fruit juices which could be due to loss or changes in volatile compounds. Papaya fruit is very sensitive to thermal processes. According to previous studies, traditional pasteurization methods develop cooked flavor in papaya juice; this is one of the reasons why it is often mixed with other fruits to mask the off flavors (Argaiz and Lopez-Malo, 1995; Tiwari, 2000). Heat treatment has been combined with irradiation to minimize the heat impact on the juice flavor and for potential microbial inactivation (Parker et al., 2010). However, irradiated samples were perceived sweeter than the non-irradiated during informal sensory test in our laboratory. Gomez et al., (2002) suggested that changes in texture of papaya fruit (i.e., softening) could be responsible for enhancing sweetness perception since this would enable the liberation of sugar from the papaya cell.

The objective of this study was to evaluate the impact of irradiation on the flavor compounds of papaya nectar. Our goal was to identify flavor compounds present in irradiated and non-irradiated samples, with the aim of identifying specific compounds that could be responsible for the enhanced sweetness perception detected in the irradiated samples. This would help understand the cause of enhancement of sweetness in irradiated papaya nectar samples and resolve whether or not this was due to flavor components.

MATERIALS AND METHODS

Materials

Carica papaya L. Rainbow variety was graciously donated by the Hawaii Papaya Industry Association and the Hawaii Department of Agriculture. Papayas were heat treated (47°C) with water vapor for 4 hr. at 90 % humidity to eliminate fruit fly infestation and cooled 1 hr prior to shipment by 2-d air. Fruit were stored at controlled room temperature until they were 100 % yellow before pulping.

Sample preparation

Papayas were halved, seeds were manually removed and flesh was pulped using a Kitchen Aid K5SS with pulper attachment (Hobart Corporation, Troy, OH). Pulp Brix was measured using a Westover Scientific RHB-32ATC hand-held refractometer (Mill Creek, WA). Pulp was diluted to 8 Brix using double deionized water. Nectars were divided into 250 mL glass bottles and frozen (-20ºC) overnight. Frozen nectars were irradiated in a Gammacell 220 Excel with a cobalt-60 source (MDS Nordion, Ottawa, ON, Canada) in the irradiation facility at the University of Illinois. After irradiation samples were exposed to brief heat exposure (80°C, 5 min) for the purpose of inactivation of enzymatic activity (based upon Parker et al., 2010), remaining samples served as unheated controls. Samples were stored at -20ºC until used for volatile isolation.

Volatile isolation by Solvent-Assisted Flavor Evaporation (SAFE)

Samples were thawed overnight at 4ºC. They were diluted with deodorized water (double deionized water that was boiled until it was reduced by two-thirds of its original volume) to 500

g and mixed with 5 μL of an internal standard solution (containing 1 mg/ mL each of 6-undecanone, 2-methyl-3-heptanone, and 2-ethyl butyric acid diluted in methanol). The combined nectar and standards were sieved through nylon mesh (Trimaco Co; Durham, NC). Distillation started immediately using the solvent assisted flavor evaporation (SAFE) apparatus according to the operating procedures described by Rotsatchakul et al. (2007). SAFE instrument consisted of a transfer head, 1 L round bottom flask kept at 40°C in a water bath, and two nitrogen cooled traps (receiving trap and waste trap) were held separately in Dewar flasks containing liquid nitrogen at all times.

Small aliquots (100 mL) of homogenate were fed into the SAFE apparatus every 15 min followed by an additional 3.0 hr of distillation. Volatiles traveled through the outlet line of the separation head to the receiving tube where they were condensed and frozen. The total extraction was completed under vacuum condition (~ 10^{-5} Torr) in approximately 4.5 hrs. The receiving tube was removed and diethyl ether (50 mL) was added to the cryogenic trap containing the papaya nectar volatiles. The trap was then thawed overnight in a Dewar flask. The aqueous phase was washed three times in a 1 L separatory funnel with 50 mL diethyl ether, and the aqueous phase was discarded. The aroma (ether) extract was separated into acidic and neutral-basic fractions. For this, the ether extract was first concentrated by distillation to 30 mL using a Vigreux column in a water bath at 43ºC. The extract was placed in a 125 mL separatory funnel and washed three times with 20 mL of sodium bicarbonate (0.5 M). The neutral-basic extract (ether layer) was washed twice with 10 mL saturated sodium chloride solution and concentrated by distillation to 10 mL using a Vigreux column at 43ºC. This dried over anhydrous sodium sulfate, concentrated to 2 mL in water bath (43ºC) using a Vigreux column and then concentrated under a gentle nitrogen gas stream to 200 μL.

