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EVALUATION OF GREEN TEA EXTRACT AS A GLAZING MATERIAL FOR SHRIMP FROZEN BY CRYOGENIC AND AIR-BLAST FREEZING

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Food Science

By
Srijanani Sundararajan
B.Tech.,Vellore Institute of Technology University, 2008
December 2010

Dedicated to

Mom, Dad and Jegan

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ABSTRACT

Green tea (*Camellia sinensis*) extract (GTE) is rich in polyphenolic compounds, especially catechins that are potent antioxidants. The antioxidant property of GTE may make it ideal for use as a glazing solution for suppressing lipid oxidation in shrimp (*Litopenaeus setiferus*) during frozen storage. In this study, GTE was evaluated as a glazing material for shrimp frozen by air-blast (BF) and cryogenic freezing (CF). Two percent, three percent and/or five percent green tea extract solutions (2GTE, 3GTE, 5TGE) were used for glazing. Distilled water glazed (GDW) and non-glazed (NG) shrimp were used as controls. The GTE was characterized by measuring color, pH, ^{o}Brix, total phenols, % antiradical activity and the individual catechins were identified by HPLC. The freezing time, freezing rate and energy removal rate for freezing shrimp by both freezing processes was estimated. The frozen shrimp samples were stored in a freezer at -21°C for 180 days. Samples were analyzed at 1, 30, 90 and 180 days for pH, moisture content, glazing yield, thaw yield, color, cutting force and thiobarbituric acid reactive substances (TBARS). The HPLC analysis of the GTE revealed the presence of catechin and its isomers and the total polyphenol content determined by HPLC was 148.1 ± 2.49 g/l. The freezing time for air-blast and cryogenic freezing was 48.67 ± 2.30 min and 4.83 ± 0.29min respectively. Cryogenic freezing had an energy removal rate (836.67 ± 78.95 J/s) nearly ten times higher than blast freezing (80.26 ± 3.82 J/s). The pH of all samples increased with increase in frozen storage time and there was no significant difference among treatments. Glazed samples had higher moisture content compared to NG shrimp after 180 days storage. Glazing improved the thaw yield of shrimp. GTE was effective in controlling lipid oxidation in shrimp. Cryogenically frozen shrimp glazed with 5 % green tea extract (CF5GTE) had the lowest lipid oxidation at the end of 180 days. Glazing with GTE affected a* and b* values, but had no significant effect on the L*

values of shrimp. The cutting force of all NG shrimp was significantly lower than that of glazed shrimp. This study showed that green tea extract could be successfully applied as an antioxidant glaze for frozen storage of shrimp.

CHAPTER 1 INTRODUCTION

World shrimp production has increased from 3.4 billion pounds in 1980 to 13.4 billion pounds in 2005 (Keithly and Poudel, 2008). Shrimp is the most widely consumed seafood in the United States and US landings of shrimp are valued at 370.2 million dollars. It also tops the list of seafood imported by the USA in value ($4.1 billion) and volume (1.2 billion pounds) (NMFS 2009). The Gulf region is the top producer of shrimp in the United States and accounts for about 80 % of the nation's total, producing, on an average, 241 million pounds. Louisiana is the top producer among the Gulf States with production of nearly 109.8 million pounds (NMFS 2009). About 220 to 280 million pounds of shrimp are harvested in the southeastern USA annually and hence has an impact on the economy of this region.

Shrimp (*Litopenaeus setiferus*) is low in saturated fat and is a very good source of protein, selenium, and vitamin B12. Raw shrimp is extremely perishable and hence normally sold frozen. Frozen shrimp is a product of high commercial value which enjoys increasing demand due to competitive price, extended shelf life (Tsironi and others 2008) and consumer interest in individually quick frozen (IOF) product for which the price is considerably higher than its block frozen counterparts (Jacobsen and Fossan 2001). Lipid oxidation, protein denaturation and dehydration can occur during frozen storage and can result in off flavors and odors, toughening of muscle, and loss of juiciness (Boonsumrej and others 2007). These undesirable biochemical and physical changes that occur during frozen storage can be reduced by glazing. Glazing is the application of a thin layer of ice on the surface of a frozen product (Londahl 1992). The glaze acts as a barrier and keeps oxygen from reaching the fat, and it also protects against dehydration as the glaze evaporates in place of tissue water from the shrimp (Jacobsen and Fossan 2001).

Though studies in the past have shown glazing to be effective, there are few studies comparing the effect of glazing along with different freezing methods.

Green tea is a popular beverage and known for its polyphenols contents which can act as natural antioxidants. Green tea extract is rich in catechins, the polyphenolic constituents of green tea. The main catechins found in green tea are epigallocatechin gallate, epigallocatechin, epicatechin, and epicatechin gallate (Yang and Koo 1997). It has been reported that the antioxidant activity of green tea is comparable to synthetic antioxidants and hence can be used in place of synthetic antioxidants which are potentially harmful (Cao and others 1996). Recently consumers are showing less interest in purchasing products containing artificial additives. In the last few years research has shown increasing consumer demand for natural products (Diana and others 2008). Green tea is known to have the highest antioxidant capacity compared to other teas (Vinson 2000) and has greater antioxidant effect than vitamin C, vitamin E, BHA (Butylated hydroxyanisole), and BHT (Butylated hydroxytoluene) (Wiseman 1997; Wanasundara and Shahidi 1998). In addition, it is reported to have many other health benefits including anti-inflammatory effects (Cheng 2003), cardio protective effects (Pillai 1999) and anti-obesity effects (Murase and others 2002). Green tea has been used to extend shelf life of lettuce (Diana and others 2008), to improve functional properties of pork patties (Jo and others 2002; Kang and others 2005), and in fermented sausages (Bozkurt 2006). However there are few studies that evaluate green tea as a glazing material for shrimp.

The purpose of this study was to evaluate green tea extract as a glazing material for shrimp, and to determine its effect on quality during frozen storage using two freezing methods, air-blast freezing and cryogenic freezing.

CHAPTER 2 LITERATURE REVIEW

2.1 General Introduction: Production and Consumption of Shrimp

Shrimp are decapod crustaceans. They are a common type of seafood and can be found in temperate or tropical saltwater and freshwater. Shrimp are broadly categorized as warm water and cold water shrimp, the warm water shrimp are further classified based on color and include the white, pink and brown shrimp (Hui and others 2004). *Litopenaeus setiferus* or white shrimp is a type of warm water shrimp, harvested in the Gulf States. Shrimp are low in saturated fat and are a very good source of protein, selenium, and vitamin B12 but are also high in cholesterol compared to most other seafood (Hui and others 2004). Shrimp is one of the most important seafoods traded worldwide (Ooaterveer 2006), with at least 50 countries having large commercial fisheries including the USA, Thailand and China which are the leading nations of haul and processing (Jacobsen and Fossan 2001). Shrimp is the most widely consumed seafood in the United States. The USA is not only a major producer of shrimp but it is also the world's largest importer. Over three hundred million pounds of shrimp, valued at around 370.2 million dollars were landed in the USA in 2009. The Gulf States account for 80 % of the nations landings (241 million pounds) and Louisiana is the top producer among the Gulf States with a total production of 109.8 million pounds (NMFS 2009). Shrimp tops the list of seafood imported by the USA by value and volume (NMFS 2009) (Figure, 2.1). Shrimp imports have been growing in the USA since 1997. World shrimp production has increased from 3.4 billion pounds in 1980 to 13.4 billion pounds in 2005 (Keithly and Poudel 2008). Market forms include fresh chilled shrimp, IQF frozen shrimp (glazed or nonglazed) and frozen solid pack shrimp. Frozen shrimp is a product of high commercial value with increasing demand due to competitive pricing, extended shelf life (Tsironi and others 2008) and also consumer interest in individually

quick frozen (IOF) product(the price for which is considerably higher than block frozen counterparts) (Jacobsen and Fossan 2000).

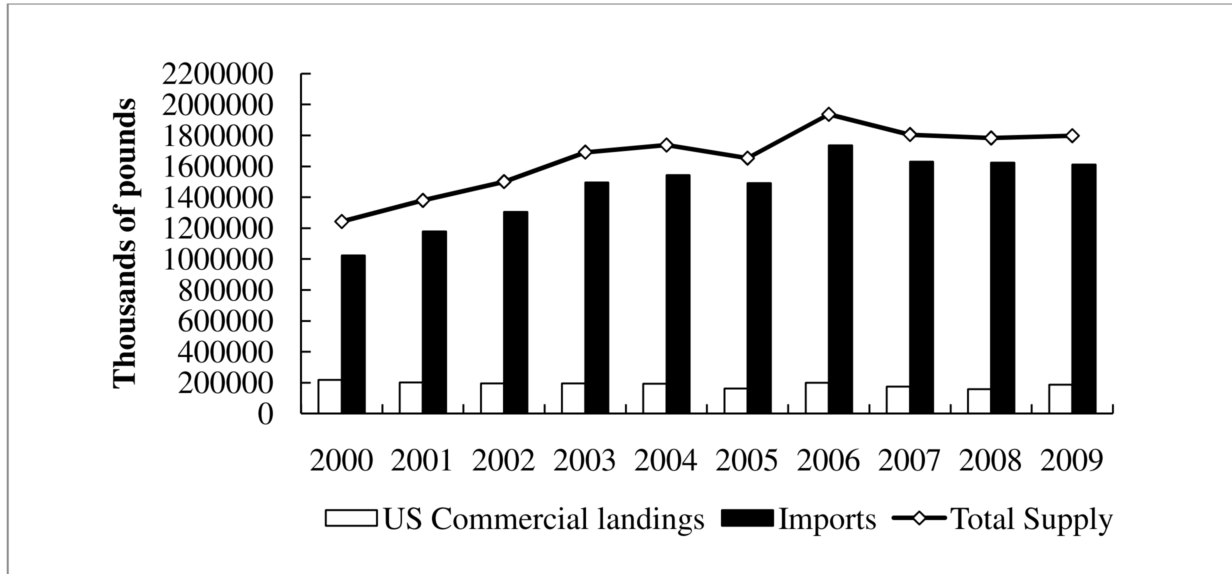


Fig 2.1: US Supply of Shrimp. (Source: NMFS 2009)

2.2 Freezing Methods

Raw shrimp contains 76-80 % water, 18-20 % proteins and approximately 1-2 % lipids, (Londahl 1992), the latter being the main reason for the perishable nature of raw shrimp. Freezing is a common preservation method used to control or decrease biochemical changes in seafood. Freezing does not improve the quality of the product; however, proper freezing and subsequent frozen storage can help retain quality (Makarios-Laham and Lee 1993; Gonclaves and others 2008). Frozen preservation of shrimp ensures year round supply. The final quality depends on the quality of the seafood at the time of freezing as well as other factors during freezing, cold storage and distribution. If freezing and frozen storage are carried out properly they can yield a shelf life of longer than a year (Regenstein and Regenstein 1991; Johnston and others 1994;

Chevalier and others 2001). Freezing preserves the taste, texture and nutritional value of food better than other preservation methods (George, 1993).

2.2.1 Air -Blast Freezing

In blast freezing, cold circulating air is used to remove heat from the product. Fans are normally used to increase the airflow and hence the rates of heat transfer. In its simplest form a blast freezing operation is one in which food arranged on trays is placed in a trolley and wheeled into the freezer cabinet (Smith 2003). Blast freezing can be done as a continuous or batch operation. Blast freezing is versatile and can accommodate many different product sizes and shapes. A major disadvantage is desiccation or dehydration of product surfaces caused by the air flow. A moisture loss of 1-2 % is common and in poor operations this can be up to 5 % (Kolbe and Kramer 2007). It may take 6-8 hrs to freeze 8-10lb of fish in a blast freezer (Pigott and Tucker 1990). Longer freezing time will affect product quality as it will result in formation of larger ice crystals and increased drip loss on thawing.

2.2.2 Cryogenic Freezing

Cryogenic freezing is a rapid freezing method involving the use of refrigerants such as liquid CO_2 and liquid N_2. In cryogenic freezing the refrigerant is directly sprayed on the product, it absorbs heat from the product and vaporizes. Liquid N_2 vaporizes at -320°F at 1 atm, while liquid CO_2 first forms a snow and then vaporizes at -108°F (Kolbe and Kramer 2007). The direct contact of the refrigerant, its phase change and its very low temperature are responsible for high heat transfer rate, which considerably reduces the freezing time. The heat transfer rate when using liquid N_2 primarily depends on the droplet size of the refrigerant and surface temperature at the food/droplet interface (Awonorin 1989). The advantages of cryogenic freezing include

rapid freezing rates, high product quality and throughput, minimum required floor space and flexibility, and low capital entry (George 1993). Cryogenic freezing is suited for products that are considered value-added foods and for products that have a high surface area to volume ratio, such as shellfish, meat slices, pizza and extruded products (Geroge 1993; Erickson and Hung 1997). Additionally, the high freezing rates in cryogenic freezing can result in the formation of a frozen crust at the food surface and this can prevent dehydration during freezing and it can also help with food safety, as the optimum temperature range for growth of pathogenic microbes is passed quickly (Kolbe and Kramer 2007; George 1993; Leeson 1993).

2.3 Quality Deterioration During Frozen Storage

Although freezing is an effective long term preservation method some changes or loss in quality occur during frozen storage. The quality deterioration in frozen shrimp is mainly due to oxidation, sublimation and recrystallization of ice (Londahl 1992), and dehydration (Chandrasekaran 1994). These changes can result in the development of off flavors, rancidity, loss in juiciness, weight loss, drip loss, toughening, increase in volatile basic nitrogen (Riaz and Qadri 1990; Yamagata and Low 1995), and reduced water binding capacity (Bhobe and Pai 1986; Hui and others 2004; Gonclaves 2005; Boonsumrej and others 2007). The rate of freezing and thawing, storage temperature, temperature fluctuations and freeze-thaw abuse during storage and transportation influence the extent of quality loss (Licciardello 1990; Hui and others 2004; Boonsumrej and others 2007). Shrimp and fish share similarities in the composition of flesh, and the mechanism observed in fish undergoing freezing or frozen storage may be comparable to those in shrimp under similar circumstances.

2.3.1 Oxidation

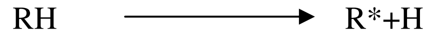
Lipid oxidation is the primary problem occurring during frozen storage of seafood, and results in

development of objectionable off flavors. The product also losses nutritional value due to oxidation (Huang and Weng 1998). In addition, the products formed from the breakdown of lipids can interact with proteins, leading to alterations affecting texture and functionality (Erickson and Hung 1997). Oxidation is studied more in seafood, as it is a more serious problem here than in poultry, pork or beef because of the unsaturated lipids present. Even though it has been thought that lipid oxidation is more of an issue in fatty fish; it can be a very serious problem in lean fish. Most of the lipids that are present in the lean fish are phospholipids present in the membranes and these are more unsaturated and hence more susceptible to oxidation compared to adipose triglycerols (Fenemma 1996). Shrimp is mostly sold as a frozen product and the freezing process may facilitate oxidation due to concentration effects that occur during freezing. The extent of oxidation will depend on the presence of prooxidant, the fatty acids present, the degree of unsaturation of the fatty acids, the presence of enzymatic systems that may convert iron to active ferrous form, temperature, water activity, and exposed surface area. In general the rate of oxidation increases with increase in the degree of unsaturation of the lipids. The lipoxygenase enzyme, when present, promotes oxidation. Shrimp contains significant amounts of C_{20} and C_{22} polyunsaturated fatty acids (Krzeczkowski 1970)) and hence these will affect the rate of oxidation. Oxidation occurs when oxygen from the food surface diffuses into the muscle (Huang and Weng 1998). Proper packaging, low temperature storage, the presence of antioxidants and glazing may help retard oxidation (Brannan and Erickson 1996).

Oxidation in frozen seafood is mainly thought to occur by autoxidation and to a lesser extent enzymatic oxidation. In unsaturated lipids, oxidation occurs by attack of the oxygen at or near the unsaturated center, while for a saturated lipid it can occur anywhere in the hydrocarbon chain

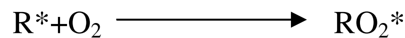
(Schultz and others 1962). The autooxidation of a substance occurs as described below (Adapted from Ingold 1962).

Initiation

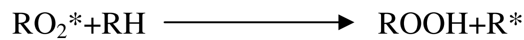


$$RH \longrightarrow R^{*}+H$$

Propagation

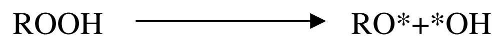


$$R^{*}+O_2 \longrightarrow RO_2^{*}$$



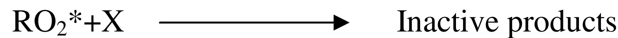
$$RO_2^{*}+RH \longrightarrow ROOH+R^{*}$$

Hydroperoxide decomposition



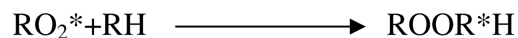
$$ROOH \longrightarrow RO^{*}+^{*}OH$$

Termination



$$RO_2^{*}+X \longrightarrow \text{Inactive products}$$

RO_2^{*} is a preoxy radical, ROOH is a hydroperoxide and X can be either a free radial or a free radical inhibitor. The inhibitor(X) converts the peroxy radical to hydroperoxide and becomes resonance stabilized, thus terminating the chain. Mayo and others (1985) reported propagation in which polyperoxides are formed from compounds with conjugated double bonds.



$$RO_2^{*}+RH \longrightarrow ROOR^{*}H$$

2.3.2 Dehydration

Dehydration is caused by the diffusion of water from the inner tissue of the food to the surface. The water on the surface eventually evaporates leaving behind a shrunken and tougher product.

Dehydration leads to weight loss which directly translates to loss of value. This weight loss is directly proportional to the amount of exposed surface area (Gonclaves and others 2008). Smaller food items that have a high area to volume ratio will be affected more by moisture loss (Erickson and Hung 1997). Freezer burn can result from dehydration. This appears as a gray patch on the surface of the muscle. Freezer burn affects the quality of a product and can reduce consumer acceptability. In addition, freezer burn may accelerate the rate of oxidation due to larger exposed surface area. During frozen storage recrystallization of ice occurs and the average size of the ice crystals increase (Blanshard and Franks 1987; Reid 1994). Larger ice crystals can lead to higher drip losses which can cause increased dehydration, and result in tougher meat (Kolbe and Kramer 2007). Drip loss occurs when water migrates out of the tissue upon thawing. It has been reported that around 95 % of water is present within fibers, of which around 71 % is within myofibrils and 24 % between myofibrils. Relocation of water from these areas will result in higher drip loss. Water gets relocated on freezing accounting for more drip loss, and the extent of relocation will depend on the freezing rate (Erickson and Hung 1997; Voyle 1974; Anon and Calvelo 1980).

