PROCESSING OF COCONUT WATER WITH HIGH PRESSURE CARBON DIOXIDE TECHNOLOGY

By

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To my Mom and Dad

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Coconut water, the clear liquid inside immature green coconuts, is highly valued due to its nutritional and therapeutic properties. It has been successfully used in several parts of the world for oral rehydration, treatment of childhood diarrhea, gastroenteritis and cholera. This juice is mostly consumed locally as fresh in tropical areas since it deteriorates easily once exposed to air. Commercially, it is thermally processed using ultra high temperature (UHT) technology. However, coconut water loses its delicate fresh flavor and some of its nutrients during heating. A non-thermal process is desirable to protect the fresh flavor and nutrient content of coconut water, which would increase marketability of this healthy drink and availability to consumers throughout the world. This study evaluated the effects of dense phase CO_2 (DPCD) pasteurization on sensory, physical and chemical quality of a coconut water beverage. The coconut water beverage was formulated by acidification with malic acid to pH around 4.30, sweetened with Splenda (0.7% w/w) and carbonated at 1.82 atm CO_2 at 4^{o}C. Microbial reduction was

quantified as a function of pressure, temperature and % CO_2 level. Optimum DPCD treatment conditions for microbial inactivation were determined to be 13% CO_2, 25^{o}C, 34.5 MPa for 6 min. Quality attributes such as pH, ^{o}Brix, % titratable acidity (%TA) and color of DPCD treated, fresh and heat pasteurized (74^{o}C for 15 s) coconut water beverages were measured and compared throughout refrigerated storage (4^{o}C for 9 weeks). DPCD treatment did not cause a change in pH or ^{o}Brix. The color of coconut water eventually turned pink during storage, independent of treatment. Sensory panels showed that DPCD treated coconut water was liked as much as fresh coconut water; whereas heat pasteurized coconut water was significantly less liked (α=0.05) at the beginning of storage. Flavor compounds of immature coconut water were identified. Flavor profiles showed that heat treated coconut water had more aroma active compounds than DPCD treated coconut water.

This study showed that a fresh-like tasting coconut water beverage can be produced by DPCD technology with an extended shelf-life of more than 9 weeks at 4^{o}C.

CHAPTER 1
INTRODUCTION

Coconut water, as a tropical fruit juice, is highly valued and consumed in tropical areas since it is tasty and has desirable nutritional and therapeutic properties. The total world coconut cultivation area was estimated in 1996 at 11 million hectares (ha), and around 93% was found in the Asian and Pacific regions (Punchihewa and Arancon 2005). Indonesia, the Philippines, and India are the largest producers of coconut in the world. Coconut (*Cocos nucifera* Linn.) fruit is filled with the sweet clear liquid "coconut water" when the coconut is about 5 to 6 months old. Coconut water has been called the "fluid of life" due to its medicinal benefits such as oral rehydration, treatment of childhood diarrhea, gastroenteritis and cholera (Kuberski 1980, Carpenter and others 1964). It is high in electrolyte content and has been reported as an isotonic beverage due to its balanced electrolytes like sodium and potassium that help restore losses of electrolytes through skin and urinary pathways. Coconut water was claimed as a natural contender in the sports drink market with its delicate aroma, taste and nutritional characteristics together with the functional characteristics required in a sports drink (Food and Agricultural Organization [FAO] 2005).

The constituents of coconut water are water 94% (w/v), sugars such as glucose, fructose and sucrose around 5% (w/v), proteins around 0.02% (w/v) and lipids only about 0.01% (w/v). It is rich in minerals such as potassium, calcium, magnesium and manganese, and low in sodium.

Most coconut water is consumed fresh in tropical coastal areas due to its short shelf-life. Once exposed to air, it loses most of its sensory and nutritional characteristics and deteriorates. Commercially, juice production is carried out mostly in Indonesia, the Philippines, and Thailand using ultra high temperature (UHT) sterilization while some of coconut water's nutrients and its delicate flavor are lost during this thermal processing (FAO 2005), which limits the product's marketability.

Usually juices are pasteurized by a low temperature long time (LTLT) process at about 145^{o}F (63^{o}C) for 30 min or a high temperature short time (HTST) process at about 162^{o}F (72^{o}C) for 15 s. Resulting shelf-life is about 2 to 3 weeks under refrigeration (lower than 7^{o}C). Heat treatment can cause significant reduction in physical, nutritive and sensory quality of foods. Flavor changes in foods due to heating have been reported by many studies (Shreirer and others 1977, Shaw 1982, Bell and Rouseff 2004). Non-thermal processing methods have been receiving an increasing interest as alternative or complementary processes to traditional thermal methods because they minimize quality degradation by keeping the food temperature below the temperatures used in thermal processing.

Dense phase CO_2 (DPCD) technology is a non-thermal method emerging as an alternative to traditional thermal pasteurization. It is a cold pasteurization method that does not use heat to destroy microorganisms and enzymes, but instead uses the molecular effects of CO_2 at pressures lower than 50 MPa. Therefore, DPCD pasteurized foods are not exposed to adverse effects of heat, and are expected to retain their fresh-like physical, nutritional and sensory qualities.

The lethal effects of CO_2 under high pressure on microorganisms have been investigated since the 1950's. Carbon dioxide is suitable for use in foods since it is a non-toxic, non-flammable, and an inexpensive gas. It is a natural constituent of many foods, and has generally recognized as safe (GRAS) status. The study of Fraser (1951) is the first research showing that CO_2 can inactivate bacterial cells under high pressure. Since then many researchers investigated effects of DPCD on microorganisms (pathogenic and spoilage organisms, vegetative cells and spores, yeasts and molds), enzymes, and quality attributes of foods. Within the last two decades, the number of research studies and patents has increased, and commercialization efforts intensified. DPCD is one of the emerging non-thermal technologies that satisfied FDA's requirement of 5 log pathogen reduction for juice manufacturers.

DPCD technology has a great potential for use in the fruit juice industry especially for tropical fruits that have limited availability to consumers throughout the world. This study evaluated the use of DPCD technology with coconut water regarding microbial inactivation, and physical, chemical and sensory quality evaluation. Objectives of this study included quantification of microbial inactivation as a function of DPCD treatment conditions, evaluation of beverage quality during storage, comparison of DPCD treated coconut water beverage with fresh and heat treated coconut water beverages, and finally the identification of flavor compounds in coconut water and comparison of flavor profiles for heat treated and DPCD treated beverages. The demonstrated quality retention and shelf-life extension in coconut water with DPCD technology would increase its marketability and availability to the consumer.

CHAPTER 2
LITERATURE REVIEW

Coconut Water: Composition and Characteristics

The coconut (*Cocos nucifera* Linn.) fruit, egg-shaped or elliptic, consists of a fibrous outer layer called coconut husk (mesocarp), which covers a hard layer called shell (endocarp). Inside the shell is a kernel (endosperm), which is considered the most important part of the fruit. It is the source of various coconut products such as copra, i.e., the dried meat of mature fruit with 5% water content, coconut oil, coconut milk, coconut water and coconut powder. The cavity within the kernel contains coconut water (Figure 2-1) (Woodroof 1979). This part begins to form as a gel when the coconut is about 5 to 6 months old, becomes harder and whiter as coconut matures, and the inside is filled with coconut water (Oliveira and others 2003). An immature coconut between 6 to 9 months contains about 750 mL of water that eventually becomes the flesh (FAO 2005).