To obtain the acid compounds the acidic fraction (bottom or aqueous layer) was acidified to pH ~ 2.0 with hydrochloric acid (18 %) and extracted three times with 20 mL ethyl ether. The ether layer containing the acidic volatiles was washed twice with 15 mL saturated sodium chloride and subjected to the same concentration procedures used for neutral-basic fraction. Both neutral-basic and acidic fractions of each treatment (control, irradiated, and irradiated + heat) were individually stored in a 1.5 mL septum-capped Target DP vial (National Scientific, Rockwood, TN) at -70ºC until GC-MS and GCO analysis.

Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS system consisted of a 6890 GC/5973N mass selective detector MSD (Agilent Technologies Inc.). Aroma extract (1 μL) was injected into a cool on-column inlet (+3°C oven tracking mode). Separations were performed using a nonpolar column SAC-5 (30 m x 0.25 mm i.d.; 0.25 μm film thickness; Supelco) and a polar column RTX®-Wax (30.0 m x 0.25 mm i.d. 0.25 μm film thickness; Restek, Bellefonte, PA). Helium served as carrier gas at rate of 1.0 mL/min constant flow mode. Initial oven temperature was programmed at 35°C (5 min) to final temperature 225°C (20 min) at a rate of 4°C/min. MSD conditions were: capillary direct interface temperature 280°C; ionization energy 70 eV; mass range 35 to 300 amu; electron multiplier voltage (Autotune + 200 V); scan rate 5.27 scans/s.

Gas Chromatography Olfactometry (GCO)

The GCO instrument consisted of a 6890 GC (Agilent Technologies Inc.) equipped with a flame ionization detector (FID), an on-column injector, and a sniff port (DATU Technology Transfer, Geneva, NY). To evaluate the aroma-active compounds each fraction was injected (1

μL) by cool on-column mode (+3°C oven tracking mode) into either of two columns of different polarities: nonpolar RTX-5MS (15m x 0.32 mm i.d.; 0.5 μm film thickness, Restek) or a polar RTX®-Wax column (15m x 0.32 mm i.d.; 0.5 μm film thickness, Restek). Helium was the carrier gas at 2.2 mL/min constant flow. The GC oven temperature was programmed from 40 to 225ºC at 5ºC /min, with initial and final holding times of 5 and 40 min, respectively. FID and transfer line were held at 200ºC. Other GCO conditions have been previously described by Cadwallader et al. (1995).

Compound identification

Tentative compound identification was done by comparing retention indices (RI) from 2 GC column phases and mass spectra of the unknown compounds with literature values and the mass spectra database from the National Institute of Standards and Technology (NIST, 1995). Retention indices were determined using a series of n-alkenes that were analyzed following the same chromatographic conditions (Van Den Dool, et al., 1963). Specific compounds were selected from GCO and GC-MS to determine quantitative changes across treatments. Identifications of selected compounds were confirmed by use of authentic standard compounds (linalool, delta-octalactone, benzyl isothiocyanate, (*Z,Z)*-3,6-nonadienal, alpha- ionone, butyric acid, 4-hydroxy-2, 5-dimethyl 3(2H)-furanone).

RESULTS AND DISCUSSION

The objective of this study was to evaluate the impact of processing on the flavor of papaya nectar and determine whether the difference in sweetness perception was due to changes in flavor volatiles resulting from irradiation. To accomplish this objective volatile compounds

from ripe papaya nectar processed (irradiated and irradiated plus heat) and non-processed, were isolated using high vacuum distillation by SAFE followed by solvent extraction.