2.3.3 Protein Changes

Proteins are denatured during freezing and frozen storage. The denaturation of myofibrillar proteins is more pronounced than sacroplasmic and stromal proteins (Erickson and Hung 1997). This freeze denaturation can lead to changes in texture, water holding capacity, solubility and emulsifying properties (Shahidi and Botta 1994). Fish muscle proteins are very sensitive to denaturation (Reid 1986). Jiang and others (1991) reported that solubility of actomyosin decreased with increase in frozen storage time in grass prawn. Several theories attempt to explain denaturation of proteins during freezing. One possible explanation is that proteins in

their native state have some hydrophobic side chains facing inward to the protein molecule and some exposed at the surface of the molecule. The exposed hydrophobic side chains will have water molecules surrounding them which helps mediate hydrophobic/hydrophilic interactions between protein molecules and also lowers energy at the oil/water interface. Ice is formed from these water molecules during freezing and hence the structure is destabilized and the hydrophilic and hydrophobic regions may form crosslinks when exposed to the new environment (Hui and others 2004; Matsumoto 1979; Lewin 1974). The increase in trimethylamine, the interaction of lipid oxidization products and the breakdown of trimethylamine oxide (TMAO) have all been associated with the denaturation of proteins during frozen storage (Shahidi and Botta 1994; Kolbe and Karmer 2007). Formaldehyde formed from the breakdown of trimethylamine oxide can cause cross-linking and aggregation of proteins (Ang and Hultin 1989; Regenstein and others 1982). Enzymes such as lipases, phospholipases, proteinases (cathepsins, calpins) and trimethylamine oxide demethylase also affect quality during frozen storage. Lipases can cause hydrolysis of triglycerides and are still functional during frozen storage. Their activity can lead to free fatty acid formation in frozen fish (Alford and Pierce 1961; Erickson 1993). Cathepsins can cause degradation of z-lines and also damage sacromeres (Smolinska and Abdul-Halim 1992). This can lead to degradation of myosin in shrimp. Trimethylamine oxide demethylase catalyzes the breakdown of TMAO to dimethylamine and formaldehyde (Erickson and Hung 1997). Enzymes may be released by rupture of lysosomes during freezing. Slower freezing rates and temperature fluctuations cause more damage to lysosomes (Geromel and Montgomery 1980) compared to fast freezing methods. It has also been suggested that the increase in salt concentration during freezing can lead to protein denaturation (Love 1966; Dyer and others 1961).

2.3.4 Color and Flavor Changes

Color of the product is one of the ways consumers may evaluate a product before purchase and hence is an important quality attribute. Color degradation during frozen storage can be caused by oxidation of the pigments (carotenoids can be degraded), freezer burn (leaves behind grayish spots), and/or lipolysis (yellow coloration of fat). The size of ice crystals present in a frozen product can also affect the perception of its color; larger ice crystals will scatter less light and make the product appear pale (Hui and others 2006). Melanosis is the discoloration of shrimp caused by the enzyme polyphenol oxidase (PPO) and is more a problem during refrigerated and ice storage of shrimp. PPO is inherently present in shrimp, however post mortem, PPO oxidizes the phenolic substrates into o-benzquinones which by further oxidation and non-enzymatic polymerization form melanins, the pigments responsible for the dark color in shrimp (Benjakul and others 2009).

Flavor changes during frozen storage are primarily attributed to lipid oxidation. Hydroperoxides formed during lipid oxidation breakdown to aldehydes and ketones leading to off-flavors (Erickson and Hung 1997). Aldehydes such as cis-4-heptenal found in shrimp (Kubota and Kobayashi 1988) are usually responsible for “cold store” flavor (Fennema 1996). Reduction in fat content can lead to flavor loss due to flavor volatility, and triglycerides are believed to lower vapor pressure of lipophilic flavor compounds (Hui and others 2004, Leland 1997). Denatured proteins can bind sulfur containing flavors. Products of lipid oxidation may interact with side chain groups of certain amino acids, leading to an increase in the hydrophobicity of proteins and reduce their flavor binding capacity (Hui and others 2004; Adams and others 2001; Shenouda, 1980). Alcohols such as 5Z-octa-1, 5-dien-3-ol and 1-penten-3-ol have been identified in shrimp

and may be responsible for metallic and rancid flavors (Whitfield and others 1982; Sakakibara and others 1988).

2.4 Glazing

Glazing refers to a process in which a thin layer of ice is applied to the surface of the frozen product, either by dipping or spraying (Londahl and Nilsson 2003). The main objective of glazing is to protect the product against quality deterioration that can occur during frozen storage. Glazing can protect against dehydration, freezer burn and oxidation (Jacobsen and Fossan 2001). Of all the changes occuring during frozen storage, sublimation of ice or dehydration can be considered the most important as this affects other chemical reaction such as oxidation of lipids, denaturation of proteins and discoloration (Londahl and Astrom 1972). Sublimation is more pronounced at higher storage temperatures and during storage at fluctuating temperatures. Products with high surface area to volume ratio will be affected the most (Londahl and Astrom 1972). The glaze provides a uniform surface that will occupy a lesser area compared to the ice crystals in a product and hence reduced sublimation (Londahl and Nilsson 2003). Additionally, moisture loss can come from the added glaze, instead of the tissue water (Londahl and Nilsson 2003; Jacobsen and Fossan 2001). If monitored, glazing may be reapplied as necessary to protect the frozen product from any moisture loss during the period of frozen storage. The glaze will also prevent oxygen from reaching the fat, and hence can protect against lipid oxidation. It acts as a barrier by slowing diffusion of moisture from the food and oxygen from the environment. Glazing with water is the most common, however studies in which glazing was done using sugar or salt solutions, or with the addition of corn syrup solids, or cellulose gums or pectinates to glazing solutions have been reported (Ijichi 1978; Mathen and others 1970). The thickness of the glaze will depend on glazing time, core temperature of

product, temperature of the glazing solution, and size and shape of product (Johnston and others 1994; Jacobsen and Fossan 2001; Gora and others 1972). Higher glazing time and lower product temperature resulted in an increase in glaze deposition for shrimp (Goncalves and others 2009). The amount of glaze typically varies from 8-12 % of the gross weight, but excessive coatings up to 25-45 % have been reported (Jacobsen and Fossan 2001). Since glazing will add to the weight of the raw material, excessive coatings will affect consumer, as they may pay for the added glaze instead of the raw material. Londahl and Astrom (1972) reported that IQF shrimp are very sensitive products and have a very short storage life even with vacuum packaging in the absence of glazing. The addition of an 8 % glaze increased the shelf life considerably. Vanhaecke and others (2010) reported 6-10 % glaze was effective in protecting fish fillets from dehydration, oxidation and quality loss, and less than 6 % can lead to hampered protective function. Due to abusive glazing in certain cases, the Food Advisory committee has recommended that 'All glazed fish products should bear an indication of net weight of fish core prior to glazing' (FAC 1987). There are a number of methods for determining the amount of glaze, most important are the AOAC and CODEX-standard methods both of which determine the weight percentage of the glaze. The CODEX method developed by the FAO is widely used. The difference between frozen product weight and deglazed weight gives the amount of glaze based on weight percentage. The glaze percentage is calculated as weight of deglazed shrimp over weight of frozen shrimp, multiplied by 100. The deglazed weight is determined by thawing the product in running water or luke warm water. Recently, Jacobsen and Fossan (2001), investigated an enthalpy method, for estimation of glaze percentage. They suggested that with information on glazing water temperature, and product, water and ice thermodynamic properties, an estimate in product temperature elevation can be used to determine glaze percentages. Glazing can be

considered a cheap alternative to packaging. Most packaging may not cover the product as tight as a glaze and sublimation can still occur from the product to the package in the absence of glaze. Glazing is cheap and can be easily applied and for the consumer it can be of value in maintaining quality under cold storage and home freezing (Jacobsen and Fossan 2001). Storage without glazing may be suitable for storage of prawns for less than 2 months, but glazing is necessary for long term storage (Jacobsen and Pedersen 1997).

2.5 General Introduction: Green Tea

Tea is one of the most popular beverages in the world and is second in consumption only to water (Koo and Cho 2004; Cheng 2006; Clement 2009; Chen and others 2009). Drinking tea is associated with culture and has been in practice for over 4000 years (Clement 2009). Tea was first cultivated in China and then in Japan. With the travel of European traders to the east as early as the 15th and 17th centuries, commercial cultivation and large scale trading of tea started between Europe and Oriental countries. Today the annual production is around 1.8 million tons of dried leaf (Wang and Ho 2009). The tea plant, believed to have originated in Southeast Asia, is currently cultivated in over 30 countries. Tea comes from the leaves of the plant *Camellia sinensis* a member of the *Theaceae* family and is broadly categorized into three types green, oolong and black tea based on the level of fermentation. During production of Green tea, unlike the other two varieties, the leaves are rolled immediately after harvest with heat to inactive the polyphenol oxidase that is capable of oxidizing tea catechins and thus it is less fermented (Cheng 2006). Oolong is partially fermented tea while black is fully fermented tea.

Green tea denotes the product obtained from the fresh leaves of the *Camellia sinensis* plant and processed to prevent oxidation of the polyphenolic constituents (Graham 1992). Since green tea is less fermented it has a higher level of catechins, the polyphenolic compounds present in tea.

Green tea has been reported to have higher antioxidant activity and a greater level of polyphenols compared to black tea (Koo and Cho 2004). Compared to black and oolong tea, less green tea is produced, accounting for only about 20 % of production. The major countries involved in production are Japan and China (Graham 1992). A list of the major tea producing countries can be found in Table 2.1.

Table 2.1: Principal Tea Producing Countries and Production Amount (tons) in 2007.

China	1.186.500
India	949.220
Kenya	315.000
Sri Lanka	304.600
Indonesia	192.000
Turkey	191.605
Vietnam	153.000
Japan	95.000
Argentina	72.000
Bangladesh	58.500
Malawi	39.000
Tanzanya	31.000
World production	*3.871.339*

Source: FAO, 2009

2.6 Composition

In recent years there have been numerous studies on the potential benefits of green tea. Green tea has been studied extensively for its antioxidant activity and numerous studies have also reported green tea to contain anticarcinogenic, antimicrobial, cardiovascular and cerebrovascular protective effects, anti-obesity, and anti-hypercholestolemic effects (Si and others 2006; McKay

and Blumberg 2002; Rietveld and Wiseman 2003; Cooper and others 2005; Clement 2009; Cheng 2006). Many of these benefits have been attributed to the polyphenolic content of green tea.

The polyphenols are one of the major constituents of tea, and the flavanols and flavonols are most abundant in green tea (Figure, 2.2). Polyphenols have been reported to account for up to 40 % of the dry weight (FeneClement 2009). Catechins (flavan-3-ols) are the most predominant polyphenols present in green tea (Marcia and others 2007) and the six main catechins present are epicatechin(EC), epigallocatechin-3- gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) , gallocatechin and catechin (Roberts 1953) and these collectively account for over 60 % of the total catechins (Yang and Koo 1997). Catechins are isomers and most of them are found to occur in the "epi" form, the gallo catechins are those that contain three hydroxyl groups on the B ring (Fig 2.3), when the OH group on the pyran ring is esterified with gallic acid, catechingallates are formed. The catechins are colorless, astringent and water soluble (Graham 1992, Marcia and others 2007). The flavonols found in tea are mainly quercetin, kaempferol, myricitin and their glycosides (Balentine and others 1997; Del Rio and others 2004). Horzic and others (2009) compared the polyphenol and methylxanthine content of different varieties of teas and herbal infusions. Their study found that green tea had the highest total phenol and flavonoids content compared to oolong and black tea. The green tea phenol content was also higher than linden and chamomile infusion. However, the composition of tea leaves are subject to change with the variety, climate, season, horticultural practice and age of plant (Graham 1992)

Apart from polyphenols tea has many other compounds. These include alkaloids, amino acids, proteins, minerals, glucides, volatile compounds and trace elements (Stagg and Millin 1975). The purine alkaloids in green tea are caffeine and theobromine (Del Rio and others 2004).

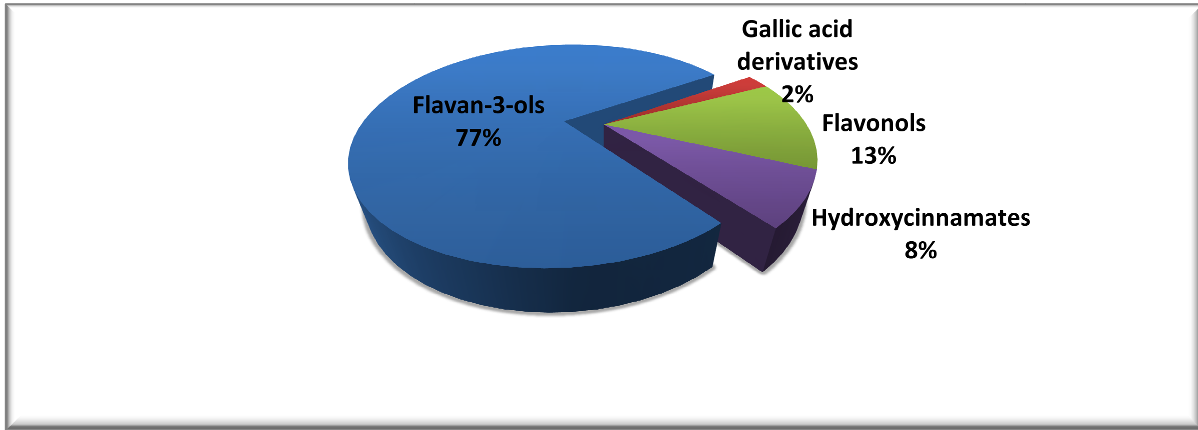


Fig 2.2: Green Tea Phenolics. (Source: Del Rio and others 2004)

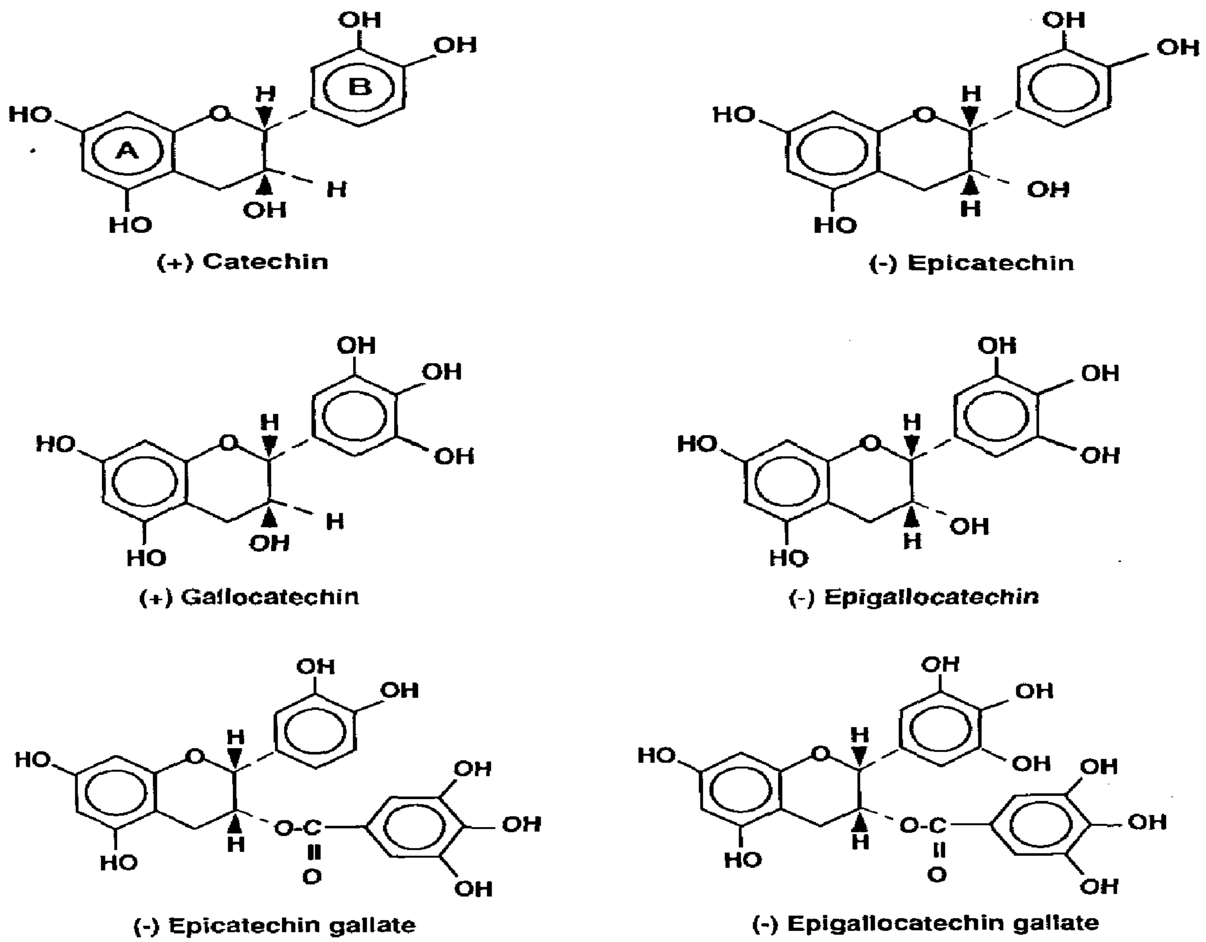


Fig 2.3: Polyphenols (Catechins) in Green Tea. (Source: Graham 1992)

Caffeine has been detected in tea and is considered important for the popularity of tea (Lin and others 1998; Wang and others 2000). Marcia and others (2007) reported 141-338 mg/l of caffeine in green tea infusion. Free gallic acid is also present in tea (Sakata and others 1985; Del Rio and others 2004). Theanine has been identified as a unique amnio acid found in tea (Cartwright and others 1954). Proteins comprise about 15 % (dry weight basis) of tea and these include enzymes such as polyphenol oxidase (catalyses the oxidation of catechins), glucosidase (catalyses hydrolysis of aroma compounds), lipooxidase (catalyse generation of volatile aldehydes) and enzymes responsible for methyl xanthine synthesis (Owuor 1986; Yano and others 1990; Graham 1992). Tea plants accumulate minerals; calcium, fluoride, manganese and chromium are some of the important minerals that have been identified (Xie 1998; Fung and others 1999; Matsuura and others 2001; Marcia and others 2007). Volatiles including alcohols, carbonyls, esters, and cyclic compounds have been identified in tea (Yinfang and others 1982). Pigments that are present in tea include chlorophyll and carotenoid as the major pigments, but violaxanthine, β-carotene, neoxanthin and lutein have also been identified in tea (Venkatakrishna and others 1976).