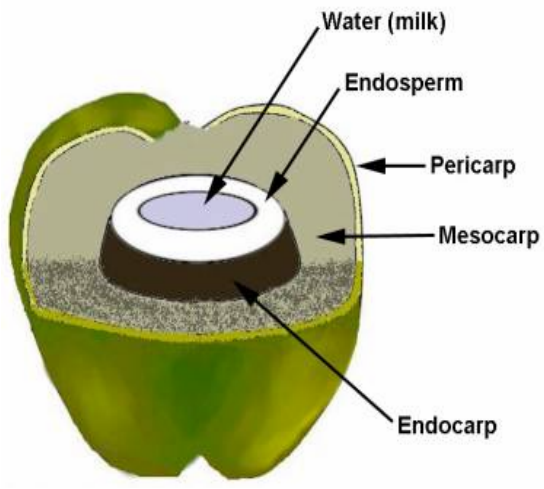


Figure 2-1. Cross section of coconut (*Cocos nucifera*) fruit

Total world coconut cultivation area in 1996 was estimated at 11 million hectares (ha), and around 93% is found in Asian and Pacific regions (Figure 2-2) (Reynolds 1988). The two biggest producers, Indonesia and the Philippines, have about 3.7 million ha and 3.1 million ha, respectively. India is the third largest producer. In the South Pacific countries, Papua New Guinea is the leading producer. In Africa, Tanzania is the largest producer while in Latin America Brazil accounts for more than one half of the total coconut area for that region (Punchihewa and Arancon 2005).

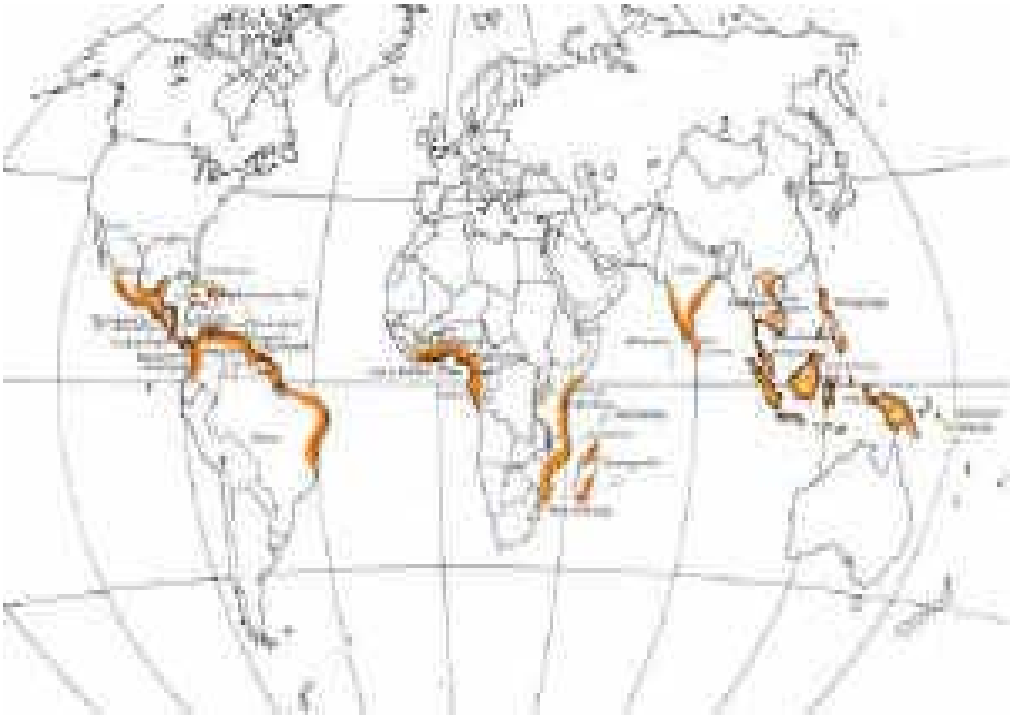


Figure 2-2. Coconut producing areas of the world

Coconut water has been called the "fluid of life" in many parts of the world due to its medicinal benefits. It has been reported as a natural isotonic beverage due to electrolytes like sodium and potassium, and its isotonic properties are demonstrated by its osmol (the number of moles of osmotically active particles; 1 mole of glucose, which is not ionizable, forms 1 osmol, 1 mole of sodium chloride forms 2 osmols) concentration, which lies in the range of 300-330 mOsmol/kg (Gomes and Coelho 2005). With its high

electrolyte content, it has been studied for its potential use as an oral rehydration solution. Comparison of coconut water with a "carbohydrate electrolyte beverage" resulted in similar rehydration indices (SDcoconut 2005). There are many reports of its successful use in gastroenteritis or diarrhea (Kuberski 1980). It is suggested as a readily available source of potassium for cholera patients (Carpenter and others 1964). Coconut water resembles blood plasma in its contents. Its successful intravenous use has been documented (Falck and others 2000). During the Pacific War of 1941-45, coconut water was siphoned directly from the nut to wounded soldiers for emergency plasma transfusions (FAO 2005). Although its glucose, potassium, magnesium and calcium levels are higher and sodium content is lower than blood plasma, studies on its intravenous infusion show no allergenic or sensitivity reactions (Fries and Fries 1983). A summary of the contents of coconut water and normal blood plasma is given in Table 2-1.

Campos and others (1996) determined the chemical and physicochemical composition of a pool of coconut water from 30 green coconuts. They measured water content, total solids, soluble solids, total sugars, reducing sugars, ash, protein, lipids, total phenolics, total titratable acidity and turbidity (Table 2-2). Carbohydrates are the main constituents of coconut water, and glucose and fructose are the most abundant soluble solids in green coconuts, while sucrose is the main one in ripe coconuts (Oliveira and others 2003).

Table 2-1. A summary of contents for coconut water and human blood plasma

Study	Specific gravity	pH	Na^{+} meq/ L	K^{+} meq/ L	Cl^{-} meq/ L	Glucose g/L	Ca^{+2} meq/ L	PO_4 meq/ L	Mg^{+2} meq/L
Pradera and others 1942*	1.018	----	5.0	64	45.5	1.2	17	2.8	----
Elseman 1954*	----	5.6	4.2	53.7	57.6	1.8	9	2.4	17
Rajasurya 1954*	1.02	4.8	----	38.2	21.3	----	14.5	4.4	----
DeSilva 1959*	1.02	4.9	----	----	----	----	----	----	19
Olurin 1972*	1.02	5.6	0.7	81.8	38.6	----	3.6	3.2	25
Iqbal 1976*	1.019	5.6	5.0	49	63	2.1	12	8	4.7
Kuberski 1979*	----	----	4.0	35.1	41	2.8	13.1	4	5.2
Msengi 1985*	1.023	6.0	2.9	49.9	----	----	5.3	----	13.4
Atoiffi 1997*	----	4.2	9.7	43.1	39.8	1.73	----	----	----
Normal plasma	1.027	7.4	140	4.5	105	0.1	5.0	2.0	1.8

(* Cited in Falck and others 2000)

Table 2-2. Chemical and physicochemical composition of green coconuts

Water (g/100 mL)	94.2±1.90
Total solids (g/100 mL)	5.80±0.12
Soluble solids (Brix, 20^{o}C)	5.27±0.11
Total sugars (g/100 mL)	5.30±0.21
Reducing sugars (g/100 mL)	4.90±0.20
Non-reducing sugars (g/100 mL)	0.40±0.04
Ash (g/100 mL)	0.50±0.01
Protein (mg/100 mL)	19.50±0.50
Lipids (mg/100 mL)	11.00±0.60
Total phenolics (mg catechin/100 mL)	6.86±0.55
Total titratable acidity (mg citric acid/100 mL)	131.20±2.80
pH	5.20±0.10
Transmittance (%)	81.00±1.70

(Campos and others 1996)

Coconut water is rich in mineral composition (Table 2-3). It is high in potassium, calcium, magnesium, and manganese, and low in sodium. Coconut water is low in fat and proteins. It is rich in many essential amino acids such as lysine, leucine, cystine, phenylalanine, histidine and tryptophan (Pradera and others 1942). Its arginine, alanine, cysteine and serine percentage is higher than those of cow's milk (Maciel and others 1992). It contains ascorbic acid and B complex vitamins. Ascorbic acid content of coconut water from a 7-9 month coconut has been reported to be 2.2 to 3.7 mg/100 mL (Mantena and others 2003). Coconut water is low in calories with a caloric value of 17.4 kcal/100 g (Woodroof 1979).

Table 2-3. Mineral composition of tender coconut water

Minerals	(mg /100 mL)
Copper	26
Potassium	290
Sodium	42
Calcium	44
Magnesium	10
Phosphorous	9.2
Iron	106

(Krishnankutty 2005)

Coconut water is mostly consumed fresh in tropical coastal areas today. In addition, commercial juice production is carried out in Indonesia, the Philippines and Thailand by heating with Ultra High Temperature (UHT). Although thermal processing eliminates bacteria, it causes loss of the delicate flavor and some nutrients of coconut water.