Gas chromatography olfactometry

Predominant odor active compounds were identified by gas chromatography-olfactometry (GCO). A total of 44 compounds were detected in the neutral/basic and acidic fractions combined (Tables 5.1 and 5.2). Thirty five compounds were detected in the neutral/basic fractions (Table 5.1) from which 30 were tentatively identified. Nine compounds were detected in the acidic fraction and 8 were tentatively identified (Table 5.2). The odorant that showed the highest odor intensity in the neutral/basic fraction across all treatments was linalool, with a characteristic aroma described as floral, fresh, lavender-like (Flath and Forrey, 1977; MacLeod and Pieris, 1983). Previous studies on Hawaiian papaya Solo variety have reported linalool and benzyl isothiocyanate as the most significant aroma contributors and abundant compounds in papaya fruit (Chan et al., 1973; Flath and Forrey, 1977; Flath et al., 1990). In our study it was observed that benzyl isothiocyanate was not one of the most significant contributors of papaya aroma as indicated by GCO. Important aldehydes identified in our papaya nectar were: (*E,Z*)-2,6 nonadienal (cucumber), (*Z,Z*)-3,6-nonadienal (watermelon), and methional (potato). Some lactones were also among the potent odorants present in our samples such as delta-octalactone and gamma-octalactone (both coconut aroma); the presence of these lactones in papaya have been previously reported by other researchers (Flath and Forrey, 1977; Macleod and Pieris, 1983; Flath et al., 1990; Pino et al., 2003).

The following compounds: 1-octen-3-ol (mushroom), p-cresol (stable), and vinylguaiacol (cinnamon) were only detected in the irradiated + heat sample; 2-acetyl-2-thiazoline (popcorn)

was detected only in both processed samples (irradiation, and irradiation + heat); while (*E*)-2-nonenal (hay) was present only in the control sample. Methional (potato) and alpha-ionone (floral) increased in odor intensity by two-fold in the heated sample (Table 5.1). In addition, linalool and benzyl isothiocyanate slightly decreased in odor intensity after irradiation. The most potent odorant in the acidic fraction (Table 5.2) was butyric acid (cheesy aroma), followed by phenyl acetic acid (rosy). Flath et al. (1977) also reported butyric acid as the most abundant acidic compound in papaya Solo variety.

This is the first report of the following flavor compounds being identified in papaya nectar: (*E,Z*)-2,6 nonadienal (cucumber), (*Z,Z*)-3,6-nonadienal (watermelon), methional (potato), and (*Z*)-1,5-octadien-3-one (geranium).

Gas Chromatography-Mass Spectrometry results

Selected ions were used to determine the concentration of the most abundant compounds detected by GC-MS (Table 5.1 and 5.2); benzyl isothiocyanatomethyl was the most abundant component. The second most abundant was a group of terpene alcohols (linalool, (*Z*)-linalool oxide, (*E*)-linalool oxide, epoxy linalool, epoxylinalool oxide), with (*Z*)-linalool oxide being the most abundant. Linalool was the strongest odorant detected by GCO in our papaya nectar; however, GC-MS indicated that it was present in low concentrations. Previous research on Hawaiian papaya Solo variety reported linalool as the most abundant flavor (67% w/w) compound (Flath and Forrey, 1977). This dissimilarity in compound abundance between both experiments could be attributed to the different papaya variety used; Rainbow variety was used in our experiment. MacLeod and Pieris, (1983) also reported low concentrations of linalool among volatiles of Sri Lankan papaya; the most abundant compounds were esters (53% w/w

sample). Similar findings were observed in Maradol papaya (Pino et al., 2003). Studies on papaya volatiles indicated that benzyl isothiocyanate and benzyl nitriles are formed after cell disruption by degradation of benzyl glucosinolate by thioglucosidase enzyme action (Tang, 1971; Heidlas et al., 1984).

High variability between replications was observed in our results from GC-MS, making it difficult to draw conclusions about the impact of processing on papaya flavor compounds. For this reason the replications have been reported in separate tables (Tables 5.3 and 5.4). However, this variability between replications significantly decreased when the percentage of the concentration of each compound was calculated (Table 5.5). This indicates that variability in concentration of compounds present in the different treatments was due to internal standard variation and not to the treatments. Only two compounds, benzyl nitrile and beta ionone were more abundant in proportion in irradiated samples than in control samples.

Previous researchers calculated concentrations of compounds expressed in percentages to show the abundance of compounds in the sample (Flath et al., 1977; MacLeod and Pieris, 1983; Pino et al., 2003). Although this seems to be a useful tool to eliminate variation due to the internal standard in our samples (Table 5.5, 5.6, and 5.7), it is not considered a reliable basis from which to draw conclusions, because each percentage is based upon the concentration of the other components in the sample, thus a compound's percent abundance is dependent upon the abundances of other compounds.