Table 2.2: Composition of Green Tea Leaf (in % dry weight).

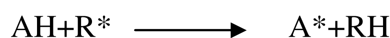
Polyphenols	36	Carbohydrates	25
Methylxanthines	3.5	Protein	15
Aminoacids	4	Lignin	6.5
Organicacids	1.5	Lipids	2
Carotenoids	<0.1	Chlorophyll	0.5
Volatiles	<0.1	Ash	5

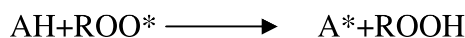
Source: Graham (1992)

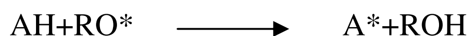
2.7 Antioxidant Activity

The US Food and Drug administration (FDA) defines antioxidants as “substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation”. Green tea extract has been noted for its antioxidant activity; due to its polyphenolic content. Antioxidants in food can be primary, secondary or synergistic antioxidants. Green tea extract may be considered as both a primary and a synergistic antioxidant. The mechanism of oxidation has been explained previously (Section 2.3.1). Green tea extract can act as antioxidant by quenching free radicals, inhibiting formation of free radicals and/or interrupting the propagation of free radicals and by acting as cheltators for metal ions (Graham 1992; Chen and Chan 1996). The hydroxyl group present on the polyphenolic compounds in green tea can interrupt propagation of free radical autooxidation chain by donating a hydrogen atom to stabilize the free radical (Kaur and Kapoor 2001). Thus its action can be compared to that of a primary antioxidant. The mechanism of a typical phenol antioxidant is illustrated in Figure 2.4.

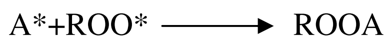
Green tea can prevent or retard the initiation step (Nanditha and Prabhasankar 2009)

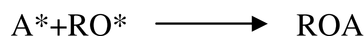






A* is the antioxidant free radial formed as a result of reaction, this can prevent chain propagation reactions due to formation of peroxy antioxidant compounds.





Green tea can also be thought of as a synergistic antioxidant. Green tea can act as a scavenger of reactive oxygen and nitrogen species. EGCG can bind metal ions and thus can act as chelator. Tea has also been shown to enhance the expression of endogenous antioxidants such as glutathione, glutathione reductase, glutathione peroxidase, catalase and quinone reductase (Khan and others 1992; Morel and others 1994; Valerio and others 2001; Hider and others 2001). Green tea may also inhibit enzymes like lipoxygenase in fish which may be responsible for formation of hydroperoxides that initiate autooxidation (Banerjee 2005). Liu and Pan (2004), showed green tea extract was effective in inhibiting lipoxygenase and hemoglobin catalyzed oxidation of arachidonic acid and linoleic acid. Frankel and others (1997) studied the effect of green tea on different lipid systems and suggested that green tea catechins may act as antioxidants by inhibiting alkoxy radicals formed as precursors of aldehydes.

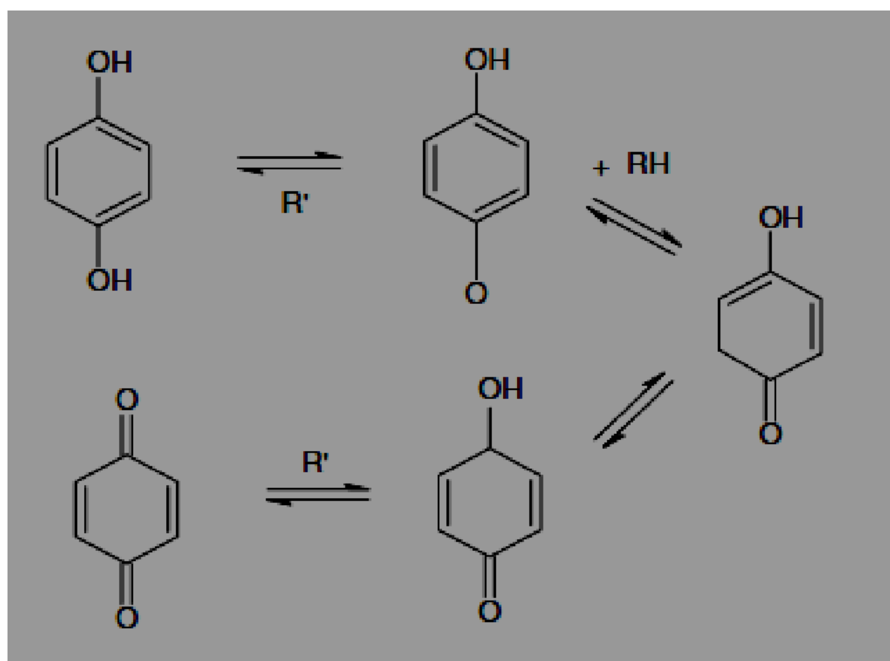


Fig 2.4: Mechanism of Action of Phenol Antioxidants. (Source: Nanditha and Prabhasankar 2009).

2.7.1 Applications in Food

Many studies in the past have indicated green tea to be a potent antioxidant. The antioxidant properties find application in the food industry as well as in pharmacological studies, where it has been found to reduce LDL oxidation, as well as prevent/retard the progress of atherosclerosis and thrombosis (Frankel and others 1993; Kinsella and others 1993). The catechins present in green tea are readily oxidizable, and this makes them useful antioxidants in food (Graham 1992). Chen and Chan (1996), reported that green tea catechins (GTC) obtained from jasmine tea were very potent antioxidants and were capable of protecting canola oil from lipid oxidation even better than BHT (Butylated hydroxytoluene) and the thermal degradation of GTC was less than BHT in heated canola oil (95°C). Ana and others (2008) reported that green tea extract was effective in extending the shelf life of fresh-cut lettuce and in preserving the ascorbic acid and carotenoid content of the lettuce. Depending on concentration of catechins, green tea extracts have been reported to have higher antioxidant activity compared to BHT, BHA (Butylated hydroxyanisole), TBHQ (Tertiary butyl hydroquinone), and Vitamin E (Wanasundara and Shahidi 1998). Chen and others (1998) reported green tea extract to be much more effective than rosemary extract against oxidation in canola oil, pork lard and chicken fat. They also reported green tea extract to have higher antioxidant activity than BHT. Jo and others (2003) showed that lipid oxidation was effectively controlled in cooked pork patties containing irradiated freeze-dried green tea extract and that sensory properties of the patty were not affected. Lin and Lin (2005) showed glazing with tea extracts enhanced the storage quality of bonito fillets and that green tea was better than black tea. In a study by Bozkurt (2006), it was found that green tea extract was more effective than BHT and *Thymbra spicata* oil in preventing lipid oxidation in Turkish dry fermented sausage. All of these studies showed that green tea extract could be

considered as a replacement for synthetic antioxidants. Since it is a natural antioxidant it will be more acceptable to consumers. Recently consumers have shown less interest in purchasing products containing artificial additives. Research has shown increasing consumer demand to buy natural products (Ana and others 2008). Also studies have shown some synthetic antioxidants could be potential carcinogens and have been banned in several countries (Madavi and Salunkhe 1995; Wanasundara and Shahidi 1998).

2.8 Anti-browning, Antidiscoloration and Deodorizing Properties

Green tea extract has been reported to have anti-browning effects. Soysal (2008) studied the effect of green tea extract on apple Polyphenol oxidase (PPO), and found green tea extract to be effective in inhibiting browning of apple slices. He reported that green tea extract (30mg/ml), decreased PPO activity by 42 %. The catechin in green tea has a similar structure to the PPO substrate of shrimp and hence it may be capable of inhibiting melanosis by competitive inhibition (Soysal 2008; Benjakul 2009). The antidiscoloring effect of tea polyphenols was studied in beverages, margarine and fish samples. The tea polyphenols were effective in preserving the color of beverages and were better than L -ascorbate. Margarine samples with tea polyphenols retained their yellow color after exposure to UV radiation. Tea polyphenols also suppressed the discoloration of fish samples (Yamamoto and others 1997). Green tea extract has also been shown to have deodorizing effects. The deodorizing effect of green tea extract was tested on methyl mercapton, trimethylamine, ammonia and tobacco smoke. On all the compounds tested, green tea extract proved to be a better deodorizing agent than sodium copper chlorophyllin (Yamamoto and others 1997). Candies and chewing gum made with green tea extract have been studied for their effect on halitosis and were effective in considerably reducing halitosis (Yamamoto and others 1997; Yasuda and others 1995). Dohi and others (1990)

reported green tea polyphenols were effective as a deodorant against smell generated in fish and meat products during processing.

2.9 Other Functional Properties

Green tea and green tea extracts have been studied extensively for their health benefits. Green tea is believed to have anti-cancer (Sun and others 2006; Jian and others 2004;Yang and Wang, 1993), cardiovascular and cerebrovbascular protective effects (Taubert and others 2007; Arab and others 2009;Yang and others 2004), antihypercholestolemic activity (Hooper and others 2008; Nantz and others 2009; Nagao and others 2007), anti-obesity effect (Maki and others 2009; Dulloo and others 1999), anti-diabetic effect (Zeyuan and others 1998; Wang and others 2002; Wu and others 2004), anti-bacterial and anti-viral effects (Hui and others 2001; Mukoyama and others 1991). Green tea catechins can lower oxidation of LDL (Low density lipoprotein) cholesterol, and this will help prevent atherosclerosis, and hence the occurrence of myocardial infarction and stroke (Clemet 2009; Hooper and others 2008). The ability of green tea in inhibiting cell proliferation due to its antioxidant activity has been attributed to its anti-inflammatory and anti-cancer properties (Bode and Dong 2002; Chen and others 1998; Fujiki and others 1999). It has been shown that epigallocatechin gallate (EGCG), is a potent nitric oxide synthase and cyclooxygenase-2 inhibitor and by suppressing these enzymes, the release of prostaglandins which are mediators for inflammation and tumorogenesis is controlled (Chan and others 1997; Raso and others 2001; Cho and Koo 2004). The anti-bacterial effects of tea are responsible for the prevention of gastric cancers and tooth damage (Gutman and Ryu 1996; Hamilton-Miller, 2001). Green tea catechins were found to inhibit *Helicobacter pylori*, the microorganism responsible for development of gastric and duodenal ulcers. Catechins inactivate the urease enzyme, which is required for the production of ammonia that helps the bacteria

survive in the intestine (Graham and others 1992; Matsurbara and others 2003). The anti-obesity effects can be attributed to the fact that tea reduces the absorption of fat and sugar. Tea polyphenols inhibit digestive lipases and interfere with lipid-micelle formation and this causes a decrease in fat absorption, the reduction of the activity of the glucose transporter is believed to be responsible for lowering sugar absorption (Han and others 1999; Juhel and others 2000; Ikeda and others 1992; Shimizu 1999; Cho and Koo 2004). The insulin like properties of green tea polyphenols (Wang and others 2002) and the reduction in sugar absorption may be responsible for the anti-diabetic effect.

CHAPTER 3 MATERIALS AND METHODS

3.1 Color, pH, Total Solids and Refractive Index of Green Tea Extract (GTE)

Alcohol free green tea extract (GTE) was obtained from theVitamin Shoppe, North Bergen, NJ, USA. The color was determined using a Hunter lab Labscan XE colorimeter (Hunter Lab, Reston, VA) and reported as L*, a* and b* values. The pH of GTE was determined using a Thermo Scientific Orion 4 star benchtop pH meter (Thermo Fisher Scientific Inc, Waltham, MA). The total solids (^{o}Brix) and refractive index of GTE were determined using a digital handheld refractometer (AR 200, Reichert, Inc., Depew, NY, USA).

3.2 Total Phenols, Antiradical Activity and Polyphenolic Compounds of GTE

3.2.1 Total Phenols

Total phenolic content was analyzed according to the Folin-Ciocalteu method (Singleton 1977). Twenty micro liters of the tea extract was mixed with 100 µl of Folin-Ciocalteu reagent (purchased from Sigma Chemical Co, St. Louis, MO, USA) was diluted with 1.58 ml of distilled water. Then 300 µl of sodium carbonate was added for color development. The absorbance of the derived product was read at 765 nm, using a U-3000 spectrophotometer (Hitachi, Tokyo, Japan). Gallic acid was used as a standard for the calibration curve (Appendix 1). The total phenolic content was expressed as grams per liter of gallic acid equivalents.

3.2.2 Antiradical Activity

The antiradical activity of the GTE was determined based on reduction of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical by the antioxidant in methanolic solution. In the presence of the extract the purple color of the DPPH solution is reduced to yellow and the absorbance of this solution can be measured spectrophotometrically (Blois 1958). The assay was done according to

the method described by Molan and others (2009) with some modifications. Fifty micro liters of the green tea extract was mixed with 5ml of DPPH solution (0.25g DPPH in 1l of methanol) and incubated at 37°C for 30 min and the absorbance was measured at 550nm, using a U-3000 spectrophotometer (Hitachi, Tokyo, Japan). The % antiradical activity was calculated as [(absorbance of control -absorbance of the green tea extract)/absorbance of control]*100.

3.2.3 Polyphenolic Compounds

The polyphenolic compounds in GTE were analyzed using HPLC with some modification to one of the methods described by Nishitani and Sagesaka (2004). The HPLC analysis of the green tea extract was performed using an Alliance Water 2690 separation module. An Agilent C18 column was used along with a guard column and a Waters 996 PDA (Photodiode array detector). The Millennium 32 software was used for data analysis. One milliliter GTE was diluted with 9 ml of distilled water and filtered using a 0.2 µm Whatman PTFE (polytetrafluoroethylene) filter; 20 µl of the diluted GTE was injected. Standards including (-)- Catechin, (-)- Catechin gallate, (-)- Epicatechin, (-)- Epicatechin gallate,(-)- Epigallocatechin,(-)- Epigallocatechingallate and (-)- Gallocatechingallate were prepared at concentrations of 1000-250 ppm, filtered and 20 µl of standards was injected. All of the standards were purchased from Sigma Aldrich (St. Louis, MO, USA) and purity was > 95 %, except (-)-Epigallocatechingallate which was >80 %. The mobile phase composition was water/methanol/formic acid (83:17:0.3). Gradient flow elution was used with flow rate set at 1.00 ml/min for 25 mins and then flow rate was ramped to 1.70 ml/min from 25-30 min and finally held at 1.7 ml/min up to 80 min. The column temperature was set at 30°C. Absorbance was reported at 275nm.

3.3 Proximate Analysis and Fatty Acid Methyl Esters Profile of Fresh Shrimp

Fresh white shrimp (*Litopenaeus setiferus),* of extra large size (26/30) was obtained from a local

seafood store in Baton Rouge, LA. Four batches of shrimp were transported on ice to the Food Processing Pilot Plant, Louisiana State University Agricultural Center, where they were stored in the refrigerator room at 4°C for 12 hours before use. The shrimp were manually de-headed and de-shelled.

The de-headed and de-shelled shrimp samples were analyzed for moisture, protein, lipid, ash and fatty acid methyl ester content. The moisture content was determined by drying in a draft oven at 105°C for 24 hrs (AOAC 1995). Protein content was determined by the Dumas combustion method using the TruSpec nitrogen analyzer from Leco (Michigan,USA) at the Soil Testing and Plant Analysis Laboratory, Louisiana State University Agricultural Center. Protein content was determined from the nitrogen content using a conversion factor of 6.25. The ash content was determined using Thermolyne Type 6000 muffle furnace (Thermo Scientific, Lawrence, KS) at 549 ºC. Lipid content was determined by the Bligh and Dyer method (1959).

The fatty acid profile was determined as fatty acid methyl esters (FAMEs) at the W.A. Callegari Environmental Center, Louisiana State University Agricultural Center. About 25 mg of lipid extracted from shrimp, was diluted in 10 ml hexane, in a test tube with a screw cap. Three ml of 2N potassium hydroxide in methanol was added to the test tube and the mixture was vortexed and centrifuged. The clear supernatant was used for the analysis. The analysis was done using a GC/MS system. The Varian 450 GC (Gas Chromatograph) was coupled to a Varian 240-MS (Mass spectrometer) detector for analysis. A CP-8400 auto sampler was used. The column used was a Varian, four factor capillary column,VF-WAXms, 30 mm X 0.25 mm ID. The injector temperature was 240°C and a 1 µl injection volume was used. Helium was used as a carrier gas. The oven temperature was programmed to be 100 ^{o}C for 2 min, then ramped to 255 ^{o}C at the rate of 12 ^{o}C/min and finally held at 255 ^{o}C for 8.08 min. Hexadecanoic acid -2 -hyroxy methyl ester

was used as an internal standard at a concentration of 4ppm. The results were expressed as % total fatty acids.

3.4 Freezing of Shrimp

The deheaded and deshelled shrimp samples were frozen using either cryogenic or air-blast freezing. Shrimp were arranged on aluminum trays which were previously covered in aluminum foil and a freezer paper was used to cover the shrimp. A cabinet type cryogenic freezer with liquid nitrogen (Air liquid, Houston, Texas) was used for cryogenic freezing and a mechanical freezer was used for blast freezing. Thermocouples (Comark®, Comark Limited, Stevenage, Herts, UK) connected to a data logger were used to monitor the temperature changes during cryogenic freezing. A U12 stainless temperature logger (Homo®, Onset Computer Corporation, Bourne, MA) was used to record the temperature changes during the air -blast freezing. The thermocouples were inserted at the center of the second abdominal segment of the shrimp and the freezing was done till the shrimp reached a core temperature of -21 ^{o}C. Shrimp were weighed before and after the freezing process.