Flavor Analysis

Introduction

Flavor is a combination of the perceived aroma, taste and trigeminal sensations (Fisher and Scott 1997). Taste sensation has four major categories; sweet, sour, bitter,

and salty. Umami is included as the fifth category by some scientists. Trigeminal sensations give the pungency, cooling or astringency. Taste and trigeminal components of flavor are polar, non-volatile and water-soluble compounds. Aroma, on the other hand, is created by the volatile compounds. A summary of the volatile and non-volatile compounds and the examples of their sensory descriptors are given in Tables 2-4 and 2-5 (Fisher and Scott 1997).

Fruit flavors are a combination of sweet and sour tastes and the characteristic aroma compounds. Sugars such as glucose, fructose and sucrose are responsible for the sweetness of the fruit. Organic acids such as malic, citric, tartaric, etc. give sourness. These compounds are common in most fruits. Most volatile constituents in fruits contain aliphatic hydrocarbon chains, or their derivatives such as esters, alcohols, acids, aldehydes, ketones and cyclic compounds such as lactones. These compounds are reported as ripening products that develop from two different sources including fatty acids by several lipid oxidation pathways, and amino acids via amino acid metabolism. Generally, aromas of citrus fruits are created by terpenoids while that of non-citrus fruits consists of esters and aldehydes (Fisher and Scott 1997).

Table 2-4. Volatile compound classes and their sensory characteristics

Compound class	Sensory character	Examples
Aldehydes	Fruity, green, oxidized, sweet	Acetaldehyde, hexanal, decanal, vanillin
Alcohols	Bitter, medicinal, piney, caramel	Linalool, menthol, α-terpineol, maltol
Esters	Fruity	Ethyl acetate, ethyl butyrate
	Citrus	Geraniol acetate
Ketones	Butter, caramel	Diacetyl, furanones
Maillard reaction products	Brown, burnt, caramel, earthy	Pyrazines, pyridine, furans
Phenolics	Medicinal, smokey	Phenols, guaiacols
Terpenoids	Citrus, piney	Limonene, pinene, valencene

(Fisher and Scott 1997)

Table 2-5. Non-volatile compound classes and their sensory characteristics

Compound class	Sensory character	Examples
Acids:		
Amino acids	Sweet, sour, bitter	
Organic acids	Sour	Citric, malic, tartaric
Polyphenolic acids	Astringent, bitter	Chlorogenic, caffeic
Flavonoids	Astringent, bitter	Flavonols, anthocyanins
Phenolics	Medicinal, smokey	Guaicols, phenols
Sweeteners:		
Sugars	Sweet, body	Sucrose, glucose, fructose
High intensity sweeteners	Sweet	Aspartame, acesulfame-K

(Fisher and Scott 1997)

Instrumental Analysis

Gas chromatography/olfactometry (GC/O)

Gas chromatography (GC) is typically the method of choice for the analysis of flavor compounds. Initial studies of flavor analysis were conducted using packed column GC, which gave poor analytical results compared to today's capillary column GC. Combining GC with mass spectrometry (GC/MS) allowed separation and identification of numerous volatile compounds (Mistry and others 1997). It is possible to identify more than 6900 volatile compounds by using these techniques. However, not all of these volatiles have odor impact, only a few give the characteristic odor of the foods. GC olfactometry (GC/O) is an important analytical tool in flavor research to characterize the odors emerging from a sniffing port. GC/O allows the separation of odor active chemicals from the volatile chemicals with no or minimal odor response.

Due to the complexity of the food matrix and aromas, and low concentration levels of aroma compounds, typically in the parts per million (ppm), parts per billion (ppb) or parts per trillion (ppt) ranges, generally isolation and concentration of the flavors are needed prior to the analysis with GC. The most commonly used techniques are solvent extraction, headspace sampling, and distillation methods. Each method has advantages

and disadvantages. For example, in headspace sampling, analytes are removed from the sample without the use of an organic solvent. However, this method usually has low sensitivity and can give poor quantitative results (Reineccius 1984). Headspace isolates can be concentrated by the use of cryogenic or adsorbent traps. In cryogenic traps, water is the most abundant volatile isolated from the food and should be removed by additional steps that may cause sample contamination. Adsorbent traps offer advantage of water-free isolates, but differential affinity of analytes for adsorbent can result in low sensitivity for some chemicals. Solvent extraction is an accurate qualitative and quantitative method, however, it can be laborious and its use is limited to fat-free foods. Although distillation is an effective method, it takes a long time and impurities from the system components or thermally induced chemical changes can be a problem. Recently, solid phase microextraction (SPME) has found applications and is recommended as a convenient method for sample preparation before GC analysis (Wardencki and others 2004).

Solid phase microextraction (SPME)

SPME is a relatively new sample preparation technique for rapid and solvent-free extraction or pre-concentration of volatile compounds before analysis with GC. The key component of SPME is the fused silica fiber coated with an adsorbent polymeric material. This is an equilibrium technique and utilizes the partitioning of organic compounds in the sample between the aqueous or vapor phase and the thin adsorbent film coating. Adsorbed materials are thermally desorbed in a GC injection port. SPME is a simple, rapid, solvent-free and inexpensive method when compared with other sample preparation techniques such as solvent extraction, purge-and-trap, simultaneous distillation/extraction and conventional solid-phase extraction (Yang and Peppard 1994). Each additional step in the analytical procedure increases the possibility of analyte loss,

sample contamination and analytical error. SPME minimizes the number of steps used in sample preparation by combining extraction and concentration steps. For volatile/semi-volatile and non-polar/semi-polar analytes, SPME can reach detection limits of 5-50 pg/g, with an approximate sample preparation time of 15-60 min (Wardencki and others 2004). The effectiveness of the SPME depends on many factors such as type of fiber, sample volume, temperature, extraction time, mode of extraction and desorption of analytes from the fiber. The most commonly used commercially available fibers are non-polar Polydimethylsiloxane (PDMS), semi-polar PDMS/divinylbenzene and polar Carbowax/divinylbenzene.

Yang and Peppard (1994) used SPME liquid sampling and solvent extraction with dichloromethane to extract flavor compounds of a fruit juice beverage and analyzed the compounds by GC/MS. They showed comparable or higher sensitivity than solvent extraction method for most esters, terpenoids and γ-decalactone. They also analyzed a vegetable oil for butter flavor by SPME headspace sampling and found that this technique was effective in detection of diacetyl, δ-decalactone and δ-dodecalactone. They reported that conventional headspace sampling method generally was more sensitive for highly volatile compounds while the SPME headspace method picked up more of the less volatile compounds.

Sensory Analysis

Flavor research studies the effect of changes in foods on flavor, and characterizes these changes. Consumer acceptability or likeability of products developed with the new technologies is a major tool for commercialization. Sensory evaluation of food provides

guidance for the maintenance, optimization and improvement of these products (Lawless and Heymann 1998).

Sensory methods commonly used are separated into three groups: discriminant, descriptive and affective methods. The method of choice depends on which questions are to be asked about the product during the test. Discrimination methods answer whether any difference exists between products, while descriptive tests answer how products differ in specific sensory characteristics and provides quantification of these differences (Lawless and Heymann 1998). Once differences are observed by discrimination type tests, then descriptive tests can provide further information on the reasons for the differences found. Affective tests are conducted to find out how well the products are liked or which products are preferred. Examples of discrimination tests are triangle, duo-trio and paired comparison tests. In some cases, difference-from-control test is used instead of triangle or duo-trio tests, when the magnitude of difference from a control is important (Miller and others 1998). This test not only assesses difference but also quantifies the magnitude of difference.