CONCLUSIONS

From the GCO data it can be concluded that processing did not have a major impact on papaya flavor volatiles. There were three compounds present only in the irradiated + heat sample

(1-octen-3-ol, p-cresol, and vinylguaiakol); their development could be attributed to the heat process. Irradiation fostered formation of 2-acetyl-2-thiazoline (roasted, popcorn note) and degradation of (*E*)-2-nonenal (green, hay aroma). However, based upon previous reports of flavor contributions of these two compounds, one would not expect them to be responsible for the sweeter taste detected in the irradiated nectar (Buttery et al., 1994).

High variability in GC-MS replications could be attributed to storage time, different people conducting the extraction, concentration problems, variation in application of the method, or internal standard problems. When variability due to the internal standard was eliminated, the variation between replications also disappeared.

It was not possible to determine from the GC-MS data whether irradiation has an impact on one or more flavor compounds responsible for the sweeter flavor detected in irradiated samples. Gomez et al. (2002) found that ripe and intermediately ripened papayas were perceived sweeter than mature green papaya even at the same total soluble sugar content (glucose, fructose, and sucrose). They suggested that this could be due to changes in texture (softer texture) which would enable the sugar liberation from the papaya cell for the sensory perception. This would be a reasonable explanation for changes in our samples after irradiation. Informal testing in our laboratory revealed smoother texture and smaller particle size in irradiated nectars than the control, which may have an impact on the perceived sweeter taste of the irradiated samples.

TABLES

Table 5.1. Odor-active compounds detected by GCO in the neutral/basic fraction of papaya nectar.

Name	Odor Description	RI WAX	RI DB-5	Odor intensity[b] CTRL	IRR	IRR+H
ethyl acetate	nail polish remover, fruity	807	<700	1.0	1.0	1.0
ethyl butyrate	fruity	950	760	1.0	0.5	0.5
(*Z*)-3-hexenal	green, grassy	1140	801	1.5	2.0	1.5
unknown	dried fruit, buggy	1144	-	0.5	1.5	1.0
octanal	orange	1285	1002	0.5	2.0	1.0
1-octen-3-one	mushroom	1295	977	1.0	1.0	1.5
(*Z*)-1-5-octadien-3-one	metallic, geranium	1367	980	3.0	3.0	3.0
dimethyltrisulfide	cabbage	1374	971	0.5	1.5	0.5
3-mercapto-4-methyl-2-pentanone	catty	1376	944	1.5	0.5	2.0
1-octen-3-ol	mushroom	1402	979	n.d.	n.d.	1.0
methional †	potato	1446	908	2.0	2.5	4.0
(*Z,Z*)-3,6-nonadienal †	watermelon	1498	1093	3.5	3.5	3.0
(*Z*)-2-nonenal	hay	1504	1146	1.5	n.d.	0.5
(*E*)-2-nonenal	hay	1527	1161	1.0	n.d.	n.d.
linalool †	floral, spicy coriander	1540	1100	5.5	4.0	4.5
(*E,Z*)-2,6-nonadienal	cucumber	1579	1152	3.0	2.5	3.0
unknown	vitamin, meaty	1653	-	n.d.	1.0	n.d.
unknown	hay like, green, metallic	1716	-	3.0	2.5	3.5
2-acetyl-2-thiazoline	popcorn, corn chip	1736	-	n.d.	1.0	1.0
unknown	melon, cucumber	1764	-	1.0	n.d.	0.5
β-damascenone	cooked apple	1817	1383	1.0	n.d.	3.0
alpha-ionone	perfume, floral	1842	-	0.5	0.5	2.0
2,4,6-nonadienal isomer	oats	1861	1277	2.0	2.0	2.5
2-phenylethanol	floral, rosy	1900	1139	1.0	1.5	1.0
gamma-octalactone	coconut, herbaceous	1901	1259	2.0	2.0	2.0
delta-octalactone †	coconut, herbaceous	1956	1290	2.5	2.5	3.0
(E)-4,5-epoxy-(*E*)-2-decenal	metallic, unripe	1994	-	2.5	1.5	2.0
gamma-nonalactone	coconut, peachy	2019	-	1.0	1.0	n.d.
p-cresol (4-methylphenol)	stable, dung	2066	1091	n.d.	n.d.	1.5
benzyl isothiocyanate †	mustard, pungent	2095	1369	4.0	2.5	2.0
vinylguaiakol	cinnamon, cloves	2178	-	n.d.	n.d.	2.0
gamma-dodecalactone	coconut	2365	-	0.5	n.d.	1.0
lauric acid	waxy, liver	2463	1570	2.0	3.0	2.5
unknown	grape, plastic	-	1371	n.d.	n.d.	2.0
gamma-decalactone	grapy, peachy, coconut	-	1469	1.0	1.0	2.0