3.4.1 Energy Removal Rate and Freezing Rate Determination

Energy removal rate (Q) is the amount of heat that has to be removed from the shrimp in a particular time to change its internal temperature form T_o to T_{if}. T_o and T_{if} represent the initial and final temperature of the shrimp (See Figure, 3.1)

The amount of heat removed from the product was calculated based on product heat load (Δh) and the rate at which the heat was removed was calculated using equation (1) :

$$Q = \frac{\Delta h}{t} \qquad [1]$$

where Q = the rate of heat transfer expressed in Kilojoules per second (kJ/s), t = time taken to reduce temperature to -21 ^{o}C in s, and Δh is the product heat load (kJ). Freezing time was determined based on the freezing curve (Figure, 4.3 in section 4.4) and internal temperature of the shrimp.

Δh is the product heat load (kJ) calculated as,

$$\Delta h = m[C_{pu}(T_o - T_{if}) + L + C_{pf}(T_{if} - T_f)] \ldots\ldots\ldots\ldots [2]$$

where, m = weight of shrimp in kilogram (kg), T_o = the initial temperature of the shrimp in Kelvin (K), T_{if} = the initial freezing point temperature of shrimp and T_f is the final freezing temperature (K). Latent heat (L) was calculated as $L = x_i L'$,where L' is the latent heat of fusion of water (333.6 kJ/ kg) and x_i is the weight fraction of ice given by $x_i = (x_{wu} - Bx_S)\left(\frac{T_{if} - T_f}{T_o - T_f}\right)$, where x_{wu} is the weight fraction of water, x_s is the weight fraction of solute, B is kg bound water/kg solute, expressed as $B = b - 0.5\frac{M_w}{M_s}$, where M_w and M_s is the molecular weight of water and molecular weight of solutes and b is the constant (b = 0.32 was reported for fish by Schwartzberg(1976), Pham(1987), Murakami and Okos(1989)). The molecular weight of solutes was calculated as $M_s = 18.02\left(\frac{X_w(1 - x_{wu})}{x_{wu}(1 - X_w)}\right)$, and X_w is the mole fraction of water of shrimp. X_w is calculated as, $ln(X_w) = -18.02\left(\frac{L'(T_o - T_{if})}{RT_o^2}\right)$ where R is the Gas constant = 8.314 kJ/ k mol K and temperature is expressed in K.

This calculation was based on the following approximations. Water is the only substance that was freezing. The internal temperature measured is a representative of the overall temperature of

the shrimp (Figure, 3.1). All the ice crystals were formed at the internal freezing temperature (T_{if}).

The specific heat capacity of the unfrozen and frozen shrimp was calculated using Siebel (1892) equations.

$$C_{pu} = 0.837 + 3.349X_w \quad [3]$$

$$C_{pf} = 0.837 + 1.256\ X_w \quad [4]$$

where C_{pu} = specific heat capacity unfrozen shrimp(kJ/kgK); C_{pf} = specific heat capacity frozen shrimp (kJ/kgK); X_w = moisture content of raw shrimp.

Shrimp was weighed before and after the freezing process and the freezing rate (°C/min) was estimated as [(Final freezing temperature, T_o (°C) - Initial freezing temperature, T_{if} (°C))/Total freezing time (min)].

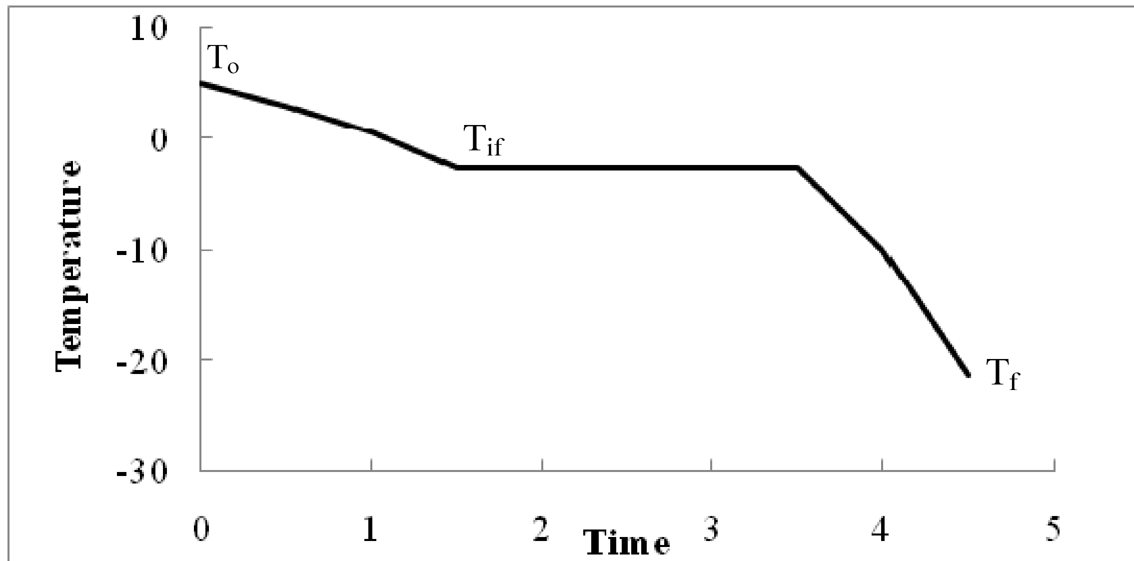


Fig 3.1: An Ideal Freezing Curve.

3.5 Glazing of Shrimp

The cryogenically and blast frozen shrimp were glazed at the Food Processing Pilot Plant, Louisiana State University Agricultural Center. The shrimp were glazed as described in Figure 3.1. Distilled water (DW), 2 % green tea extract solution (2GTE), 3 % green tea extract solution (3GTE) or 5% green tea extraction solution (5GTE) were used for glazing. The 2 %, 3 % and 5 % GTE solutions were prepared by diluting the GTE in distilled water at room temperature About 100g of shrimp were separately glazed by dipping in the appropriate solution at room temperature for 30sec and then allowed to drip for 5 sec before packing in Ziploc bags. The shrimp were weighed before and after glazing and stored at -21°C in the blast freezer, for 1, 30, 90 and 180 days.

3.6 Glazing Yield and Thaw Yield of Shrimp

The glazing yield and thaw yield % were determined as described by Sathivel and others (2007). The glazing yield was calculated as (weight of glazed shrimp (g)/weight of non-glazed raw shrimp (g))*100. The thaw yield was calculated as (weight of thawed glazed shrimp (g)/weight of non-glazed raw shrimp (g))*100.

3.7 Color and Texture of Shrimp

The surface color of the shrimp was measured with a Minolta spectrophotometer CM-3500d (Konica Minolta Inc, NJ, USA) using the Minolta Spectramagic software version 1.00 (Cyberchrome Inc, Minolta Co,Ltd) and recorded as L*, a* and b* values. Calibration was done using a standard white calibration plate (CM-A120) and zero calibration box (CM-A124). Triplicate samples were analyzed. For each sample six measurements were taken throughout the entire surface of the shrimp and the average of these measurements was reported. Texture was measured using a TA-TDI texture analyzer (Stable Micro Systems, Scarsdale,NY) equipped with

a knife blade and a 25kg load cell, according to the method described by Boonsumrej and others (2007). Thawed glazed and non-glazed shrimp were used for the texture analysis. The maximum force needed to cut the second abdominal segment of the shrimp was recorded as the cutting force (N).

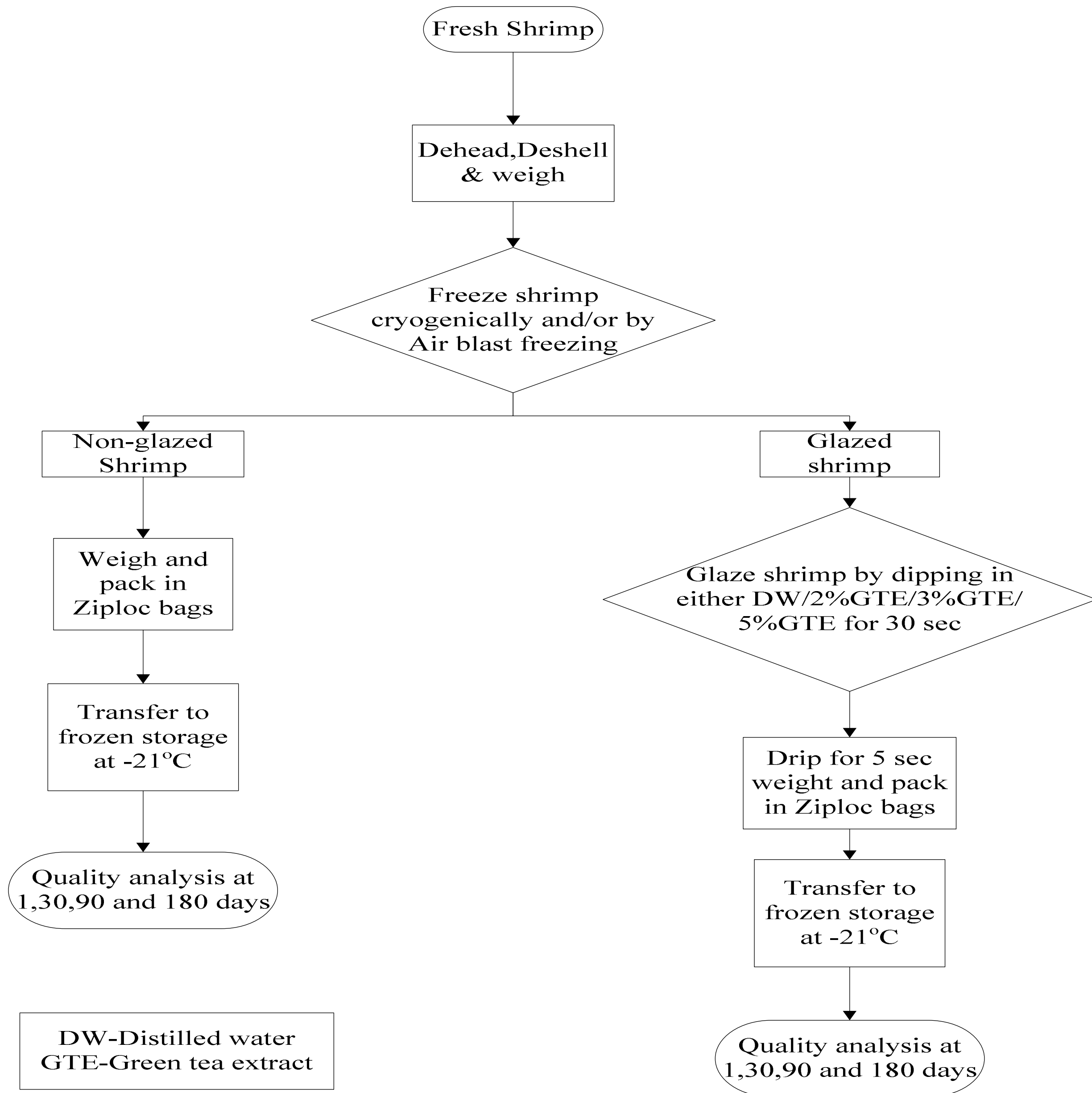


Fig 3.2: Flow Diagram for Preparation of Non-glazed, Distilled Water Glazed and Green Tea Glazed Shrimp for Freezing, Frozen Storage and Analysis.

3.8 pH, Moisture Content, and Lipid Oxidation of Shrimp

The shrimp samples were analyzed before freezing and after 1, 30, 90 and 180 days of frozen storage. The samples were thawed at 4 ^{o}C and ground using a Electric Meat Grinder (Northern Industrial, Inc, Burnsville, MN) with an inlet diameter (cm) of 6.35 at the top and 4.45 at the bottom, an outlet Diameter (cm) of 6.35 and Plate Diameter (cm) of 6.99. The ground samples were used for the analysis of pH, moisture content, and thiobarbutric acid reactive substances (TBARS). Triplicate samples were analyzed for moisture content by drying in a draft oven at 105 ^{o}C for 24 hrs (AOAC, 1995). The samples were removed from the oven and weighed. Moisture content % was calculated as [(weight of initial sample-weight of dry sample)/initial weight of sample]*100. The pH of the non-glazed and glazed shrimp after frozen storage was evaluated as described by Sathivel (2005) with slight modification. Ten grams of ground shrimp sample were placed in a 150-mL beaker and homogenized with 40 mL distilled water for 1 min at 4°C using an ultrasonicator (Cole-Parmer Inc, Vernon hills, USA). The pulser was set at 2 sec and the amplitude was 82 %. The pH of homogenized sample was measured with a Thermo Scientific Orion 4 star Benchtop pH meter (Thermo Fisher Scientific Inc, Waltham, MA). TBARS analysis was done according to the method of Lemon (1975). The extraction solution was prepared by dissolving 7.5 % trichloroacetic acid, 0.1 % propyl gallate and 0.1 % ethylene diaminetetraacetic acid (EDTA) in deionized water. Thiobarbutric acid (TBA) reagent (0.02M) was prepared by dissolving 2.88 g TBA in 1l of deionized water. All reagents were purchased from Sigma Chemical Co, St. Louis, MO, USA. Ground shrimp (15g) was mixed with the extracting solution (30ml), homogenized and filtered using Whatman no 1 filter paper. The filtrate was then mixed with the TBA solution, placed in boiling water for 40 min, cooled and absorbance was measured at 530 nm using a U-3000 spectrophotometer (Hitachi, Tokyo, Japan).

TBARS values were calculated from the standard curve of Malonaldehyde (MDA) and expressed as mg MDA/kg sample.

3.9 Statistical Analysis

Means values from six measurements and/ or triplicate analysis were reported. Statistical analysis was done using the SAS (Statistical Analysis System) software (version 9.2) (SAS Institute Inc., Cary, NC, and U.S.A). Data was analyzed by Analysis of variance (ANOVA) following Tukey's studentized range test ($p < 0.05$).

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Color, pH, Total Solids and Refractive Index of Green Tea Extract (GTE)

The L*, a* and b* values of the GTE were 0.17 ± 0.06, 0.68 ± 0.06, and 0.29 ± 0.04, which indicated that the GTE was dark brown in color. The pH of the green tea extract (GTE) was found to be 4.98 ± 0.01. The total solids (^{o}Brix) and refractive index of GTE was 58.63 ± 0.06 and 1.28 ± 0.00 respectively. The high value for ^{o}Brix indicates that the extract was concentrated (Table 4.1).

Table 4.1: Physicochemical Properties of Green Tea *(Camellia sinensis)* Extract.

Color	
L*	0.17 ± 0.06
a*	0.68 ± 0.06
b*	0.29 ± 0.04
pH	4.98 ± 0.01
Total solids (^{o}Brix)	58.63 ± 0.06
Refractive index	1.44 ± 0.00
Total phenols(g/L)	175.58 ±19.13
%ARA(Antiradical activity)	92.48 ± 0.78

4.2 Total Phenols, Antiradical Activity and Polyphenolic Compounds of GTE

The total phenols of GTE was higher (175.58 g/L) than that reported by Horzic and others (2009) for total phenols (1.83 g/L) in green tea obtained by brewing 2 g of tea leaves in 200 ml distilled water at 100 ^{o}C, this indicated that the GTE was concentrated. The % antiradical

activity was found to be 92.48 ± 0.78 and this represents the antioxidant activity of the extract. This value is higher than reported by Horizic and others (2009) (88.32 ± 9.9).

The HPLC analysis of the GTE revealed the presence of catechin and its isomers (Figure 4.1). The compounds identified in GTE were Catechin(C), Catechin gallate(CG), Epicatechin(EC), Epicatechingallate (ECG), Epigallocatechin (EGC), Epigallocatechingallate (EGCG) and Gallocatechingallate (GCG). The chromatograms of the standards used for identification are shown in Figure 4.2. The total polyphenol content of these catechin isomers was 148.10 ± 2.49 g/l (Table 4.2). The HPLC chromatogram of GTE also showed several other peaks. The other peaks could be compounds such as caffeic acid, coumaric acid, coniferin, chlorogenic acid, caffeine, theaflavin, and theaflavin gallate normally found in tea extracts (Friedman and others 2005; Nishitani and Sagesaka 2004). The chromatogram of sample obtained in this study was comparable to that reported by Nishitani and Sagesaka (2004). EGCG was the predominant catechin found in the GTE used in this study. Chen and Chan (1996), reported the antioxidant activity of GTE to be in the order of EGC>EGCG>EC>ECG, while Amarowicz and Shahidi (1995) reported the order of antioxidant activity as ECG>EC, EGCG>EGC for green tea catechins. It has been reported previously that EGCG is the major component among tea polyphenols and shows the strongest antioxidant activity (Chen and Ho 1995). The antioxidant capacity of the GTE could be attributed to the presence of all these catechins, particularly EGCG.

However it should also be noted that the composition of tea leaves are subject to change with differences in variety, climate, season, horticultural practice and age (Graham 1992) and these differences will result in compositional differences among tea extracts also.