Coconut Flavors

The desirable flavor of coconut water is sweet and slightly astringent, with a pH around 5.6 (Maciel and others 1992). There are a limited number of studies on the analysis of coconut flavor compounds. Lin and Wilkens (1970) identified 15 aroma compounds in coconut meat by GC/MS analysis. Among these, δ-C8 and –C10 lactones were the major volatile components and were described as buttery, tropical-fruity and coconut-like. The other aroma compounds were octanal, 2-heptanol, 2-octanol, 2-nonanol, 2-undecanol, hexanol, octanol, 2-phenylethanol, benzothiazole, ethyl decanoate

and dodecanoic acid, that were described mostly as fruity and also as nutty, rancid, green, lemon and rose aromas.

Jayalekshmy and others (1991) determined aroma compounds of roasted coconut meat by GC/MS. They suggested that roasting of coconut meat led to the formation of heterocyclic aroma compounds, especially pyrazines. The δ-lactones, alcohols, esters and fatty acids also contributed to the overall roasted coconut flavor. They isolated acid, neutral and basic fractions from roasted coconut by selective solvent extraction and pH adjustment. They identified pyrazines and other heterocyclic compounds, which gave the roasted aroma, in the basic fraction. There were twenty different types of pyrazines identified, and their amount increased with time of roasting. The GC profile of neutral fraction was dominated by δ-lactones, and their amount decreased from 80% to 60% during roasting. The basic and acid fractions were dominated by pyrazines and short chain fatty acids, respectively.

Jirovetz and others (2003) identified aroma compounds in the coconut milk and meat of ripe coconuts from Cameroon. They extracted headspace volatiles by SPME, and identified more than thirty compounds using GC/MS. The main components of coconut aroma were nonanal, nonanol, heptanal, ethyl octanoate, heptanol and 2-nonanol, while coconut meat was rich in δ-octalactone, ethyl octanoate, nonanal, nonanoic acid, decanol, decanal and nonanol. Other short chain alcohols, aldehydes, ketones, lactones, acids and esters were present in lower concentrations. They did not detect any γ-lactones or δ-C14 lactone that were reported in coconut meat by previous researchers. Although there are a few studies regarding the flavor compounds in coconut meat or milk, there is no flavor study with coconut water from immature fruit.

Thermal Processing Methods

Pasteurization

Pasteurization is a mild heat treatment for high-acid foods such as juices and beverages, and low-acid refrigerated foods such as milk and dairy products. It is used in order to inactivate vegetative cells of pathogenic microorganisms. Usually foods are pasteurized by a low temperature long time (LTLT) process at about 145^{o}F (63^{o}C) for 30 min or a high temperature short time (HTST) process at about 162^{o}F (72^{o}C) for 15 s (David and others 1996). The resulting shelf-life of the product is about 2 to 3 weeks under refrigerated (lower than 7^{o}C) conditions. The pasteurization process does not intend to inactivate all spoilage bacteria or any heat-resistant spores, thus the product is not commercially sterile after pasteurization (David and others 1996).

Ultrapasteurization

The objective of ultrapasteurization is similar to pasteurization but it is done at higher temperatures with shorter exposure times and extends the shelf-life about 6 to 8 weeks under refrigeration. Foods are ultrapasterized at 280^{o}F (138^{o}C) or above for 2 s or longer (David and others 1996). This process is usually used for dairy products, juices and non-dairy creamers.

Ultra High Temperature (UHT)

Commercially sterile products are obtained by a UHT process at temperatures in the range of 265 to 295^{o}F (130 to 145^{o}C) and holding times between 2 and 45 s. The product is aseptically packaged after UHT processing in order to obtain a shelf stable product with a shelf life of 1 to 2 years at ambient temperatures.

Heat Pasteurization of Juices

Common thermal processes used for juices and soft drinks are flash pasteurization, hot filling, in-pack pasteurization and aseptic filling (Tompsett 1998, Lea 1998). Usually flash pasteurization is done by passing juice rapidly through heated plates by HTST treatment at 96^{o}C for 4 s, or by standard process at 80^{o}C for 20 s. In hot filling, the product is heated in a heat exchanger above 80^{o}C (typically 87^{o}C) , sent to the filler while hot, filled into containers and held for about 2 min. Hot fill process is adequate for acidic beverages to obtain a shelf stable product with a shelf-life of 6 to 12 months. In-pack pasteurization is achieved by passing completely filled closed packs through a heating and a superheated zone, and then through a pasteurizing zone for the desired period of time, and finally through a cooling zone. Typical in-pack processing is done at 74^{o}C for 17 min. A special in-pack process is possible by heating the product above 100^{o}C in a retort and then cooling (Lea 1998, Tompsett 1998). Aseptic filling may involve HTST pasteurization or UHT sterilization, depending on the high-acid or low-acid character of the juice, which is then filled into sterile containers in a sterile environment (David and others 1996).

The choice of pasteurization method depends on the level of microbial contamination of the raw materials and packaging, the ability of the product to withstand heat, growth potential of microorganisms and the pH of the product. In orange and tangerine juice processing, pasteurization does not only kill microorganisms but also inactivates pectinesterase. Normally, temperatures above 71^{o}C are enough to kill pathogens and spoilage bacteria in orange juice. However, temperatures between 86 and 99^{o}C are required to inactivate pectinesterase. In commercial practice, orange and tangerine juices are flash pasteurized by heating the juice rapidly to about 92^{o}C for 1 to

40 s (Nordby and Nagy 1980). In lemon juice the pectinesterase enzyme can be inactivated at lower temperatures (69 to 74°C), and commercial pasteurization is done at 77°C for 30 s.

Thermal processing methods have been shown to change the flavor of foods. For example, the delicate flavor of fresh orange juice is easily changed by heat treatment. Citrus processors and flavorists search for methods to make processed orange juice and orange-flavored beverages taste more like fresh orange juice (Shaw 1982). Shreirer and others (1977) reported that some volatile compounds such as α-terpineol and carveol, which are formed by the oxidation of d-limonene, increased and the amount of terpene hydrocarbons decreased in heat pasteurized orange juice.

Bell and Rouseff (2004) determined changes in the flavor of grapefruit juice after heat processing. Sensory analysis of juices processed at 100ºC for 10 min indicated formation of a heated, pineapple, metallic, and cooked off-flavor, while the initial unheated juice had a typical fresh grapefruit character. Analysis of flavor compounds by GC/O showed that there was at least a 45% decrease in levels of volatile compounds associated with fresh grapefruit juice after heat processing. A corresponding increase in compounds associated with flavor degradation such as furaneol and methional was observed after heating.

Non-thermal Processing Methods

Non-thermal processing methods have gained increasing interest in recent years and several emerging technologies are under intense research to evaluate their potential as alternatives to traditional thermal methods. Traditionally, most foods are preserved by subjecting to temperatures between 60°C to 100°C for a certain period of time (Barbosa-Cánovas 1998). The large amount of energy transferred to food during heat treatment

may initiate unwanted reactions and result in undesirable changes in the physical, sensory and nutritional quality of food.

Quality degradation is minimized using non-thermal technologies since the food temperature is held below the temperatures used in thermal processing. Among the emerging non-thermal technologies are ultra high pressure (UHP), high intensity pulsed electric fields (PEF), irradiation, oscillating magnetic fields, pulsed high intensity light, and dense phase CO_2 (DPCD). UHP and irradiation are being used in commercial operations. One of the most important issues in the commercialization of non-thermal technologies is regulatory approval. Processes must comply with pasteurization or sterilization requirements of Food and Drug Administration (FDA) and also ensure the safety of equipment operators and consumers. Each of these technologies can be used for specific food applications; some are more suitable for liquid products whereas some are appropriate for solids. It is important to determine the quality of non-thermally processed foods, especially in cases where the nature of the food precludes use of thermal methods. Evaluation of sensory, nutritional and physical changes resulting from non-thermal processes is essential (Barbosa-Cánovas 1998).

Several studies evaluated the quality of fruit juices processed by non-thermal technologies. PEF treated orange juice had significantly higher ($P<0.05$) ascorbic acid, flavor compounds and color than thermally processed orange juice (Hye and others 2000, Min and others 2003). Jia and others (1999) showed that there was 10 to 40% loss in the major orange juice flavor compounds after heat pasteurization while 0 to 5% losses occurred for the same compound with PEF processing. Ayhan and others (2002) reported that PEF processing did not alter sensory evaluation of flavor and color of fresh orange

juice. Similarly, Min and others (2003) reported higher sensory scores for flavor and overall acceptability of PEF treated tomato juice compared to heat pasteurization. Apple juice retained fresh like ascorbic acid levels and color after PEF processing (Akdemir and others 2000, Liang and others 2003).