[a]RI = retention index, DB-WAX and DB-5 = polar and non-polar columns, [b]Odor intensity determined using DB-WAX and DB-5 columns: CTRL= control sample, IRR = irradiated sample, IRR+H = irradiated + heat. [b]Odor intensity: 1= very weak, 2= weak, 3= medium, 4= medium strong, 5= strong, 6= very strong. † Identified compounds.

Table 5.2. Odor-active compounds detected by GCO in the acidic fraction of papaya nectar.

Name	Odor Description	RI WAX	RI DB-5	Odor intensity CTRL	Odor intensity IRR	Odor intensity IRR+H
acetic acid	vinegar	1434	n.d	1.0	1.5	1.5
butyric acid †	cheesy, fecal	1603	828	5.5	5.5	5.5
isovaleric acid	cheesy	1654	878	1.0	2.0	1.0
4-hydroxy-2,5-dimethyl-3(*2H*)- furanone †	burnt sugar	2010	n.d	1.0	-	1.0
octanoic acid	sweaty	2020	n.d	2.0	1.0	1.0
decanoic acid	waxy	2185	n.d	0.5	-	1.0
Unknown	waxy, candle	2234	n.d	2.0	-	1.5
phenyl acetic acid	rosy, honey like	2523	n.d	1.0	3.0	2.0
vanillin	vanilla	2511	1412	1.0	0.5	1.5

[a]RI = retention index, DB-WAX and DB-5 = polar and non-polar columns, CTRL= control sample, IRR = irradiated sample, IRR+H = irradiated + heat. [b]Odor intensity: 1= very weak, 2= weak, 3= medium, 4= medium strong, 5= strong, 6= very strong.† Identified compounds.

Table 5.3. Relative concentration of flavor compounds present in papaya nectar measured by GC-MS in the neutral/basic fraction using DB-Wax column, replicate I.

Compound name	CTRL I (ug/Kg sample) Conc. Comp	IRR I (ug/Kg sample) Conct. Comp	IRR + H I (ug/Kg sample) Conct. Comp
(*Z*)-linalool oxide	133	442	458
(*E*)-linalool oxide	30.4	106	110
Linalool	24.8	51.1	60.8
epoxy linalool	38.9	213.1	206
epoxy linalool oxide	3.50	0.00	0.00
benzyl alcohol	15.3	75.6	66.8
benzyl nitrile	6.06	114	203
benzene isothiocyanate	410	106o	103o
gamma- octalactone	14.1	53.3	55.5
delta-octalactone	7.04	38.1	33.5
β-ionone	1.20	4.10	19.6
Total compound amount	684	216o	224o

[b]CTRL I = control sample replica 1, IRR I = irradiated samples replica 1, IRR+H I = irradiated + heated samples replica 1.

Table 5.4. Relative concentration of flavor compounds present in papaya nectar identified by GC-MS in the neutral/basic fraction using DB-Wax column, replicate II.

Compound name	CTRL II (ug/Kg sample) Conc. Comp	IRR II (ug/Kg sample) Conct. Comp	IRR + H II (ug/Kg sample) Conc. Comp
(*Z*)-linalool oxide	155	133	138
(*E*)-linalool oxide	35.5	30.8	39.4
linalool	29.1	22.8	19.74
epoxy linalool	46.8	46.4	51.6
epoxy linalool oxide	0.00	3.58	0.00
benzyl alcohol	15.4	17.8	16.7
benzyl nitrile	31.5	45.2	100
benzene isothiocyanate	409.7	402	276
gamma-octalactone	15.9	12.2	16.1
delta-octalactone	1.52	4.52	4.33
β-ionone	2.19	2.19	4.84
Total compound amount	743	721	667

[b]CTRL I = control sample replica 1, IRR I = irradiated samples replica 1, IRR+H I = irradiated + heated samples replicate 1.