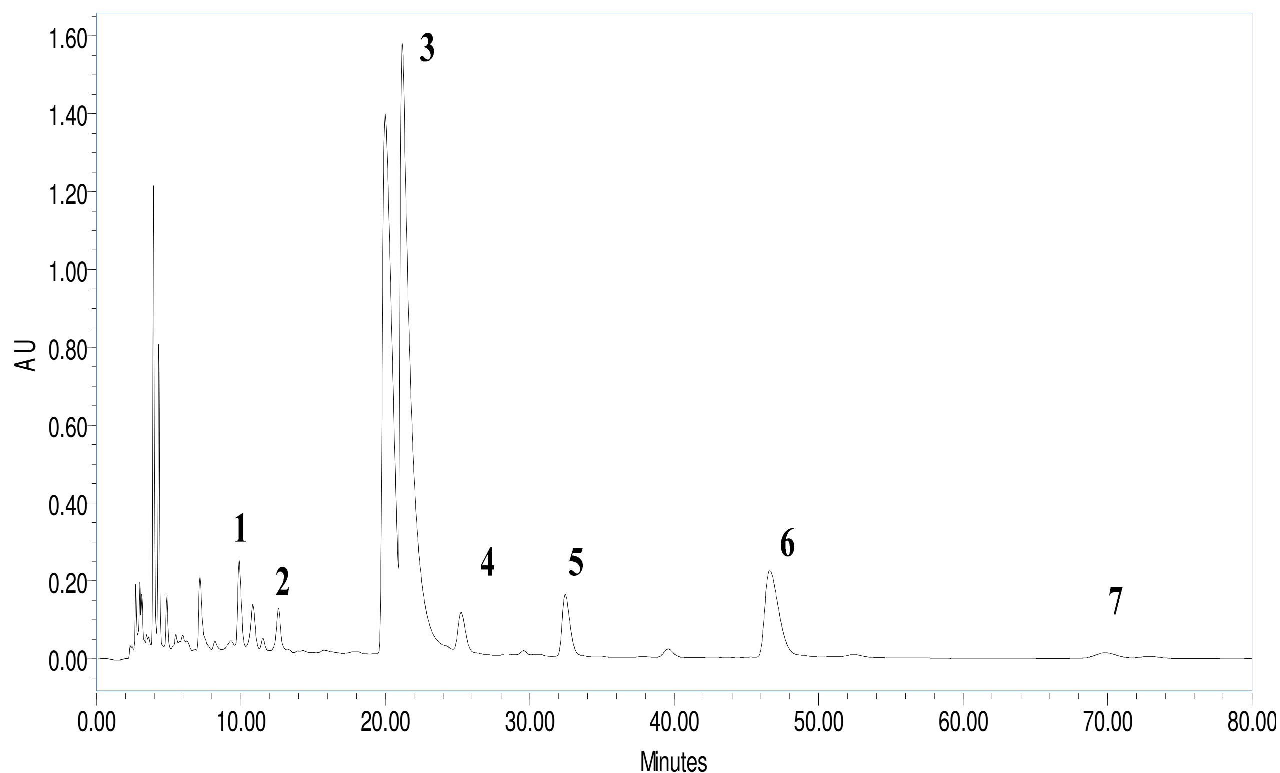


Fig 4.1: HPLC Chromatogram of Green Tea Extract. 1 = Epigallocatechin, 2 = Catechin, 3 = Epigallocatechingallate, 4 = Epicatechin, 5 = gallocatechingallate, 6 = Epicatechingallate 7 = Catechingallate.

Table 4.2: Major Catechins Present in Green Tea Extract (g/l).

C(Catechin)	4.31 ± 0.03
CG(Catechingallate)	1.70 ± 0.11
EC(Epicatechin)	5.14 ± 0.55
ECG(Epicatechingallate)	19.30 ± 0.02
EGC(Epigallocatechin)	6.22 ± 0.05
EGCG(Epigallocatechingallate)	103.20 ± 1.54
GCG(Gallocatechingallate)	8.23 ± 0.19
Total Polyphenols	148.10 ± 2.49

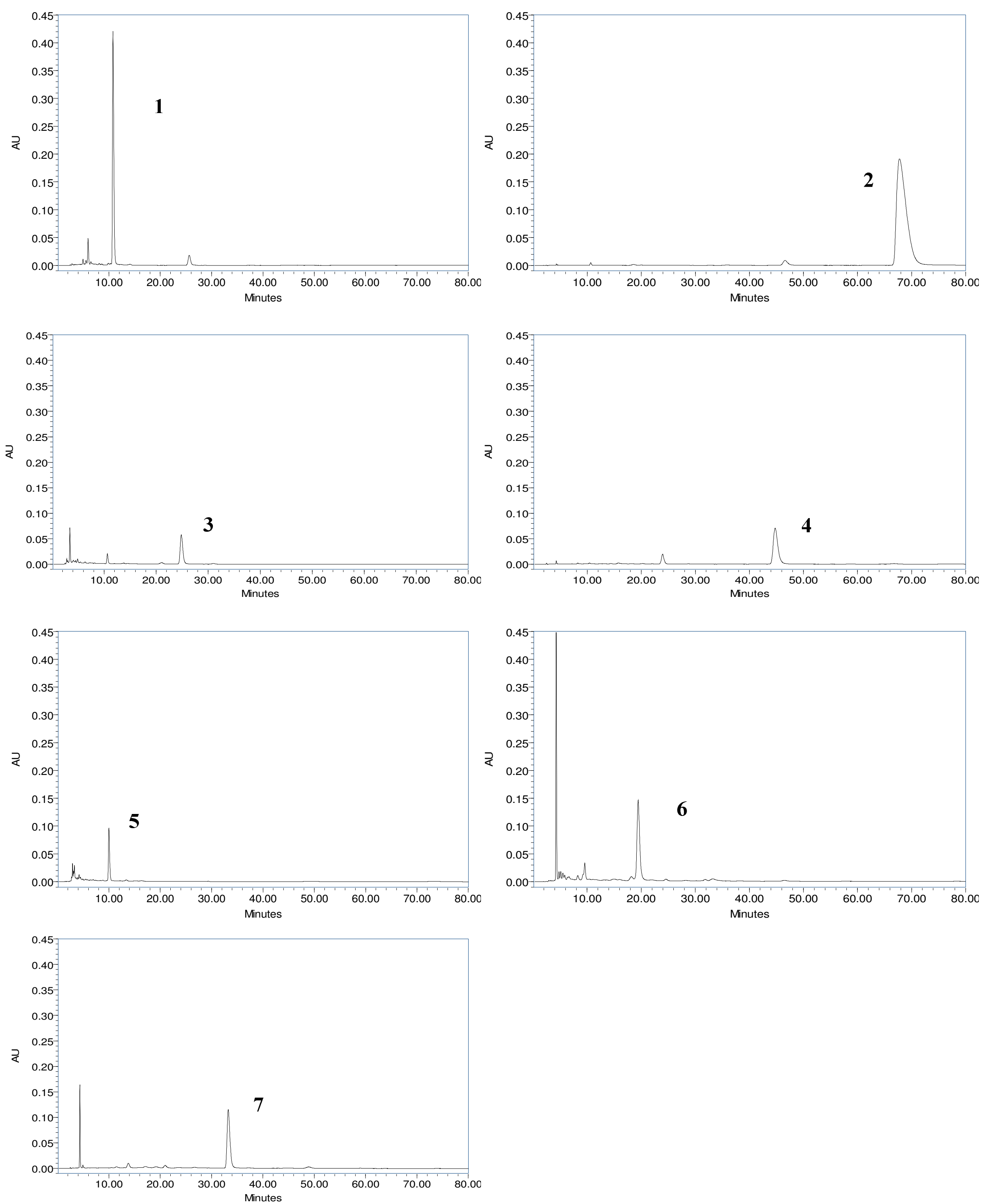


Fig 4.2: HPLC Chromatograms of Standards.1-catechin, 2-catechingallate, 3-epicatechin, 4-epicatechingallate, 5-epigallocatechin, 6-epigallocatechingallate, 7-gallocatechin

4.3 Proximate Composition and Fatty Acid Methyl Esters Profile of Fresh Shrimp

The proximate composition of deheaded and deshelled white shrimp is shown in Table 4.3.The moisture and protein content of fresh shrimp were found to be 77.36 ± 1.78 % and 18.69 ± 1.25 %, respectively. These values were similar to that reported by Sriket and others (2007) for deheaded, deshelled and deveined white shrimp. The ash content was found to be 2.10 ± 0.07 %. Rosa and Nunes (2003) reported that the ash content of shrimp ranged between 1.9-2 % and Sriket and others (2007) reported the ash content of white shrimp to be 1.47 %. The ash content obtained in this study is slightly higher compared to the articles cited, however it has been reported that the ash content of white shrimp may be slightly higher compared to other types and there may also be a seasonal variation. Rodde and others (2008) investigated the seasonal changes and the chemical composition of shrimp and found the lowest protein concentration (January-October) occurred when the ash content was high. The lipid content of shrimp was found to be 1.02 ± 0.1 %. This is lower than the valued reported by Sriket and others (2007) (1.30 ± 0.09). The proximate composition of shrimp is dependent on a number of factors including size and seasonal changes.

Table 4.3: Proximate Composition of White Shrimp *(Litopenaeus setiferus)*.

Composition(% wet weight)	
Moisture	77.36 ± 1.78
Protein	18.69 ± 1.25
Ash	2.10 ± 0.07
Lipids	1.02 ± 0.1

Values are means of triplicate measurements.

The fatty acid methyl ester profile of the shrimp showed that around 49.86% was saturated fatty acids and around 50.72 % was unsaturated (Table 4.4). The polyunsaturated fatty acids (PUFA) account for 32.23 %. The major PUFA were EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) accounting for 17.45 and 9.11 % of total fatty acids respectively. The major saturated fatty acids were found to be, Lauric acid and Palmitic acid which accounted for 17.32 % and 14.41 % of the fatty acids. Palmitoleic acid was found to be the major monounsaturated fatty acid (10.77 %). Rosa and Nunes (2003) reported the PUFA fraction to be 48.4 % of total lipids for red and pink shrimp. Sriket and others (2007) reported the PUFA fraction of white shrimp to be around 42.2 % of the total lipids. EPA and DHA were the major polyunsaturated fatty acids identified in previous studies (Rosa and Nunes 2003; Sriket and others 2007) and this is in accordance with our study. The type of shrimp, location and seasonal variation may have resulted in the difference in results.

Table 4.4: Fatty Acid Methyl Esters, FAME (% total fatty acids) of White Shrimp *(Litopenaeus setiferus).*

Dodecanoic acid (C12:0)	17.32 ± 0.09
Tetradecanoic acid (C14:0)	8.89 ± 0.03
Tetradecenoic acid (C14:1)	0.55 ± 0.01
Pentadecanoic acid (C15:0)	0.75 ± 0.00
Hexadecanoic acid (C16:0)	14.41 ± 0.01
Hexadecenoic acid(C16:1)	10.65 ± 0.03
Heptadecanoic acid (C17:0)	1.34 ± 0.00
Heptadecenoic acid(C17:1)	1.07 ± 0.00
Octadecanoic acid (C18:0)	7.11 ± 0.00
Cis-9-Octadecenoic acid (C18:1)	5.68 ± 0.01
Cis-9,12-Octadecadienoic acid (C18:2)	0.27 ± 0.00
Cis-9,12,15-Octadecatrienoic acid (C18:3)	0.39 ± 0.00

Table 4.4 continued	
Cis-6,9,12-Octadecatrienoic acid (C18:3)	0.84 ± 0.01
Cis-11,14-Eicosadienoic acid (C20:2)	0.54 ± 0.00
Cis-5,8,11,14-Eicosatetraenoic acid (C20:4)	3.38 ± 0.01
Cis-5,8,11.14,17-Eicosapentaenoic acid (C20:5)	17.45 ± 0.03
Docosanoic acid (C22:0)	0.04 ± 0.00
Cis-13,16-Docosadienoic acid (C22:2)	0.25 ± 0.00
Tetracosanoic acid (C24:0)	0.02 ± 0.00
Cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6)	9.11 ± 0.01

Values are means of triplicate measurements.

4.4 Energy Removal Rate, Freezing Rate and Freezing Time

The initial temperature of the shrimp was 4.37 ± 0.9 ^{o}C and 5.10 ± 0.3 ^{o}C for cryogenic and blast freezing, respectively. The operational temperature for cryogenic and blast freezing was -120 ^{o}C and -23 ^{o}C, respectively. It took 4.83 ± 0.29 min to reduce the temperature of the shrimp to -21.3 ± 0.40 ^{o}C by cryogenic freezing and 48.67 ± 2.3 min by air- blast freezing to reduce the temperature to -21.07 ± 0.49 ^{o}C. The energy removal rates for cryogenic and blast freezing were 836.67 ± 78.95 and 80.26 ± 3.82 J/s for 1kg of shrimp respectively. The freezing rates of CF and BF were -5.23 ± 0.28 and -0.54 ± 0.03 ^{o}C/min respectively (Table 4.5)

Table 4.5: Energy Removal Rate, Freezing Rate and Freezing Time of Shrimp.

	Cryogenic Freezing (Liquid N_2)	Blast Freezing (Air Velocity: 1.15m/s)
Product heat load (kJ)	247.54 ± 6.73	234.01 ± 1.52
Specific heat capacity of unfrozen shrimp, C_{pu} (kJ/kgK)	3.48 ± 0.02	3.47 ± 0.01
Specific heat capacity of frozen shrimp, C_{pf} (kJ/kgK)	1.83 ± 0.01	1.82 ± 0.01

Table 4.5 continued		
kg Bound water per kg solute, B	0.21 ± 0.00	0.18 ± 0.00
Weight fraction of ice	0.57 ± 0.01	0.52 ± 0.01
Latent heat (kJ/kg)	190.70 ± 2.37	173.23 ± 1.97
Freezing time (min)	4.83 ± 0.28	48.67 ± 2.30
Freezing rate (^{o}C/min)	-5.23 ± 0.28	- 0.54 ± 0.03
Energy removal rate (J/s)	836.67 ± 78.95	80.26 ± 3.82

On comparing the freezing time, freezing rate and energy removal rate, it can be seen CF was about ten times faster than air-blast freezing. The faster freezing time in CF is attributed to the contact of boiling liquid N_2 directly with the sample which results in a higher heat transfer rate (Awonorin 1989) and to the lower ambient temperature in the cryogenic freezer, whereas in blast freezing cooled air is used and this has a lower heat transfer rate. The air velocity in the blast freezer was low, 1.15m/s, and the ambient temperature was -23°C, and this resulted in longer freezing time.

The freezing curves of shrimp frozen by CF and BF are shown in Figure, 4.3. The regions T_o to T_{if} and T_o'to Ti_f' on the freezing curve correspond to the sensible heat region during which heat is lost from the product to the medium. The regions T_{if} to T_{if}* and T_{if}'to T_{if}'* correspond to the latent heat region, during which most of the ice crystal formation occurs. Since most of the ice crystal formation occurs in this region, it is essential to pass this region as quickly as possible. When there is a rapid drop in temperature, the rate of ice crystal formation is higher and crystals formed evenly throughout the tissue, whereas when the freezing rate is low (blast freezing) extracellular ice crystals form first and these will grow into larger ones by drawing water from the cells (Kolbe and Kramer 2007). This leads to dehydration of the product, and shrunken cells (Love 1966). On thawing, the extracellular ice crystals melt and contribute to the drip loss and will result in a drier and tougher fish muscle (Kolbe and Kramer 2007). With higher freezing

rates nucleation is faster and will, result in formation of a large number of small ice crystals as opposed to small numbers of large crystals. In CF the rate of crystal formation dominated the rate of crystal growth. The size and the distribution of ice crystals will have an effect on the extent of cellular damage and product quality (Bello and others 1981).

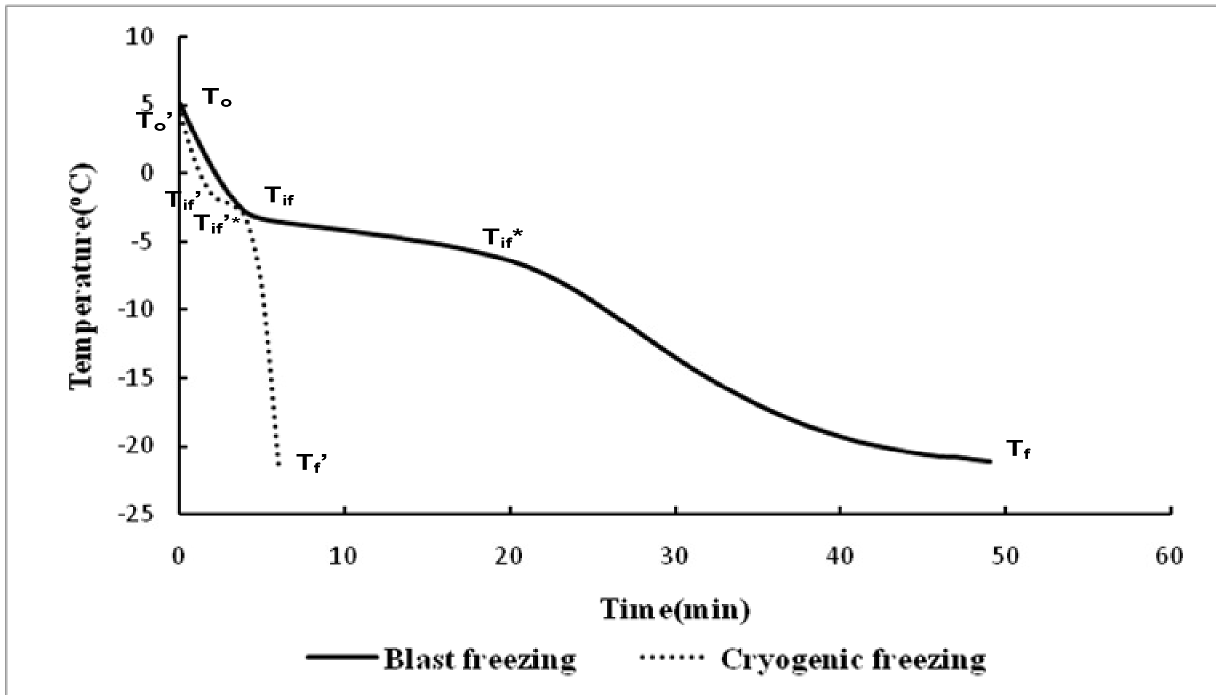


Fig 4.3: Freezing Curves of Shrimp Frozen by Cryogenic and Air-Blast Freezing.

Petrovic and others (1993) reported that the highest weight losses from freezing occurred at slow freezing rates and with increase in freezing rate there was a linear decrease in weight loss. Grujic and others (1993) studied the effect of freezing rates on the structure and ultra structure of beef muscle. They reported that during slow freezing large irregular ice crystals were formed inbetween the muscle fibers, and these crystals deformed the tissue, pushing fibers into groups and compressing the myofibrils inside the fibers, while fast freezing rates resulted in smaller sized intracellular ice crystals. With faster freezing rates the latent heat region is short, as seen in

Figure 4.3. The latent heat region can be considered a critical zone, as most ice crystals are formed here. There is also a concentration of solutes and an increase in activity of enzymes (Kolbe and Kramer 2007). Protein denaturation and breakdown of lipids are also increased at this region. Slower freezing rates cause increased lysosome rupture and this can result in release of enzymes. Pan and Yeh (1993) studied the lysosome activity of liquid N_2 frozen and air-blast frozen shrimp (*Penaeus monodon*). They report that liquid N_2 freezing caused minimal damage to muscle cells while air-blast freezing (at -35 ^{o}C) resulted in rupture of 50 % of the lysosomes, which caused an increase in cathepsin D activity. There was loss of solubility and the water holding capacity decreased with slower freezing rates (Reid and others 1986; Petrovic and others 1993).

The freezing time and energy removal rate can be important in designing and estimating the cost of the freezer. For the same freezing capacity, the size of the freezer varies depending on the freezing time. Higher energy removal rate implies production rates can be much higher per freezer size for cryogenic freezing compared to blast freezing.