UHP processing at pressures between 100 to 800 MPa has been reported to be effective in inactivation of pathogens without affecting taste or nutritional value of fresh juices (Morris 2000). UHP treated citrus juices retained a fresh-like flavor with no loss of vitamin C and a shelf-life of approximately 17 months (Farr 1990). Polydera and others (2003) compared shelf-life and ascorbic acid retention of reconstituted orange juice processed by heat at 80^{o}C for 30 s with that of UHP processed juice (500 MPa, 35^{o}C, 5 min). UHP processing resulted in 24% to 57% increase in the shelf-life compared to thermal pasteurization. Sensory characteristics of UHP pasteurized juice were rated superior and ascorbic acid retention was higher.

FDA's juice HACCP regulations require validation of 5 log pathogen reduction for juice manufacturers. Dense phase CO_2 (DPCD) is one of the emerging non-thermal technologies that conforms to this requirement and has a great potential for commercial use in juice pasteurization.

Dense Phase Carbon Dioxide Technology

Mechanisms of Microbial Inactivation by DPCD

Several hypotheses have been proposed to explain the lethal effects of DPCD on microorganisms. Although the exact means are not clear, studies show that several mechanisms may be involved. DPCD was claimed to inactivate microorganisms by:

pH lowering effect

CO_2 can lower pH when dissolved in the aqueous part of a solution by forming carbonic acid. Carbonic acid further dissociates to give bicarbonate, carbonate and H^+ ions lowering extra-cellular pH by the following equations:

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad \text{pKa} = 6.57$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{-2} \qquad \text{pKa} = 10.62$$

Meyssami and others (1992) predicted the pH of simple model liquid foods under DPCD and obtained good correlations with the experimentally measured pH values. They found that the presence of dissolved materials other than CO_2 such as acids and salts had a reducing effect on the lowering of pH by DPCD treatment (Figure 2-3).

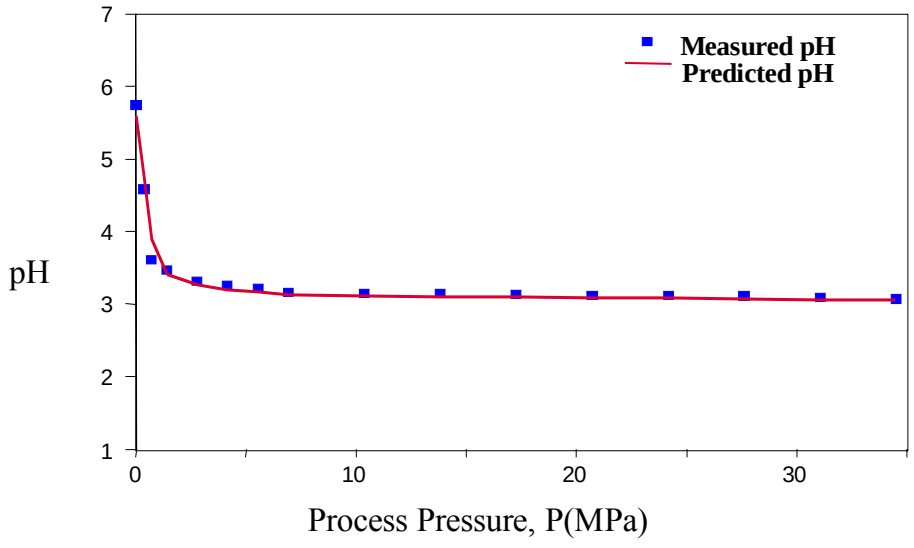


Figure 2-3. Measured and calculated pH of pure water pressurized with CO_2 up to 34.5 MPa

However, the internal pH of microbial cells, not the external pH, has the largest effect on cellular destruction. When there is a sufficient amount of CO_2 in the environment, it can penetrate through the cell membrane, which consists of phospholipid layers, and lowers internal pH by exceeding the buffering capacity of the cell. Normally, cells have to maintain a pH gradient between the internal and external environments. Cellular systems actively pump hydrogen ions from the inside to the outside of the cell. These systems can be overwhelmed with sufficient CO_2, reducing internal pH. It is believed that reduced internal pH may inactivate microorganisms by the inhibition of essential metabolic systems including enzymes (Daniels and others 1985, Ballestra and others 1996). Ballestra and others (1996) measured the activities of eight enzymes from *E.coli* cells before and after DPCD treatment (5 MPa, 15 min, 35^{o}C). These enzymes were selectively inactivated. The activity of some enzymes having an acidic isoelectric point such as alkaline phosphatase and β-galactosidase disappeared, whereas those with basic isoelectric points such as acid phosphatase were slightly affected.

Hong and Pyun (2001) treated *L.plantarum* cells by DPCD under 7 MPa at 30^{o}C for 10 min, and measured activity of 13 different enzymes. They also observed that enzymes were inactivated selectively. Some enzymes such as cystine arylamidase, α-galactosidase, α- and β--glucosidase lost their activities significantly, whereas enzymes such as lipase, leucine arylamidase, and acid and alkaline phosphatase were little affected by DPCD treatment. At the same time, cell viability of *L.plantarum* decreased by more than 90% under these conditions. They concluded that it was uncertain whether the observed inactivation of some enzymes was a primary cause of cell death. Evidence in

the literature does not specify which of the enzymes mentioned are critical for survival and therefore vital in their inactivation.

Inhibitory effect of molecular CO_2 and bicarbonate ion

Another suggestion to explain inactivation of bacterial enzymes is the inhibitory effect of CO_2 itself on some enzymes (Ishikawa and others 1995a). Weder (1990) and Weder and others (1992) claimed that under a low pH environment, arginine could interact with CO_2 to form a bicarbonate complex, and inactivate the enzyme containing this amino acid. Jones and Greenfield (1982) have shown that decarboxylases are inhibited by excess CO_2, breaking the metabolic chain (Spilimbergo and Bertucco 2003).

Ishikawa and others (1995a) obtained complete inactivation of alkaline protease and lipase at 35^{o}C and 15 MPa treatment by using a micro-bubble system. They compared residual activity of these enzymes by supercritical CO_2 ($SCCO_2$) to that of low pH (3.0) and concluded that alkaline protease could be inactivated due to pH lowering by dissolved CO_2, whereas lipase must have been inactivated by a different mechanism. They also conducted a study with glucoamylase and acid protease, showing that a higher $SCCO_2$ density resulted in lower residual activity of these enzymes. As a result, they claimed that inactivation of these enzymes could be caused by the sorption of CO_2 into the enzyme molecules.

Another mechanism proposed is precipitation of intracellular calcium and magnesium ions by the effect of carbonate (Lin and others 1993). When the applied CO_2 pressure is released, bicarbonate converts to carbonate, which can precipitate intracellular calcium, magnesium and similar ions from the cell and cell membrane. Calcium-binding proteins are known as the most important class involved in intracellular regulation (Aitken 1990). Certain types of calcium- and magnesium-sensitive proteins could be

precipitated by carbonate, depending on the binding site of the ion and chemical structure of the protein. Consequently, a lethal change to the biological system is produced (Lin and others 1993).