Table 5.5. Percentage concentration of flavor compounds present in papaya nectar identified by GC-MS in the neutral/basic fraction using DB-Wax column.

	CTRL I Conc % (w/w)	CTRL II Conc % (w/w)	IRR I Conc % (w/w)	IRR II Conc % (w/w)	IRR+H I Conc % (w/w)	IRR+H II Conc % (w/w)
(*Z*)-linalool oxide	19.4	20.8	20.4	18.5	20.4	20.7
(*E*)-linalool oxide	4.44	4.79	4.90	4.27	4.88	5.90
linalool	3.63	3.92	2.36	3.17	2.71	2.96
epoxy linalool	5.70	6.31	9.85	6.44	9.17	7.73
epoxy linalool oxide	0.51	0.00	0.00	0.50	0.00	0.00
benzyl alcohol	2.23	2.07	3.49	2.48	2.97	2.50
benzyl nitrile	0.89	4.24	5.25	6.26	9.04	15.1
benzene isothiocyanate	59.9	55.2	49.3	55.8	45.9	41.4
gamma-octalactone	2.06	2.15	2.46	1.69	2.47	2.41
delta-octalactone	1.03	0.20	1.76	0.63	1.49	0.65
β-ionone	0.18	0.29	0.19	0.30	0.87	0.73

[c]CTRL I and II = control sample replica 1 and 2, IRR I and II= irradiated samples replica 1 and 2, IRR+H I and II = irradiated + heat samples replicate 1 and 2.

Table 5.6. Percentage concentration of flavor compounds present in papaya nectar Identified by GC-MS in the neutral/basic fraction using DB-Wax column, replica I.

	CTRL I Conc %	IRR I Conc %	IRR+H I Conc %
(*Z*)-linalool oxide	19.4	20.4	20.4
(*E*)-linalool oxide	4.44	4.90	4.88
linalool	3.63	2.36	2.71
epoxy linalool	5.70	9.85	9.17
epoxy linalool oxide	0.51	0.00	0.00
benzyl alcohol	2.23	3.49	2.97
benzyl nitrile	0.89	5.25	9.04
benzene isothiocyanate	59.9	49.3	45.9
gamma-octalactone	2.06	2.46	2.47
delta-octalactone	1.03	1.76	1.49
β-ionone	0.18	0.19	0.87

Table 5.7. Percentage concentration of flavor compounds present in papaya nectar identified by GC-MS in the neutral/basic fraction using DB-Wax column, replica II.

	CTRL II Conc %	IRR II Conc %	IRR+H II Conc %
(*Z*)-linalool oxide	20.8	18.5	20.7
(*E*)-linalool oxide	4.79	4.27	5.90
linalool	3.92	3.17	2.96
epoxy linalool	6.31	6.44	7.73
epoxy linalooloxide	0.00	0.50	0.00
benzyl alcohol	2.07	2.48	2.50
benzyl nitrile	4.24	6.26	15.1
benzene isothiocyanate	55.2	55.8	41.4
gamma-octalactone	2.15	1.69	2.41
delta-octalactone	0.20	0.63	0.65
β-ionone	0.29	0.30	0.73

REFERENCES

Almora, K.; Pino, J. A.; Hernandez, M.; Duarte, C.; Gonzalez, J.; Roncal, E. Evaluation of volatiles from ripening papaya (Carica papaya L., var. Maradol roja). *Food Chem.* **2004**, *86*, 127-130.

Bakker, J. Flavor interactions with the food matrix and their effects on perception. *Food Science and Technology. NewYork. Marcel Dekker*. **1995**, 411-411.

Buttery, R. G.; Stern, D. J.; Ling, L. C. Studies on Flavor Volatiles of Some Sweet Corn Products. *J. Agric. Food Chem.* **1994**, *42*, 791-795.

Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2432-2437.

Camargo, R. J.; Tadini, C. C.; Sabato, S. F. Physical-chemical analyses of irradiated papayas (Carica papaya L.). *Radiat. Phys. Chem.* **2007**, *76*, 1866-1868.