4.5 L*, a* and b* Values of Shrimp

The color of shrimp will affect its appearance and hence consumer acceptance. The color will also have an effect on the perception of taste. The color of shrimp can be influenced by species, season, feed and environment (Yanar and others 2004). The color changes that occur during frozen storage can be attributed to lipid oxidation and pigment degradation (Daias and others 1994). In the present study, with increase in frozen storage time there were changes in a* and b* values of the shrimp, however there was no significant changes in the L* values throughout the storage period (Tables 4.6, 4.7). L* signifies the whiteness of the sample. In previous research done on salmon and cod, an increase in L* values after frozen storage was reported, however

with decrease in storage temperatures these changes were not significant (Sathivel 2005; Schubring 2004). Benjakul and others (2007) reported a slight decrease in L* values during 4 weeks frozen storage for cuttlefish but thereafter the L* values did not change significantly up to 16 weeks. In this study, on day 1 all shrimp samples had similar a* values. At the end of 180 days frozen storage the a* values of NG and GDW shrimp were significantly lower than the GTE glazed samples for blast frozen shrimp. However, 2GTE, 3GTE and 5GTE samples had similar a* values and were not significantly different from each other. CF green tea glazed samples showed a decrease in a* value after 180 days compared to day 1 but such differences were not seen for BF green tea glazed samples. The decrease in a* values, for NG and GDW can be attributed to the degradation of astaxanthin and lipid oxidation in shrimp. It has been reported in many studies that lipid oxidation causes color degradation during storage (Lanari and others 1995; Guidera and others 1997; Erickson and Hung, 1997, Hui and others 2006. However, in the present study GTE glazed samples had higher a* values compared to controls and this may be attributed to the effect of green tea polyphenols. Yamamoto (1997) studied the antidiscoloring effect of tea polyphenols on beverages and fish samples. He reported that tea polyphenols were effective in maintaining the color of the beverages, greater than L-ascorbate. Tea ployphenols were also effective in suppressing the discoloration of fish meat. Since the color in shrimp is due to carotenoids, it could be easily degraded by oxidation due to the presence of the large number of double bonds, and since the tea polyphenols have antioxidant effects they are also effective in preserving the color. In the present study, b* values of all the samples increased during storage, however with increase in b* values of green tea glazed samples being significantly higher than both controls. There were no significant differences among blast frozen green tea glazed shrimp at the end of 180 days, but CF5GTE had a higher b* values than CF3GTE. Increase in b* values

imply there was an increase in yellowness. The tea may have acted like a stain causing an increase in b* values of GTE glazed shrimp and since color was measured at the surface the difference is significant. Increase in b* value (of non-glazed shrimp) during storage was reported by Tsironi and others (2009), for shrimp stored at variable temperatures. In this study significant differences could not be seen between non-glazed and distilled water glazed shrimp with respect to color but GTE glazed shrimp differed in their color values from NG and GDW shrimp, with respect to b* values. These results indicate that green tea glazing has a significant effect on color. Other factors such as excessive dehydration, lipolysis, freeze crack and the size of ice crystals can also have an effect on color perception (Hui and others 2007). Metmyoglobin reductase found in fish muscle has also been reported to cause color deterioration (Benjakul and Bauer 2001).

Table 4.6: L*, a* and b* Values of Glazed and Non-glazed Shrimp Samples Frozen by Cryogenic Freezing.

CRYOGENICALLY FROZEN SHRIMP				
	Treatment	L*	a*	b*
Day 1	CFNG	30.04±1.70A	1.74±0.32A	-1.93±0.15G
	CFGDW	28.98±1.25A	1.76±0.25A	-1.96±0.07G
	CF2GTE	28.56±0.74A	1.44±0.21AB	-1.88±0.18G
	CF3GTE	29.65±1.13A	1.61±0.36A	-1.73±0.04FG
	CF5GTE	28.16±0.60A	1.8±0.25A	-0.76±0.01D
Day30	CFNG	30.04±1.76A	0.85±0.21BCDE	-1.71±0.15FG
	CFGDW	29.23±3.23A	1.22±0.18ABCD	-1.55±0.03F
	CF2GTE	28.96±1.50A	1.39±0.31ABC	-1.46±0.12F
	CF3GTE	29.67±1.98A	1.27±0.20ABCD	-1.06±0.07E
	CF5GTE	29.78±2.70A	1.45±0.19AB	-1.04±0.10DE
Day 90	CFNG	30.29±0.46A	0.50±0.16EF	-1.12±0.0E
	CFGDW	29.93±0.44A	0.70±0.13EF	-1.13±0.11E

Table 4.6 continued

Day90	CF2GTE	29.37±1.25A	1.07±0.26ABCD	0.28±0.07BC
	CF3GTE	29.09±0.17A	0.95±0.14BCDE	0.19±0.07C
	CF5GTE	29.81±1.05A	1.03±0.37BCDE	0.24±0.08BC
Day180	CFNG	32.64±1.79A	0.13±0.07F	-1.09±0.06E
	CFGDW	31.95±1.18A	0.49±0.09EF	-1.05±0.04DE
	CF2GTE	30.98±1.58A	0.85±0.08BCDE	0.50±0.07AB
	CF3GTE	29.96±0.35A	0.76±0.07CDEF	0.37±0.05BC
	CF5GTE	30.22±2.41A	0.92±0.05BCDE	0.72±0.07A

CFGDW = Cryogenically frozen shrimp glazed with distilled water, CF2GTE = Cryogenically frozen shrimp glazed with 2% green tea extract, CF3GTE = Cryogenically frozen shrimp glazed with 3% green tea extract, CF5GTE = Cryogenically frozen shrimp glazed with 5% green tea extract.

Table 4.7: L*, a* and b* Values of Glazed and Non-glazed Shrimp Samples Frozen by Air- Blast Freezing.

BLAST FROZEN SHRIMP				
	Treatment	L*	a*	b*
Day 1	BFNG	29.24±0.08A	0.89±0.05AB	-1.7±0.14F
	BFGDW	29.68±0.56A	1.03±0.34A	-1.33±0.33CDEF
	BF2GTE	31.48±1.35A	0.98±0.20A	-1.33±0.07CDEF
	BF3GTE	30.11±0.75A	0.86±0.07ABC	-1.41±0.10DEF
	BF5GTE	31.73±1.84A	1.01±0.32AB	-1.15±0.38CDEC
Day 30	BFNG	30.94±1.63A	0.64±0.08ABCD	-1.6±0.11CEF
	BFGDW	31.13±1.48A	0.99±0.09AB	-1.28±0.02CDEF
	BF2GTE	31.95±0.85A	0.83±0.15ABC	-1.19±0.04CDEF
	BF3GTE	30.29±0.92A	0.77±0.06ABC	-1.32±0.14CDEF
	BF5GTE	31.9±1.24A	0.97±0.16A	-0.59±0.07B
Day 90	BFNG	31.87±0.41A	0.48±0.04CDE	-1.08±0.01CD
	BFGDW	31.71±2.78A	0.44±0.08CDE	-1.15±0.01CD
	BF2GTE	30.59±0.25A	0.81±0.13ABC	0.04±0.01A

Table 4.7 continued				
Day 90	BF3GTE	30.42±1.49A	0.81±0.09ABC	0.05±0.01A
	BF5GTE	31.4±1.50A	0.96±0.07A	0.06±0.02A
	BFNG	32.62±1.66A	0.15±0.04E	-0.89±0.05BC
	BFGDW	32.92±1.84A	0.22±0.08DE	-1.01±0.02BCD
Day 180	BF2GTE	31.28±1.27A	0.75±0.09ABC	0.22±0.03A
	BF3GTE	31.39±1.46A	0.79±0.08ABC	0.23±0.15A
	BF5GTE	31.67±0.83A	0.89±0.17AB	0.28±0.04A

BFNG = Blast frozen non-glazed shrimp, BFGDW = Blast frozen shrimp glazed with distilled water, BF2GTE = Blast frozen shrimp glazed with 2% green tea extract, BF3GTE = Blast frozen shrimp glazed with 3% green tea extract, BF5GTE = Blast frozen shrimp glazed with 5% green tea extract.

4.6 Texture of Shrimp

Texture is an important physical characteristic of seafood, and a change in texture may indicate a change in quality. Freezing and thawing processes affect the texture of shrimp (Boonsumrej and others 2007; Diaz-Tenorio and others 2006). Freeze induced changes are more pronounced in fish muscle proteins compared to plant and other meat proteins (Erickson and Hung 1997) and fish with lower fat content have a higher rate of protein change (Dyer and Morton 1956). Textural changes of muscle are mostly due to changes in protein structure and functionality, especially the myofibrillar and stroma proteins. Changes such as denaturation, cross linking, loss in water holding capacity, and solubility will affect the texture (Hui and others 2006; 2004). In this study, texture was evaluated by measuring the cutting force (N), which is an indicator of hardness. On day 1, all samples had similar cutting force (Table 4.8). As, the storage time increased the non-glazed shrimp samples had significantly lower cutting force than glazed samples. After 90 days storage, both cryogenically and blast frozen shrimp had similar cutting force. It can be seen from the results that the lower cutting force obtained by cryogenic freezing initially, is lost during prolonged storage. After 180 days all the glazed shrimp samples had

higher cutting force than of non-glazed shrimp regardless of glazing materials applied on the shrimp. Significant differences were not seen between GDW and GTE glazed samples. These changes in texture can be attributed to changes in structure and functionality of proteins, lipid oxidation, and enzymatic activity. It has been reported that increased lipid oxidation can lead to protein denaturation; that is, the products of lipid oxidation may interact with proteins and cause alterations in proteins (Erickson and Hung 1997). Malonaldehyde formed during lipid oxidation has been reported to form cross-links with protein and can form insoluble protein aggregates (Buttkus 1970). In the present study there was a continuous increase in TBARS values which indicated increase in oxidation and the products of lipid oxidation may have been responsible for the texture changes. The aggregation of myofibrillar proteins caused by secondary interactions and disulfide bonds, leads to a decrease in water holding capacity (Hui and others 2006). With a decrease in water holding capacity, there will be more drip loss and this can lead to a tougher product. However, the non-glazed shrimp were more mushy than tough; this may be because of enzymatic action. Lipases and phospholipases can lead to hydrolysis of lipids and the products of these reactions can interact with proteins altering their functionality. Lipase has been reported to be active in frozen foods stored at -29 ^{o}C (Alford and Pierce 1961). The freezing process and subsequent storage can cause increased rupture of lysosomes. The rupture of lysosomes leads to increased catheptic activity (Pan and Yeh 1993). Cathepsins are a group of proteolytic enzymes endogenous to shrimp muscle, and the increase in their activity had been reported to cause mushiness during storage and after thawing (Ercikson and others 1983, Pan and Yeh 1993). Trimethyl amine oxide (TMAO) is found inherently in crustaceans (Konosu and Yamagushi 1882). The enzyme TMAO demethylase can cause breakdown of TMAO into formaldehyde and dimethylamine (DMA) (Erickson and Hung 1997; Haard and Simpson 2000). Formaldehyde,

thus formed can cross link with muscle proteins resulting in spongy texture (Santos-Yap 1995). Enzyme action might have increased in the non-glazed shrimp compared to glazed shrimp due to increased concentration of enzymes due to increased moisture loss in non-glazed shrimp during frozen storage. Higher proteolytic enzyme concentration increases the protein hydrolyzation which may lower the cutting force for non-glazed shrimp.

Table 4.8: Cutting Force (N) of Shrimp Frozen by Cryogenic and Air- Blast Freezing.

CRYOGENICALLY FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
CFNG	32.99 ± 3.31B	27.47 ± 2.49C	26.06 ± 3.03C	24.1 ± 1.96B
CFGDW	32.67 ± 4.00B	34.27 ± 3.63ABC	36.77 ± 3.88A	38.87 ± 2.72A
CF2GTE	33.57 ± 2.98AB	35.39 ± 2.92AB	36.55 ± 2.57A	38.06 ± 3.57A
CF3GTE	32.92 ± 3.01B	32.76 ± 1.92BCD	35.56 ± 2.70AB	37.61 ± 2.94A
CF5GTE	33.50 ± 0.93AB	33.48 ± 3.65ABC	36.88 ± 2.73A	35.41 ± 2.19A
BLAST FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
BFNG	38.30 ± 4.49AB	29.93 ± 2.09DC	29.90 ± 2.48BC	26.12 ± 3.44B
BFGDW	39.38 ± 4.82A	39.46 ± 3.94A	40.1 ± 3.67A	35.64 ± 4.21A
BF2GTE	36.26 ± 2.52AB	37.83 ± 3.42AB	38.72 ± 3.48A	36.12 ± 2.77A
BF3GTE	36.25 ± 2.35AB	36.34 ± 2.14AB	37.79 ± 3.66A	36.94 ± 3.96A
BF5GTE	37.46 ± 2.48AB	37.98 ± 5.15AB	38.28 ± 4.61A	36.32 ± 4.36A

CF = Cryogenically frozen, BF = Blast frozen, NG = non-glazed shrimp, GDW = Shrimp glazed with distilled water, 2GTE = Shrimp glazed with 2% green tea extract, 3GTE = Shrimp glazed with 3% green tea extract, 5GTE = Shrimp glazed with 5% green tea extract. Capital letters mean values followed by the same letters are not significant in each column.

4.7 Glazing Yield and Thaw Yield of Shrimp

The glazing yield refers to the amount of glaze present on the shrimp at a given time. In this study glazing yield is calculated as the weight of thawed glazed shrimp over weight of raw shrimp multiplied by 100. It is important to monitor the changes in glazing yield, as this can

affect thaw yield, moisture content and lipid oxidation. On day 1, all of the cryogenically frozen and blast frozen shrimp had similar glazing yield with values ranging from 111-113% (Figure 4.4, 4.5). This implies that 13% of the weight of raw shrimp is the amount of glaze present on the product.

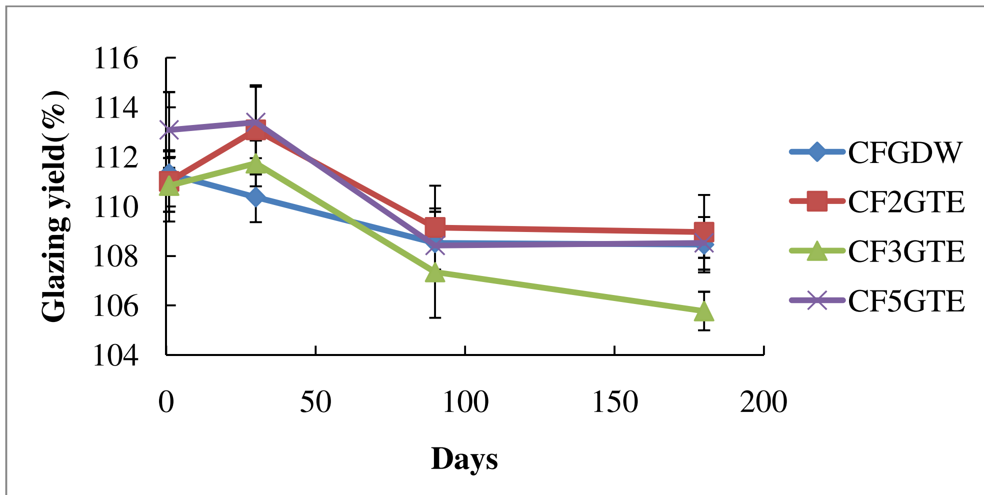


Fig 4.4: Glazing Yield of Shrimp Frozen by Cryogenic Freezing. CFGDW = Cryogenically frozen shrimp glazed with distilled water, CF2GTE = Cryogenically frozen shrimp glazed with 2% green tea extract, CF3GTE = Cryogenically frozen shrimp glazed with 3% green tea extract, CF5GTE = Cryogenically frozen shrimp glazed with 5% green tea extract.

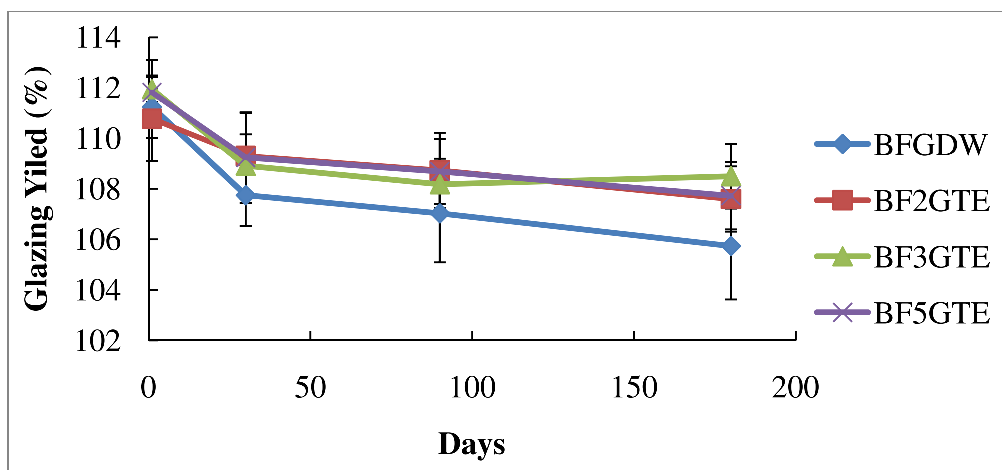


Fig 4.5: Glazing Yield of Shrimp Frozen by Air- Blast Freezing. BFGDW = Blast frozen shrimp glazed with distilled water, BF2GTE = Blast frozen shrimp glazed with 2% green tea extract, BF3GTE = Blast frozen shrimp glazed with 3% green tea extract, BF5GTE = Blast frozen shrimp glazed with 5% green tea extract.

The glazing percentages may be influenced by product size and shape, product temperature, temperature of the glazing solution and glazing time (Johnston and others 1994; Jacobsen and Pedersen 1997; Jacobsen and Fossan 2001). Glaze percentages give the amount of glaze based weight of product. Glaze percentages range from 4 to 10 % depending on product but glaze percentages of 2 to 20 % have also been reported (Johnston, 1994). Goncalves and others (2009) reported that normal glaze percentages would be between 8 and 12 %. However abusive glaze percentages from 25-45 % have also been reported (Jacobsen and Fossen, 2001; Hui and others 2004,). Londahl and Astrom (1972) reported that an 8 % glaze was found to be optimal in preserving shrimp quality. Vanhaecke and others (2010) reported 6-10 % glazing was effective in protecting fish fillet from dehydration, oxidation and quality loss. In this study a drop occurred in glazing yield % of blast frozen shrimp and cryogenically frozen shrimp after 1 month and 3 months storage respectively, after which the glazing percentages remained unchanged (105-109 %) for the rest of the storage period.