Physical disruption of cells

The first suggested mechanism of inactivation of microorganisms by DPCD was the physical disruption of cells (Fraser 1951). *E.coli* cells were almost totally killed under 50.7 MPa in less than 5 min and were thought to burst due to the rapid release of applied gas pressure and the expansion of CO_2 gas within the cell during depressurization. However, the extent of bursting of cells was determined by the Petroff-Hauser counting method that uses a microscope for direct cell counting. Therefore, it is hard to conclude if the cells were actually burst without observation with an electron microscope. Other researchers investigated the physical rupture of cells by DPCD as a possible mechanism of inactivation (Lin and others 1991, Nakamura and others 1994, Isenschmid and others 1995, Ishikawa and others 1995b, Ballestra and others 1996, Dillow and others 1999, Hong and Pyun 1999, Spilimbergo and others 2003, Folkes 2004). Lin and others (1991) claimed that yeast (*S.cerevisiae*) cells could be ruptured by pressurized CO_2 under 6.9-34.4 MPa for 5 to 15 h treatments. They measured total protein concentration in the supernatant of treated cells as an indication of cell rupture, however they did not have a direct physical observation of cells. They have shown that the amount of total proteins released in the supernatant of DPCD treated cells was about the same amount as in the supernatant of cells autolyzed by other disruption methods. The leakage of the proteins into the environment depends on the size of the breach in the cell membrane. Nakamura and others (1994) demonstrated mechanical rupture of yeast cells by DPCD treatment (4 MPa, 40^{o}C for 5 h) by scanning electron micrographs. They observed that some cells

were completely burst whereas some only lost surface smoothness and had some wrinkles or holes on the membrane surface. Folkes (2004) also observed physical disruption of yeast cells in beer by scanning electron micrographs (Figure 2-4). The process conditions in a continuous dense phase CO_2 pasteurizer were: pressure 27.5 MPa, temperature 21^{o}C, CO_2/beer ratio (10%), and residence time of 5 min.

Although cell rupture is possible during DPCD treatment, it is not necessary for cell inactivation. For instance, Ballestra and others (1996) treated *E.coli* cells at 5 MPa and 35^{o}C, and observed that more than 25% of cells had intact cell walls while the viability was only 1%. They did not observe cell rupture or burst, but only some signs of deformation in cell walls. There have been studies showing that cells were completely inactivated even when they remained intact after treatment. For example, Hong and Pyun (1999) demonstrated that *L. plantarum* cells treated with CO_2 at 6.8 MPa and 30^{o}C for 60 min were completely inactivated but SEM micrographs did not show any cell rupture. The morphological changes caused by DPCD may differ based on treatment conditions, gas release rate, or the type of microorganism. Dillow and others (1999) observed that SEM micrographs of *S.aureus* (Gram(+)), *P.aeruginosa* (Gram(-)), and *E.coli* (Gram(-)) cell walls were largely unchanged before and after DPCD treatment. However, they found that Gram(-) cells had more defects on the cell wall after treatment. They explained this by Gram(-) cells having thinner cell walls compared tocompared to Gram(+) cells.

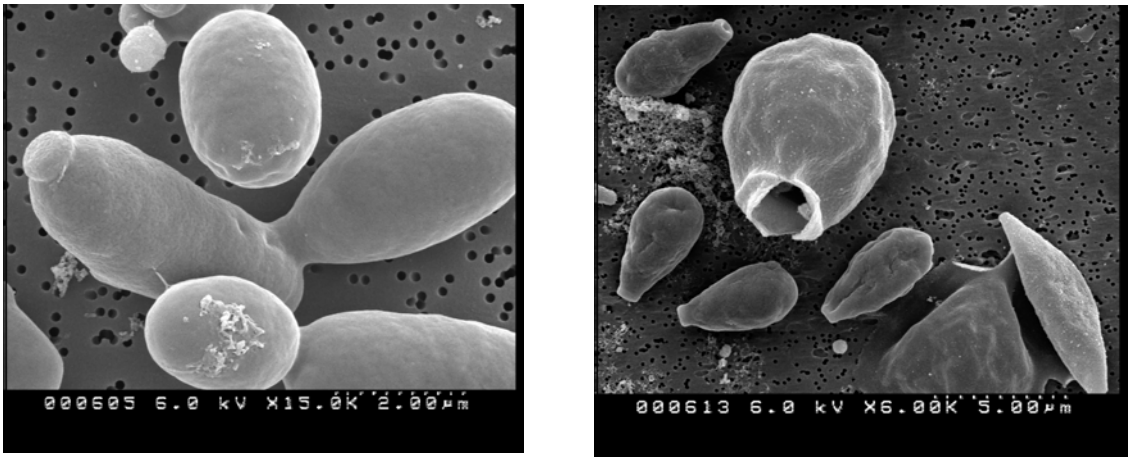


Figure 2-4. Scanning electron micrographs (SEM) of untreated (a) and DPCD treated (b) *S.cerevisiae* cells

It is important to note here that cells without any rupture, i.e., with intact cell walls could show modifications or damage in microstructural observations.

Modification of cell membrane and extraction of cellular components

Another mechanism suggested by researchers is based on the lipo- and hydrophilicity and solvent characteristics of CO_2. Kamihira and others (1987) mentioned extraction of intracellular substances such as phospholipids by DPCD as one of the possible mechanisms of microbial inactivation. Isenchmid and others (1995) proposed that molecular CO_2 diffused into cell membrane and accumulated there, since the inner layer is lipophilic. Accumulated CO_2 increased fluidity of the membrane due to the order loss of the lipid chains, also called the "anesthesia effect", and the increase of fluidity causes an increase in permeability. Lin and others (1992) suggested that once CO_2 has penetrated into the cell, it could extract cellular components and transfer extracted materials out of the cell during pressure release. Upon extraction of essential lipids or other vital components of cells or cell membranes, the cells are inactivated. These hypotheses have been investigated by several researchers either by measuring the amount

of materials in the supernatant of treated cells or by some microstructural observations on the treated cells.

Hong and Pyun (1999) have shown that although SEM observations of *L.plantarum* cells had demonstrated intact cell walls after DPCD treatment, microstructural observations by transmission electron micrographs (TEM) showed modifications in the cell membrane with possible leakage of cytoplasm (Figure 2-5). These pictures show enlarged periplasmic space between cell walls and the cytoplasmic membranes, and empty spaces in the cytoplasm. In a further study in 2001, Hong and Pyun have shown that cells treated with DPCD at 7 MPa for 10 min and 30^{o}C showed irreversible cellular damage including loss of salt tolerance, leakage of UV-absorbing substances, release of intracellular ions and impaired proton permeability. They have also used Phloxine B staining on *L.plantarum* cells as an indication of loss of cell membrane integrity, and shown that cell membrane has lost its integrity immediately after being exposed to high pressure CO_2.

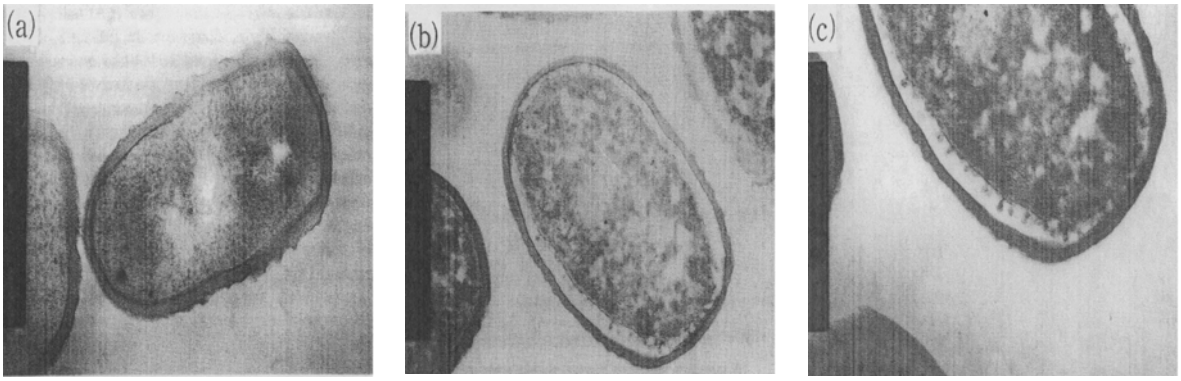


Figure 2-5. Transmission electron micrographs (TEM) of untreated (a) and DPCD (b,c) treated *L.plantarum* cells at 7 MPa, 30^{o}C, 1 h

Although the strongest effect of the above mechanisms on microbial destruction by DPCD is still in question, researchers agree in the governing role of CO_2. Several researchers have concluded that CO_2 has a unique role in inactivation of cells (Haas and

others 1989, Wei and others 1991, Lin and others 1992, Nakamura and others 1994, Ballestra and others 1996, Dillow and others 1999, Hong and Pyun 2001).