Chan Jr, H. T.; Flath, R. A.; Forrey, R. R.; Cavaletto, C. G.; Nakayama, T.; Brekke, J. E. Development of off-odors and off-flavors in papaya puree. *J. Agric. Food Chem.* **1973**, *21*, 566-570.

Flath, R. A.; Forrey, R. R. Volatile components of papaya (Carica papaya L., Solo variety). *J. Agric. Food Chem.* **1977**, *25*, 103-109.

Flath, R. A.; Light, D. M.; Jang, E. B.; Mon, T. R.; John, J. O. Headspace examination of volatile emissions from ripening papaya (Carica papaya L., Solo Variety). *J. Agric. Food Chem.* **1990**, *38*, 1060-1063.

Gomez, M.; Lajolo, F.; Cordenunsi, B. Evolution of soluble sugars during ripening of papaya fruit and its relation to sweet taste. *J. Food Sci.* **2002**, *67*, 442-447.

Gonsalves, D. Control of papaya ringspot virus in papaya: a case study. *Annu. Rev. Phytopathol.* **1998**, *36*, 415-437.

Idstein, H.; Schreier, P. Volatile constituents from guava (Psidium guajava, L.) fruit. *J. Agric. Food Chem.* **1985**, *33*, 138-143.

Heidlas, J.; Lehr, M.; Idstein, H.; Schreier, P. Free and bound terpene compounds in papaya (Carica papaya, L.) fruit pulp. *J. Agric. Food Chem.* **1984**, *32*, 1020-1021.

MacLeod, A. J.; Pieris, N. M. Volatile components of papaya (Carica papaya L.) with particular reference to glucosinolate products. *J. Agric. Food Chem.* **1983**, *31*, 1005-1008.

Manshardt, R. M. Production requirements of the transgenic papayas 'UH Rainbow' and 'UH SunUp'. University of Hawaii, College of Tropical Agriculture and Human Resources. *New Plants for Hawaii-2*. **1998**, 4.

Morton, J. F.; Dowling, C. F. Fruits of warm climates. **1987**, 9-13.

NASS. USDA National Agricultural Statistics Service. **2009**. (accessed April 2/2011). http://www.nass.usda.gov/hi.

Lobo, M. G.; Cano, M. P. Preservation of hermaphrodite and female papaya fruits (Carica papaya L., Cv Sunrise, Solo group) by freezing: physical, physico-chemical and sensorial aspects. *Zeitschrift für Lebensmitteluntersuchung und-Forschung.***1998**, *206*, 343-349.

Parker, T. L.; Esgro, S. T.; Miller, S. A.; Myers, L. E.; Meister, R. A.; Toshkov, S. A.; Engeseth, N. J. Development of an optimised papaya pulp nectar using a combination of irradiation and mild heat. *Food Chem.* **2010**, *118*, 861-869.

Penteado, A. L.; Leitao, M. F. F. Growth of Salmonella Enteritidis in melon, watermelon and papaya pulp stored at different times and temperatures. *Food control* **2004**, *15*, 369-373.

Pino, J. A.; Almora, K.; Marbot, R. Volatile components of papaya (Carica papaya L., Maradol variety) fruit. *Flavour Fragrance J.* **2003**, *18*, 492-496.

Rotsatchakul, P.; Chaiseri, S.; Cadwallader, K. R. Identification of characteristic aroma components of Thai fried chili paste. *J. Agric. Food Chem.* **2007**, *56*, 528-536.

Samson, J. A. Mango, avocado and papaya. *Tropical Fruits.* **1986**, 185-221.

Schreier, P.; Winterhalter, P. Precursors of papaya (Carica papaya L.) fruit volatiles. *Biogeneration of Aromas.* **1986**, 85–98.

Tang, C. S. Benzyl isothiocyanate of papaya fruit. *Phytochemistry.* **1971**, *10*, 117-121.

Tiwari, R. B. Studies on blending of guava and papaya pulp for RTS beverage. *Indian Food Packer*. **2000**, *54*, 68–72.

Van den Dool, H. A generalization of the retention index system including linear temperature programmed gas--liquid partition chromatography. *Journal of Chromatography A.* **1963**, *11*, 463-471.