During a freezing process, water is converted to ice and is often removed from its normal position to an extent that is dependent on the rate of freezing. On thawing most of this water may be released from the tissue as to drip loss. Drip can be defined as the exudate that results from thawing frozen tissue (Hui and others 2004). Hence, thaw yield can be correlated to drip loss, and a higher thaw yield implies a lower drip loss. The thaw losses are mostly caused by changes in functionality of protein during freezing and frozen storage and fish muscle protein are reportedly less stable than protein of beef, pig and poultry muscles (Dyer and others 1956). On comparing the thaw yield of glazed and non-glazed shrimp in this study, it is seen that glazed samples had significantly higher thaw yields (Figure 4.6, 4.7). The cryogenically frozen shrimp had higher thaw yield than blast frozen shrimp. This may be caused by the slower freezing rates

of blast frozen shrimp, which must have resulted in formation of large extracellular ice crystals and these result in a higher drip loss on thawing (Fennema 1973, Kolbe and Kramer 2007). Boonsumrej and others (2007) reported similar results for tiger shrimp. Alizadeh and others. (2007) reported air-blast frozen (slow freezing) salmon fillet to have higher drip loss than pressure shift frozen salmon (fast freezing).

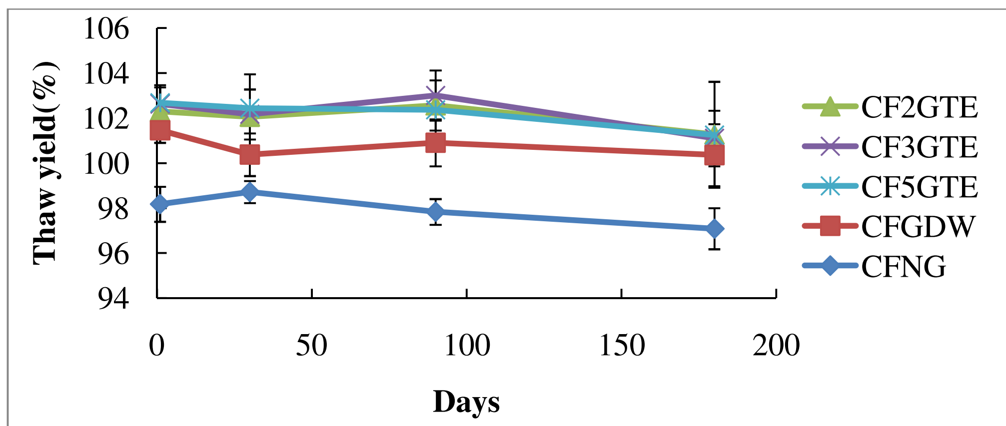


Fig 4.6: Thaw Yield of Glazed and Non-glazed Shrimp Frozen by Cryogenic Freezing. CFNG = Cryogenically frozen non-glazed shrimp, CFGDW = Cryogenically frozen shrimp glazed with distilled water, CF2GTE = Cryogenically frozen shrimp glazed with 2% green tea extract, CF3GTE = Cryogenically frozen shrimp glazed with 3% green tea extract, CF5GTE = Cryogenically frozen shrimp glazed with 5% green tea extract.

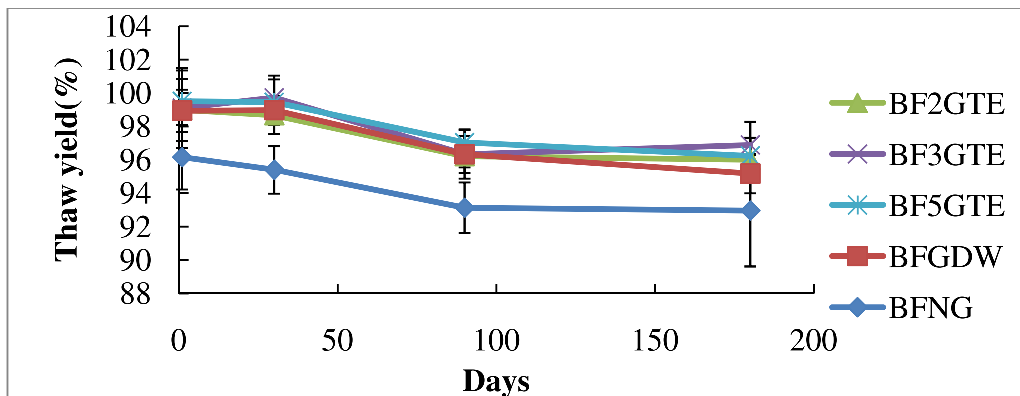


Fig 4.7: Thaw Yield of Glazed and Non-glazed Shrimp Frozen by Air -Blast Freezing. BFNG = Blast frozen non-glazed shrimp, BFGDW = Blast frozen shrimp glazed with distilled water, BF2GTE = Blast frozen shrimp glazed with 2% green tea extract,BF3GTE = Blast frozen shrimp glazed with 3% green tea extract, BF5GTE = Blast frozen shrimp glazed with 5% green tea extract.

Jul (1984) in discussing about freezing rate and drip, refers to several articles with conflicting results. While drip can be affected by freezing rates it is not always the case and the range of freezing rates considered may also play a role. It has been reported that drip losses up to 5 % weight of meat are common (Genot 2000) and slow freezing rates, result in greater drip loss than faster freezing rates (Hui and others 2006). The process can be considered slow freezing when the time for the temperature to fall from -1 ^{o}C to -7 ^{o}C exceeds 10 minutes, in the present study the blast frozen samples took around 12.18 minutes for the temperature to drop from -1 to -7 ^{o}C. The drip loss may directly indicate the ability of the protein in the fish to hold water (Ciarlo and others 1985), and during freezing and subsequent storage, the denaturation of proteins, their aggregation and an eventual decrease in water holding capacity (WHC) may be considered as the main reasons for thaw loss. The myofibrillar proteins are more susceptible to freeze denaturation compared to sacroplasmic proteins which are more susceptible than stromal proteins (Erickson and Hung 1997). In shrimp the myofibrillar proteins are the major constituent of the muscle proteins and constitute for about 60%, making them more susceptible to freeze denaturation. Higher drip loss will lead to loss in weight which will have negative impact with respect to financial value. Drip loss also will result in unattractive product and loss of nutrients. Water soluble nutrients, including proteins, minerals and B vitamins can be lost along with the thaw exudates (Pigott and Tucker 1990). Factors such as species, type of muscle, pH and storage time will also influence drip loss (Hui and others 2006). In this study there were no significant differences in thaw yields between the GTE glazed shrimp and GDW for blast frozen samples during the storage period. Cryogenically frozen GTE glazed shrimp had a slightly higher thaw yields than CFGDW on 30 days storage but after prolonged storage there was no significant difference between the GTE glazed and GDW samples. Comparison between blast frozen and

cryogenically frozen samples shows that blast frozen glazed samples had thaw yields comparable to CFNG. But BFNG had a lower thaw yield than CFNG. This suggests that glazing helped improve the thaw yield of shrimp. It should however be noted that the drip itself cannot be distinguished clearly from any superficial moisture or glaze (Hui and others 2004). The thaw yield values indicated for the glazed shrimp are high due to the presence of glaze residual that may have remained on the shrimp. A similar result for glazed/coated fish has been reported by Sathivel (2005) and Sathivel and others (2007) for salmon and pollack fillets during 3 and 4 months frozen storage.

4.8 pH of Shrimp

The pH of shrimp is generally agreed to be a good indicator of quality. pH depends on physicochemical factors and hence changes in pH may reflect other physicochemical changes that occur during storage (Riaz and Qadri 1990). In this study during the storage time, pH of all shrimp samples increased regardless of treatment and freezing method (Figure 4.8, 4.9).

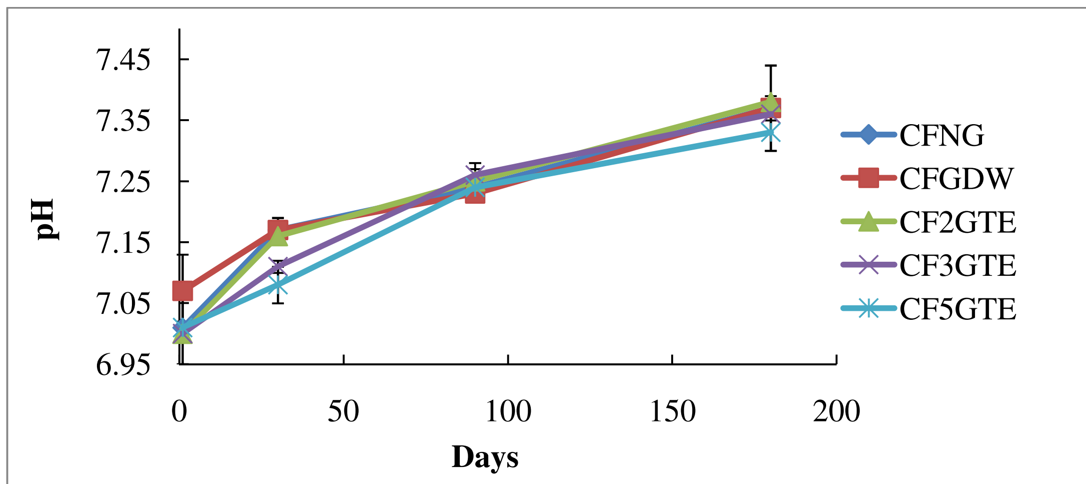


Fig 4.8: pH of Glazed and Non-glazed Shrimp Frozen by Cryogenic Freezing. CFNG = Cryogenically frozen non-glazed shrimp, CFGDW = Cryogenically frozen shrimp glazed with distilled water, CF2GTE = Cryogenically frozen shrimp glazed with 2% green tea extract, CF3GTE = Cryogenically frozen shrimp glazed with 3% green tea extract, CF5GTE = Cryogenically frozen shrimp glazed with 5% green tea extract.

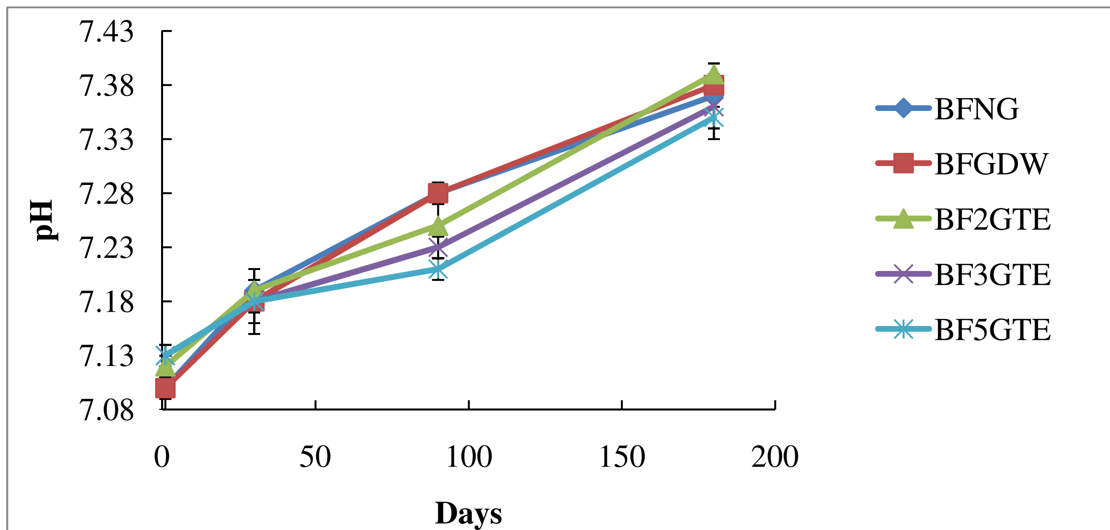


Fig 4.9: pH of Glazed and Non-glazed Shrimp Frozen by Air -Blast Freezing. BFNG = Blast frozen non-glazed shrimp, BFGDW = Blast frozen shrimp glazed with distilled water, BF2GTE = Blast frozen shrimp glazed with 2% green tea extract, BF3GTE = Blast frozen shrimp glazed with 3% green tea extract, BF5GTE = Blast frozen shrimp glazed with 5% green tea extract.

Blast frozen shrimp had a slightly higher pH compared to cryogenically frozen shrimp, but the difference may not be considered significant since the pH of the fresh shrimp used for blast freezing was slightly higher (7.11 ± 0.01) than the shrimp used for cryogenic freezing (7.00 ± 0.00). The pH of fresh shrimp is reported to be in the range of 6.5-7.00 (Mendes and others 2005). Tsironi and others (2008) have reported initial pH values of 6.95 which increased to 7.85 during 74 days of storage at -8 ^{o}C. Goswami and others (2001) reported an initial pH value of 7.2 (*Penaeus Monodon*) and this was found to increase to 7.4 during storage after liquid nitrogen freezing. He also reported similar pH before and just after liquid nitrogen freezing. These results are comparable to the results obtained in the present study. The increase in pH was similar for glazed and non-glazed samples for the cryogenically frozen shrimp; there was no significant difference among the treatments at specific storage times. At the end of 180 days storage all blast frozen samples had similar pH. Gonclaves and others (2009) reported that the glazing percentage influenced pH of shrimp and higher glazing percentages yielded lower pH

values on storage, while using water as the glazing solution. In this study, GTE glazed (3GTE and 5GTE) shrimp had slightly lower pH values than non-glazed shrimp, during 30 day storage for cryogenically frozen shrimp and during 90 days storage for air-blast frozen samples and such differences were not observed at any other time during storage. A significant difference between cryogenically frozen shrimp and blast frozen shrimp was not observed in this study with regard to pH. The increase in pH may have been caused by any of the following reasons as suggested by previous studies. Sigurgisladottir and others (2000) reported that freezing and thawing could cause changes in the pH of fish muscle. pH value of shrimp could be related to acceptability of shrimp found by sensory and chemical analysis (Shamshad and others 1990; Mendes and others 2005; Zeng and others 2005; Chung and Lian 1979). The increase in the pH of shrimp may occur due to biochemical changes that occur during frozen storage (Shamshad and others 1990; Mendes and others 2005). Microbial enzymes released before freezing can contribute to changes in pH and quality loss during frozen storage (Hui and others 2006). pH changes have been correlated to the level of indole, TMA (Trimethylamine), TVB(Total Volatile base) ,VB-N/AA-N (volatile basic nitrogen or amino acid nitrogen) ratio in previous studies (Hanpongkittikun and others 1993, Mendes and others 2005; Zeng and others 2005). TMA (Trimethylamine), can be formed from breakdown of TMAO (Trimethyamine oxide) found inherently in shrimp (Konosu and Yamagushi 1982) and this can cause an increase in the pH. Volatile amines can be produced in fish from the breakdown of amino acids. Ammonia is released by the breakdown of nucleotides by tissue enzymes, adenosine deaminase and adenosine monophosphatase (AMP) deaminase and this can lead to an increase in the pH of shrimp (Vongsawasdi and Noomhorm 2000). Increase in volatile bases produced by action of endogenous or microbial enzymes contribute to pH increase (Ruiz and others 2001; Manat and others 2005). Riaz and Qadri

(1990), found the increase in pH during frozen storage of shrimp paralled the increase in free fatty acids (FFA) and the decrease in salt soluble protein; they proposed the increase in FFA can increase denaturation of proteins which can lead to a rise in pH. The increase in FFA can also lead to an increase of TBARS value and hence this result can be compared to the result obtained in this study. A pH value of 7.8 is reported to be a critical value in determining the acceptability of shrimp (Chung and Lian, 1979), and Mendes and others (2005) found pH values in the range of 7.59-7.89 at the borderline of sensory rejection. The pH of shrimp is expected to increase during frozen storage, and values up to 7.95 may be acceptable (Goswami and others, 2001). Considering these studies, both the glazed and non-glazed shrimp in this study had an acceptable pH.

4.9 Moisture Content of Shrimp

Moisture content of non glazed samples showed a decreasing trend; while the moisture content of glazed shrimp did not change significantly during storage and was comparable to raw shrimp. The raw shrimp used for cryogenic freezing and blast freezing had similar moisture contents, 78.92 ± 0.68 and 78.53 ± 0.57, this is comparable to the value (77.21 ± 0.18), reported by Sriket and others (2007), for white shrimp. The moisture contents of shrimp frozen by CF and BF are shown in Table 4.9. The decreasing moisture content of non-glazed shrimp may be caused by evaporation of moisture. The evaporation of moisture can be affected by area exposed; surface area to volume ratio, fat covering and the presence of packaging (Hui and others 2006). Martin and others (1999) reported that water molecules are never completely immobilized even by low temperatures and hence water redistribution can occur even in frozen products. The moisture loss in food mainly occurs by diffusion (Saravacos and Maroulis 2001) and hence is affected by temperature and concentration gradients.

At the end of 1 day storage moisture contents of cryogenically frozen shrimp were not significantly different compared to blast frozen shrimp. All the BF glazed samples had similar moisture content at 30 days of storage; similar results were also observed for cryogenic freezing. The CFNG and BFNG had similar moisture content after 30 days storage. On 30 days storage the cryogenically frozen glazed shrimp and blast frozen glazed shrimp had comparable moisture contents. In this study significant differences were not observed between GTE glazing and GDW glazing with respect to moisture content. All GTE glazed samples and GDW samples had similar moisture contents and were different only form NG samples, after storage for 90 days or more within each freezing method. At 90 days of storage, it was found that BFNG and CFNG had no significant difference in moisture. However, the cryogenically frozen glazed shrimp had significantly higher moisture content than blast frozen glazed shrimp. This may have been caused by the reduction in the glazing yield of blast frozen shrimp that occurred after 1 month storage. All the glazed samples had higher moisture content that non-glazed, which indicated that the glaze protected the shrimp from dehydration during frozen storage. The glaze may offer a more uniform surface compared to a surface and thus have a total surface area for evaporation (Londahl and Nilsson, 2003). At the end of 180 days storage, significant differences could not be seen between blast frozen and cryogenically frozen shrimp with respect to moisture content, however all glazed samples had significantly higher moisture contents than non-glazed samples. After 180 days storage, BFNG had lost 5.11 % moisture and the blast frozen glazed samples on average lost 2.04 % moisture. Around 0.5-1.2 % moisture loss each month can occur when exposed meat is stored in a freezer (Genot 2000) and sublimation can double for every 10 ^{o}C rise in temperature (Cutting and Malton 1974; Pham and others 1982). The temperature fluctuation during the cycles of the blast freezer may have caused more sublimation resulting in loss of

moisture from the product. The moisture loss can affect the quality of shrimp in different ways. The decrease in moisture can concentrate the solutes present, and this may accelerate enzymatic action, lipid oxidation and protein denaturation. It has been reported that migration of water to or from the food can affect food quality, chemical reactions and microbial growth during storage (Labuza and Hyman 1998; Saravacos and Maroulis 2001). Moisture loss also has economic consequences since it is the main reason for the weight loss, the water lost will have similar economic value as the product itself (Erickson and Hung 1997).