Haas and others (1989) observed that altering external pH by acids such as phosphoric and hydrochloric did not cause as much cell inactivation as CO_2. These acids cannot enter cells easily as CO_2. This implied that the ability of CO_2 to penetrate through the cell membrane has a key role in reducing the internal pH of cells. Similarly, Wei and others (1991) added 0.1N HCl to the *Listeria* suspension to decrease pH by about 1.8 units. The same amount of pH reduction was achieved by treatment of cells with CO_2 under 6.18 MPa for 2 h. The acidification by HCl did not cause a microbial reduction whereas treatment with CO_2 caused complete inactivation.

Alternatively, Nakamura and others (1994) have shown that N_2 gas when applied under the same conditions as CO_2 (4 MPa, 40^{o}C, 4 h) did not have an effect on viability of yeast cells. Lin and others (1992) have shown that 90% of cells survived after treatment with N_2 under 6.9 MPa for 20 and 40 min whereas complete inactivation was achieved after treatment with CO_2 in less than 12 min. Similarly, Dillow and others (1999) applied tetrafluoroethane (TFE) to bacterial cells at 38^{o}C and 11 MPa for 45 min and compared the viability of cells with treatment of CO_2 under the same conditions. Although TFE did not result in reduction of viable cells, total inactivation was achieved by CO_2 treatment.

Inactivation of Vegetative Cells by DPCD

There are a number of studies showing that DPCD is effective in killing vegetative forms of pathogenic and spoilage bacteria, yeasts and molds. A summary of these studies is given in Table 2-6 including the media, treatment conditions, microorganisms, their log reduction, and the type of system used. The microbial inactivation achieved by DPCD

changed from 2 and 12 logs, pressures under 50 MPa, and temperatures between 5°C to 60°C, mostly in the 25-35°C range. Treatment times were significantly different depending on the treatment system used and could be as long as 6 h when batch systems were used, to as low as 2.5 min for continuous or semi-continuous systems.

Water activity (a_w) of treatment medium and water content of the vegetative cells were shown to have a significant role in the killing effect of DPCD. Kamihira and others (1987) compared inactivation of wet (70-90% water) and dry (2-10% water) cells of Baker's yeast, *E.coli* and *S.aureus* by DPCD treatment at 20 MPa for 2 h and 35°C. Dry cells were inactivated by less than 1 log whereas wet cells were inactivated by 5 to 7 logs. Haas and others (1989) showed that DPCD was more effective as a_w of the food increased. Kumagai and others (1997) studied sterilization kinetics of *S. cerevisiae* cells at various water contents and CO_2 pressures. The first order sterilization rate constant, k, was almost zero at water contents below 0.2 g/g dry matter, and increased with increasing water content. This increase was slight at water contents above 1g/g dry matter. Moreover, k increased with increasing CO_2 pressure at an identical water content of cells. Similarly, Dillow and others (1999) compared inactivation kinetics of *E.coli* cultures in the presence and absence of water in the cell culture when treated with DPCD at 34°C and 14 MPa. They observed that small amounts of water greatly enhanced the sterilizing effect of DPCD. It is important to note that water content of treatment medium and therefore the water content of the cells would increase CO_2 solubility in the cells, which would explain increased microbial inactivation.

The unique role of CO_2 in the inactivation of microbial cells has been shown by many researchers and the details of their studies were listed in the "Mechanisms" section.

Generally, any effect that increases the level and rate of CO_2 solubility, and therefore, penetration of CO_2 into cells in a treatment medium enhances microbial inactivation by DPCD. For instance, CO_2 solubility increases with increasing pressure, other conditions being equal. However, this increase is limited by the saturation solubility of CO_2 in the medium. Generally, inactivation efficiency increases with higher pressure, temperature and residence time. Nakamura and others (1994) demonstrated that the bactericidal effect of CO_2 treatment on baker's yeast dramatically increased by increasing pressure from 2 to 4 MPa, by increasing temperature from 20 to 40^oC, and by increasing treatment time from 0.5 h to 3 h. Hong and others (1999) achieved a 5 log reduction for *L.plantarum* by DPCD at 30^oC. It took 50 to 55 min to achieve this reduction at 6.9 MPa while it took only 15-20 min to achieve the same level of reduction at 13.8 MPa. Isenschmid and others (1995) showed that viability of *Kluveromyces fragilis, S.cerevisiae,* and *Candida utilis* decreased with increasing CO_2 pressure following a typical S-shaped curve. Sims and Estigarribia (2002) showed that once the treatment medium is fully saturated with CO_2, the killing effect of DPCD did not change significantly with the enhancing effects of pressure or temperature. For example, 7.5 MPa was nearly as effective as 15 MPa, and room temperature was as effective as 31^oC in reducing *E.coli* cells by using a membrane contactor system. This can be explained by the rapid increase of CO_2 solubility in water with increasing pressure up to 7.5 MPa, but pressure increases above 7.5 MPa result in small increases in solubility (Dodds and others 1956).

On the other hand, temperature has a more complex role in increasing microbial inactivation by DPCD. Although solubility of CO_2 decreases with increasing temperatures, higher temperatures can increase the diffusivity of CO_2 and the fluidity of

cell membrane that facilitate penetration of CO_2 into the cells. Another important effect of temperature is the phase change of CO_2 from sub-critical to supercritical conditions (Tc = 31.1^oC). The penetrating power of CO_2 is higher under supercritical conditions, and there is a rapid change in solubility and density of CO_2 by temperature at the near-critical region. Hong and Pyun (1999) observed that under a constant pressure of 6.8 MPa, microbial inactivation of *L. plantarum* increased by a log as temperature decreased from 40^oC (7 log reduction) to 30^oC (8 log reduction). They explained this less effective inactivation at 40^oC by the decrease in solubility of CO_2 in this region.

Initial pH of treatment medium is an important factor affecting microbial reduction by DPCD. Low pH environment facilitates penetration of carbonic acid, like many other carboxylic acids (Lindsay, 1976) through the cell membrane, therefore more inactivation is achieved as the medium pH decreases. For example, Hong and Pyun (1999) demonstrated that under a CO_2 pressure of 6.8 MPa at 30^oC, treatmens of 25 min in acetate buffer (pH 4.5), 35 min in sterile distilled water (pH 6.0) and 60 min in phosphate buffer (pH 7.0) were required to achieve 5 log reduction of *L.plantarum* cells.

Cell growth phase or age is another factor affecting inactivation of microbial cells by DPCD. Young cells are more sensitive than mature ones. Hong and Pyun (1999) compared inactivation of *L.plantarum* cells in log phase with those in stationary phase, and found that cells in the late log phase were more sensitive to DPCD than those in the stationary phase. They attributed this to the ability of bacteria entering the stationary phase of growth to synthesize new proteins that protect cells against adverse environmental conditions (Koltter 1993, Mackey and others 1995).

Different types of bacteria have different susceptibilities to DPCD treatment. It is hard to make comparisons since the treatment systems, solutions or conditions also differ in these studies. Referring to specific studies, it can be concluded that some microorganisms seem more affected by DPCD treatment. For example, Sims and Estigarribia (2002) showed that *Lactobacillus plantarum* cells were more resistant to DPCD than *E.coli*, *S. cerevisiae* and *Leuconostoc mesenteroides* cells. Dillow and others (1999) treated G(+) bacteria (*S. aureus*, *B. cereus, L. innocua*) and G(-) bacteria (*S. salford, P. vulgaris, L. dunnifii, P. aeruginosa and E.coli*) with DPCD. They found that *B.cereus* cells were more resistant to DPCD while *E.coli* and *P.vulgaris* were more sensitive. They suggested that the nature of the cell wall could be an important factor in the difference in sensitivity of these bacteria. Because of their thin cell walls, G(-) bacteria are expected to be more sensitive and their cell wall could be ruptured more easily than that of the G(+) bacteria. More studies need to be conducted in this area to have a clear conclusion.