CHAPTER 6

SUMMARY

The purpose of this research was to investigate the digestive properties of papaya fruit, either due to proteolytic activity or fiber content or a combination of both, and to evaluate the impact of processing on these characteristics. Papain activity in papaya fruit was evaluated through the different maturation stages, since it was previously unknown as to whether papain was active in the pulp. Active proteolytic enzymes were present in the pulp of papaya fruit throughout the different ripeness stages. Ripe papaya had the greatest enzyme activity (11.9 U/g pulp). The specific activity (U/mg protein) was higher in unripe papaya (11.3 U/ mg protein). Processing had a detrimental impact on proteolytic activity (loss of 60 % activity).

To confirm enzymatic activity, an *in vitro* experiment was used to evaluate the action of proteolytic enzymes on meat bolus displacement. The meat bolus study confirmed the proteolytic activity presence through different ripeness stages of papaya fruit. Unfortunately, this method has some limitations, and thus, it was not possible to make general inferences based on this method; it was helpful to confirm the presence of active enzyme in papaya fruit with potential for assisting human digestion.

It was unknown if the proteolytic activity present in papaya fruit and in the processed nectar would be enough to have an impact on the human GI tract, especially at the acidic pH of the stomach. To evaluate the impact of papaya on human digestive tract, *in vitro* digestion and fermentation assays were conducted. Results from the *in vitro* digestion experiment suggest that (after 6 hours incubation at 39 ^{O}C) ripe papaya has active proteolytic enzymes that might be only partially affected by the stomach acidity. Ripe papaya showed the greatest nitrogen disappearance among the substrates, indicating that ripe papaya aids to digest protein in the GI

tract. This study also demonstrated that ripe papaya produced the highest amount of short chain fatty acids (SCFA) of the three papaya substrates. SCFA are considered are good indicators for fermentability. The combination of proteolytic enzymes and fiber content found in papaya make of this fruit not only a potential digestive aid but also a good source of SCFA and all the health benefits that they provide. Processed papaya experienced the lowest protein digestibility and SCFA production among the papaya substrates. Future research studies should focus on the processing of papaya minimizing the detrimental impact on enzyme activity and SCFA production.

Our laboratory developed a processing method (irradiation and irradiation + heat) for papaya nectar that is microbiologically safe and preserves papaya nutrients and flavor. The impact of irradiation on papaya flavor compounds was evaluated. Processing did not have major impact on the papaya flavor volatiles, as noted in gas chromatography-olfactometry. There were three compounds specifically detected in the irradiated + heat sample (1-octen-3-ol, p-cresol, and vinylguaiakol); their development could be attributed to the heat processing. Irradiation was noted to promote formation of 2-acetyl-2-thiazoline and degradation of E-2-nonenal, which was present only in the control sample. These compounds are not known to provide sweetness and thus do not appear to be responsible for the sweeter taste detected in the irradiated nectar. There was high variability between replications in gas chromatography mass spectra (GC-MS), which we attributed to internal standard problems; when variability due to internal standard was eliminated, the variation between replications also decreased.

It was not possible to determine whether irradiation had an impact on one or more flavor compounds responsible for the enhanced sweetness of irradiated samples. Gomez et al. (2002) found that ripe and intermediately ripened papayas were perceived sweeter than mature green

papaya even at the same total soluble sugar content (glucose, fructose, and sucrose). They suggested that this could be due to changes in texture (softer texture) which would enable the sugar liberation from the papaya cells to influence sensory perception. This could have happen in our samples after irradiation, since it was also observed in informal testing that irradiation softens the texture of the papaya nectar. Overall irradiation plus heat had a detrimental impact on the proteolytic enzymes and SCFA production. It was observed that processed papaya impact on protein digestibility was significantly lower than ripe papaya nectar. In addition, the production of SCFA was the lowest of the three papaya samples.

The next steps for this research to determine the reason for irradiation impacting sweetness enhancement should be the use of an electronic tongue to evaluate differences in texture and sweetness perception of the irradiated samples due to the limitation in our research on the use of human sensory panels, which have not been approved for the evaluation of irradiated samples at the levels used in this experiment. Since processing had a detrimental impact on the digestive aid properties of papaya future research should be conducted to investigate other alternative methods for processing papaya, minimal impact on digestive aid properties of papaya.