Table 4.9: Moisture Content of Glazed and Non-glazed Shrimp Frozen by Cryogenic and Air- Blast Freezing.

CRYOGENICALLY FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
CFNG	77.67 ± 0.17Aa	76.95 ± 1.68BCa	74.40 ± 1.36Db	72.87 ± 0.91Bb
CFGDW	78.81 ± 0.40Aa	77.78 ± 0.78ABa	78.73 ± 0.28Aa	77.43 ± 1.47Aa
CF2GTE	78.81 ± 0.53Aa	78.55 ± 0.47Aa	78.21 ± 1.03ABa	77.84 ± 1.11Aa
CF3GTE	78.82 ± 0.39Aa	78.15 ± 0.31ABa	78.93 ± 0.87Aa	77.85 ± 0.65Aa
CF5GTE	78.83 ± 1.30Aa	78.16 ± 0.49ABa	78.64 ± 0.77Aa	77.70 ± 1.11Aa
BLAST FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
BFNG	77.84 ± 1.31Aa	76.25 ± 0.51Cab	74.21 ± 0.98Dbc	73.43 ± 1.51Bc
BFGDW	77.36 ± 0.15Aa	77.08 ± 0.48ABCa	76.30 ± 0.72Ca	76.73 ± 1.48Aa
BF2GTE	77.69 ± 1.71Aa	77.65 ± 0.41ABCa	77.01 ± 0.48BCa	76.42 ± 1.78Aa
BF3GTE	77.85 ± 0.11Aa	77.40 ± 1.71ABCa	76.71 ± 0.31BCa	76.28 ± 1.00Aa
BF5GTE	77.46 ± 0.61Aa	77.35 ± 0.49ABCa	76.70 ± 0.51BCa	76.53 ± 1.69Aa

CF = Cryogenically frozen, BF = Blast frozen, NG = Non-glazed shrimp, GDW = Shrimp glazed with distilled water, 2GTE = Shrimp glazed with 2% green tea extract, 3GTE = Shrimp glazed with 3% green tea extract, 5GTE = Shrimp glazed with 5% green tea extract. Capital letters mean values followed by the same letters are not significant in each column. Lower case letters mean values followed by the same letters are not significant in each row.

4.10 Lipid Oxidation in Shrimp

TBARS (Thiobarbutric acid reactive substances) values of fresh shrimp used for cryogenic and blast freezing were 0.47 ± 0.01 mg and 0.59 ± 0.16 mg MDA (malonaldehyde)/kg sample respectively. TBARS values of all samples increased during the storage period (Table 4.10). Increase in lipid oxidation can lead to color and nutritional quality deterioration; secondary products can cause protein damage (Erickson and Hung 1997). During lipid oxidation, unstable hydroperoxides form and decompose into shorter chain hydrocarbons and these final products are detected by the TBA test as TBARS (Benjakul and others 2005). Seafood is considered more susceptible to oxidation due to the unsaturation of fatty acids (Huang and Weng 1998). In our study 50.72 % of the fatty acids in fresh shrimp were found to be unsaturated and the PUFA fraction was 32.23 % with a major portion of it being EPH and DHA (Table 4.4). Phospholipids were reported in the edible regions (Rosa and Nunes, 2003), and this may have been the reason for continuous increase in TBARS values throughout the storage period in this study.

On day 1, all samples had similar TBARS values and the TBARS value of all samples increased, after 180 days of storage (Table 4.10). However the GTE glazed samples had significantly lower TBARS values compared to non-glazed shrimp, in both freezing methods after 30 days. CFNG and CFGDW had TBARS value similar BGNG and BFGDW respectively. Among the GTE glazed samples, the BF2GTE and CF2GTE had similar oxidation. CF3GTE and CF5GTE had similar TBARS values but were significantly lower than BF3GTE and BF5GTE only at 30 days of storage. As storage time increased BF5GTE and CF5GTE had significantly lower oxidation compared to BFNG, CFNG and BFGDW. There was no significant difference between CFGDW, CF2GTE, CF3GTE, BF2GTE and BF3GTE. The results indicate that with increase in storage time the cryogenically and blast frozen non-glazed shrimp have similar oxidation. At the

end of 180 days, it was seen that GTE was effective in significantly reducing lipid oxidation compared to controls. The BF2GTE and BF3GTE were similar to CF2GTE and CF3GTE. Though all GTE samples had similar TBARS values, the TBARS values of the 2GTE treatment may not be considered significantly different from GDW samples. CF5GTE had significantly lower oxidation compared to CFNG and CFGDW, while BF3GTE and BF5GTE had significantly lower TBARS values than BFNG and BFGDW, but they were not considerably different from CFGDW. Green tea catechins are reported to delay oxidation by inhibiting the formation of free radicals, interrupting the propagation of free radicals, acting as metal cheltaors, and inhibition of transcription factors and enzymes (Graham 1992; Tang and others 2001; Higdon and Frei 2003). Green tea catechins have similar backbone structures with differences in the number and location of hydroxyl groups (Chen and Chan 1996), and these hydroxyl groups can interrupt propagation of free radical autoxidation by donating a hydrogen atom to stabilize the free radical (Kaur and Kapoor 2001). It has been reported that the antioxidative effect of tea polyphenols are concentration dependent (Koketsu 1997). Significant differences were not observed in this study for the three different concentrations of GTE used. Since 2GTE was still comparable to GDW after 180 days, it can be said that higher concentrations may be required to see more significant differences. The antioxidant capacity of the tea polyphenols are in the order ECG>EC>EGCG>EGC (Amarowicz and Shahidi, 1995). The results indicate that green tea extract was effective in inhibiting the lipid oxidation in shrimp during the 6 months frozen storage period. Consumption limits for TBARS have been reported to be 7-8 mg MDA/kg and a TBA value less than 3 mg MDA/kg indicates a perfect quality material (Schormuller 1968; 1969). According to these values all shrimp samples remained acceptable during the 6 months storage period.

Table 4.10: TBARS Values (mg MDA/kg sample) of Shrimp Frozen by Cryogenic and Air -Blast Freezing.

CRYOGENICALLY FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
CFNG	0.51 ± 0.04Ad	1.25 ± 0.02ABc	2.07 ± 0.53Ab	2.96 ± 0.45ABa
CFGDW	0.49 ± 0.02Ad	0.84 ± 0.01DCc	1.35 ± 0.23DCb	2.34 ± 0.41BCDa
CF2GTE	0.50 ± 0.10Ac	0.73 ± 0.04DEbc	1.10 ± 0.12DCb	2.07 ± 0.52CDEa
CF3GTE	0.48 ± 0.04Ac	0.58 ± 0.02Ebc	0.91 ± 0.26DCb	1.82 ± 0.19DEa
CF5GTE	0.49 ± 0.03Ac	0.53 ± 0.02Ec	0.82 ± 0.25Db	1.69 ± 0.24Ea
BLAST FROZEN SHRIMP				
	DAY 1	DAY 30	DAY 90	DAY 180
BFNG	0.61 ± 0.10Ac	1.34±0.26Ab	2.58 ± 0.67ABa	3.04 ± 0.26Aa
BFGDW	0.52 ± 0.18Ad	1.04±0.07BCc	1.72 ± 0.55BCb	2.60 ± 0.27ABCa
BF2GTE	0.57 ± 0.21Ac	0.84±0.29DCbc	1.22 ± 0.53DCb	2.24 ± 0.16CDEa
BF3GTE	0.62 ± 0.13Ab	0.81±0.13Db	1.01 ± 0.37DCb	1.91 ± 0.33DEa
BF5GTE	0.59 ± 0.19Ab	0.79±0.10Db	0.88 ± 0.030Db	1.74 ± 0.34DEa

CF = Cryogenically frozen, BF = Blast frozen, NG = Non-glazed shrimp, GDW = Shrimp glazed with distilled water, 2GTE = Shrimp glazed with 2% green tea extract, 3GTE = Shrimp glazed with 3% green tea extract, 5GTE = Shrimp glazed with 5% green tea extract. Capital letters mean values followed by the same letters are not significant in each column. Lower case letters mean values followed by the same letters are not significant in each row.

CHAPTER 5 SUMMARY AND CONCLUSIONS

This study evaluated the effect of green tea as a glazing material for shrimp while also comparing two different freezing methods air-blast and cryogenic freezing. On comparing within the two freezing treatments, it was seen that cryogenic freezing had a much higher energy removal rate and freezing rate. However with increase in storage time, significant differences could not be found when comparing the non-glazed shrimp frozen by the two different freezing methods. This indicates that not just the freezing methods but the storage conditions are also equally important; if not stored at proper conditions a quick frozen product may lose its advantages. The results also indicate that glazing shrimp could help retain product quality compared to non-glazed product. The type of freezing to be used and subsequent storage may depend on the processor, availability and cost considerations. In absence of a cryogenic freezer, using a blast freezer, but applying a glaze may result in a better quality product than a non-glazed cryogenically frozen product.

On evaluating green tea extract (GTE) as a glazing material it was seen that, GTE were effective in controlling the lipid oxidation of shrimp. Though 2GTE was effective in controlling oxidation it was not significantly different form GDW samples. CF5GTE was significantly different from both controls. The storage period in this study was only up to 6 months, and hence the TBARS values of all the shrimp were low, so it is possible that on further storage GTE glazing may be more beneficial. It should also be noted that apart from reducing lipid oxidation the green tea glaze was similar to distilled water glaze in terms of texture, moisture retention, thaw yield, glazing yield and pH. Compared to BFNG and CFNG, the BF5GTE and CF5GTE treatments had reduced lipid oxidation by 42.76 and 48.29 % respectively. Since the glaze percentage used in this study gave a significant reduction in lipid oxidation, it can be said that by using GTE as a

glazing material, it is possible to avoid excessive glazing. It is also possible that the antioxidant was active only on the surface, since the increase in TBARS values were more pronounced with changes in glazing yield. GTE glazing affected the color of shrimp, and green tea glazed shrimp had higher a* and b* values compared to the controls. In this study the color of raw shrimp was measured at the surface, but it should be taken into account that this difference in color may not translate to a similar difference in color when the shrimp are cooked. GTE showed good antioxidant properties, however it should be noted that it is still underutilized in the food industry even though a lot of studies have shown its antioxidant properties and potential health benefits. Further research can be done to extend the applications of GTE, and it would be interesting to study the effect of GTE for iced or refrigerated storage of shrimp, and to see its effect when used as a pretreatment or dip instead of a glaze.

This study has confirmed previous research findings that use of glazing is beneficial for long term storage and has further demonstrated that green tea can be successfully applied as an antioxidant glaze.

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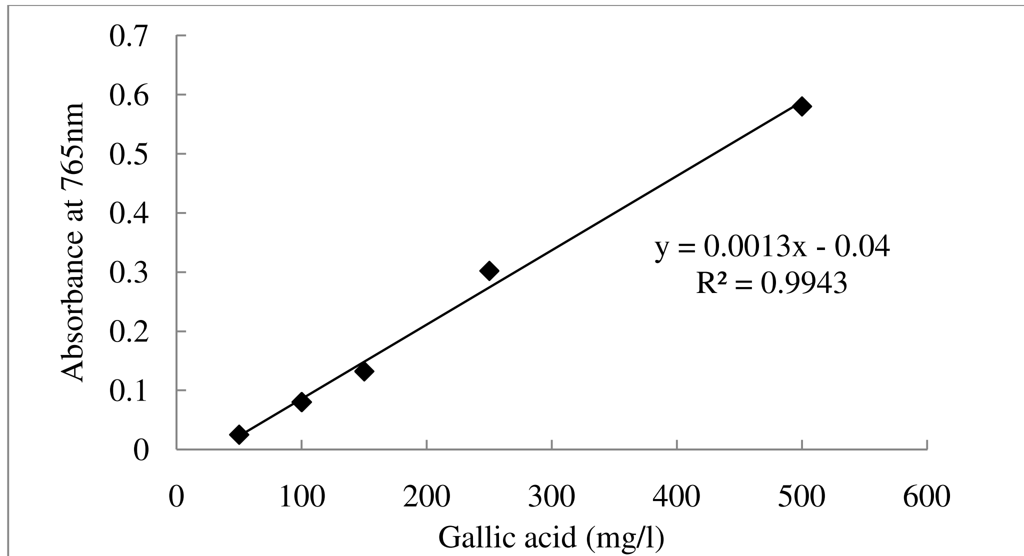
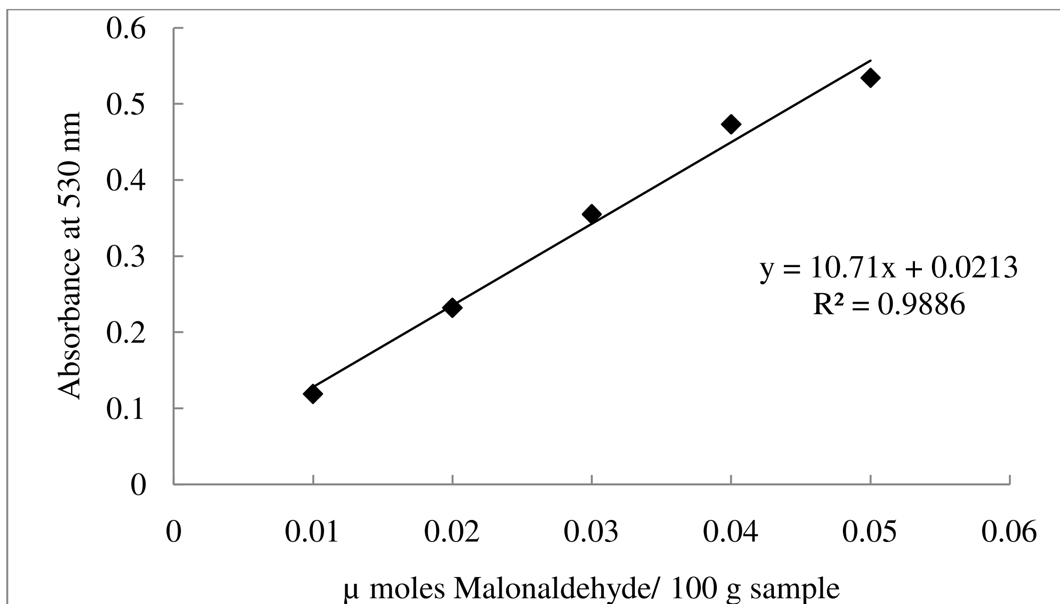
APPENDIX 1: STANDARD CURVES

1.1 Gallic Acid Standard Curve for Estimation of Total Phenols by Folin-Ciocalteu Assay



1.2 Malonaldehyde Standard Curve for Estimation of Thiobarbituric Acid Reactive Substances (TBARS)



APPENDIX 2: ESTIMATION OF ENERGY REMOVAL RATE (MODEL CALCULATIONS)

Calculation of Specific heat capacity

Above freezing

$C_{pu} = 0.837+3.349X_w$

X_w = moisture content of raw shrimp

= 0.837+3.349(0.791)

= 3.48 kJ/kgK

Below freezing

$C_{pf} = 0.837+1.256\ X_w$

= 0.837+1.256(0.791)

= 1.83 kJ/kgK

Calculation of latent heat

$L = x_i L'$

L' = Latent heat of fusion of water=333.6 kJ/ kg

$x_i = (x_{wu} - Bx_S)\left(\frac{T_{if}-T_f}{T_o-T_f}\right)$ = weight fraction of ice

Calculation of weight fraction of ice

T_0= Intial temperature of product = 277.37 K

T_{if}= Temperature at Initial freezing point = 271.40

T_f= Final product temperature = 251.67

x_{wu} = weight fraction of water in unfrozen food = 0.791

x_s = weight fraction of solutes =(1-0.791) = 0.209

B= Bound water /kg solute = $b- 0.5\frac{M_w}{M_s}$

b= constant = 0.32 for fish (Schwartzberg (1976), Pham (1987) & Murakami and Okos(1989)

M_w= Molecular weight of water = 18.02

M_s= Molecular weight of solutes = $18.02\ \left(\frac{X_w(1-x_{wu})}{x_{wu}(1-X_w)}\right)$

X_w = Mole fraction of water

$ln(X_w) = -18.02\ \left(\frac{L'(T_o-T_{if})}{RT_o^2}\right)$

= -(18.02*333.6*(277.37-271.40))/(8.314*(277.37^2))

= - 0.0561

X_w= exp (-0.0561) = 0.946

M_s = (18.02*0.946*(1-0.791))/ (0.791*(1-0.946))

= 82.59

B= 0.32-(0.5*(18.02/82.59)) = 0.211

x_i= (0.7907-(0.211*(0.2093))*((271.40-251.67)/(277.37-251.67))

= 0.57

L= (0.57*333.6) = 191.20 kJ/kg

Calculation of product heat load

Product heat load = $\Delta h = m[C_{pu}(T_o - T_{if}) + L + C_{pf}(T_{if} - T_f)$

= (1*((3.48*(277.37-271.40))+191.20+((1.83*(271.40-251.67)))

= 247.98 kJ

Calculation of energy removal rate

Q = $\frac{\Delta h}{t}$

= (247.98/270) = 0.918 kJ/s = 918.44 J/s

VITA

Srijanani Sundararajan was born in Coimbatore, India, in 1986. She obtained a Bachelor of Technology degree in biotechnology form the Vellore Institute of Technology University in 2008. She then started on her master's in food science at Louisiana State University in January 2009. Srijanani is currently enrolled as a master's student and expects to receive her degree in December 2010.