The type of system used for DPCD treatment can change the microbial inactivation rate by DPCD. Treatment systems that allow better contact of CO_2 with the treatment solution are shown to be more effective in microbial reduction because of the more rapid saturation of the solution with CO_2. Usually, batch systems require longer treatment times in order to be effective in microbial inactivation compared to continuous systems. On the other hand, it is possible to increase the inactivation rate of batch systems by agitation (Lin and others 1993, Hong and Pyun 2001). Spilimbergo and others (2003) showed that a semi-continuous system is more efficient than a batch system. Treatment of 40 to 60 min was necessary for inactivation of a wide range of bacteria with the batch system,

whereas less than 10 min was enough by using a semi-continuous system. Ishikawa and others (1995b) obtained more than 4 orders and 3 orders higher inactivation in *L.brevis* cells and *S.cerevisiae*, respectively, by using a micro-bubbling filter in their system.

Table 2-6. Summary of the studies on inactivation of various microorganisms

Solution	Microorganism	P (MPa)	Time	Temp. (°C)	System	Log redn.	Reference
[a]PS	*S.cerevisiae*	20	2 h	35	Batch	7.5 ([g]C)	Kamihira and others 1987
	E.coli	20	2 h	35		6.5 (C)	
	S.aureus	20	2 h	35		5 (C)	
	A.niger	20	2 h	35		5 (C)	
Herbs	Total bacteria count	5.52	2 h	45	Batch	5-8 (C)	Haas and others 1989
Apple juice	Total bacteria count	5.52	30 min	45		>3 (C)	
Orange juice	Total bacteria count	5.52	30 min	55		4 (C)	
Nutrient broth	*E.coli*	6.21	2 h	Room temp.		2	
	S.aureus	6.21	2 h	Room temp.		2	
	Salmonella seftenberg	6.21	2 h	Room temp.		2	
Distilled water	*L.monocytogenes*	6.18	2 h	35	Batch	9 (C)	Wei and others 1991
Egg yolk	*S.thyphimurium*	13.7	2 h	35		>8	
Orange juice	Total plate count (TPC)	33	1 h	35	Batch	2	Arreola and others 1991b
Growth medium	*S.cerevisiae*	6.9	15min	35	Batch	7 (C)	Lin and others 1992
Growth medium	*L.dextranicum*	6.9 - 21min	15-20 min	35	Batch	>8	Lin and others 1993
Sterile water	*S.cerevisiae*	4	>3 h	40	Batch	8 (C)	Nakamura and others 1994
PS	*L.brevis*	25	30 min	35	Micro-bubble	6 (C)	Ishikawa and others 1995b
	S.cerevisiae	25	30 min	35		6 (C)	
PS	*E.coli*	5	20 min	35	Batch	6 (C)	Ballestra and others 1996
Sterile Water	*S.cerevisiae*	15	1 h	40	Batch	8	Kumagai and others1997
[b]MRS broth	Lactic acid bacteria	6.9	200 min	30	Batch	5	Hong and others 1997
[c]BHIB	*S.aureus*	8	60 min	25	Batch	7 (C)	Erkmen 1997
Whole milk	Aerobic plate count	14.6	5h	25	Batch	>8	

Table 2-6 Continued

Solution	Microorganism	P (MPa)	Time	Temp. (°C)	System	Log redn.	Reference
[d]TSB w/ polymers	*B.cereus*	20.5	4 h	60	Batch	8 (C)	Dillow and others 1999
	L.innocua	20.5	0.6 h	34		9 (C)	
	S.aureus	20.5	4 h	40		9 (C)	
	S.salford	20.5	4 h	40		9 (C)	
	P.auruginosa	20.5	4 h	40		8 (C)	
	E.coli	20.5	0.5 h	34		8 (C)	
	P.vulgaris	20.5	0.6 h	34		8 (C)	
	L.dunnifi	20.5	1.5 h	40		4 (C)	
Growth medium	*L.plantarum*	13.8	30 min	30	Batch	>6 (C)	Hong and others 1999
[e]PS with broth	*L.monocytogenes*	6	75 min	35	Batch	6.98 (C)	Erkmen 2000a
PS	*E.faecalis*	6.05	18 min	35	Batch	8 (C)	Erkmen 2000b
Fruit juice-milk	*E.faecalis*	6.05	3-6 h	45		5 (C)	
PS	*Brocothirix thermosphacta*	6.05	100 min	35	Batch	5.5 (C)	Erkmen 2000c
Skinned meat	*Brocothirix thermosphacta*	6.05	150 min	35	Batch	5 (C)	
MRS broth	*L.plantarum*	7	100 min	30	Batch	>8	Hong and Pyun 2001
PS	*S.thyphimurium*	6	15 min	35	Batch	7 (C)	Erkmen and Karaman 2001
PS w/broth	*S.thyphimurium*	6	140 min	25	Batch	7 (C)	
Whole milk	*E.coli*	10	6 h	30	Batch	6.42 (C)	Erkmen 2001
Skim milk	*E.coli*	10	6 h	30	Batch	7.24 (C)	
PS	*B.subtilis*	7.4	2.5 min	38	SC[h]	7 (C)	Spilimbergo and others 2002
	P.aeruginosa	7.4	2.5 min	38		7 (C)	
Sterile water	*E.coli*	7.5	5.2 min	24	CM[i]	8.7	Sims and Estigarribia 2002
Orange Juice	*E.coli*	15	4.9 min	24		>6	
Orange juice	*L.mesenteroids*	15	<10 min	25		>6	
Orange juice	*S.cerevisiae*	15	<10 min	25		12	
Orange juice	*L.plantarum*	7.5	<10 min	35		>8	
Orange juice	*S.thyphimurium*	38	10 min	25	CF	6	Kincal and others 2005
Orange juice	*L.monocytogenes*	38	10 min	25		6	
Orange juice	*E.coli*O157:H7	107	10 min	25		5	
Apple juice	*E.coli*O157:H7	20.6	12 min	25		5.7	

Table 2-6 Continued

Solution	Microorganism	P (MPa)	Time	Temp. (oC)	System	Log redn.	Reference
Carrot juice	Aerobic plate count	4.9	10 min	5	Batch	4	Park,and others 2002
[f]WM juice	Aerobic plate count	34.4	5 min	40	CF	6.5	Lecky 2005
Mandarin juice	Aerobic plate count	41.1	9 min	35	CF	3.47	Lim and others 2006
Coconut water	Aerobic plate count	34.5	6 min	25	CF	>5	Damar and Balaban 2005

[a]PS: Physiological Saline, [b]MRS: De Man Rogosa Sharpe, [c]BHIB: Brain-Heart Infusion Broth, [d]TSB: Tryptic Soy Broth , [e]CF: Continuous flow, [f]WM: Watermelon, [g]C: Complete inactivation, [h]SC: Semi-continuous, [i]CM: Continuous membrane

Inactivation of Spores by DPCD

Spores are highly resistant forms of bacteria to the physical treatments such as heat, drying, radiation and chemical agents (Watanabe and others 2003a). A limited number of studies in the literature investigating inactivation of spores by DPCD show that the extent of inactivation achieved changes with treatment conditions, treatment systems and the type of organism (Table 2-7).

Studies suggested that processing temperature had a significant role in inactivation of spores by DPCD. Several researchers observed that a temperature threshold should be exceeded in order to achieve a killing effect on bacterial or fungal spores (Enomoto and others 1997, Ballestra and Cuq 1998, Watanabe and others 2003b). This threshold temperature can be different for different spores. Kamihira and others (1987) did not observe any killing effect of DPCD on *B. stearothermophilus* spores and observed only 53% inactivation of *B.subtilis* spores by DPCD treatment at a relatively low temperature (35^{o}C). Enomoto and others (1997) showed that there was not a significant inactivation of *B.megaterium* spores at temperatures below 50^{o}C, and survival ratio of spores decreased dramatically by increasing temperature from 50 to 60^{o}C. On the other hand, Ballestra and Cuq (1998) did not observe antimicrobial activity of DPCD treatment on *B.subtilis* spores

and *Byssochlamys fulva* ascospores below 80°C, and on *A.niger* conidia below 50°C. Similarly, Watanabe and others (2003b) observed that DPCD treatments at temperatures in the range of 35°C to 85°C did not have a killing effect on *Geobacillus stearothermophilus* spores. However, it may be possible to achieve significant amounts of spore inactivation at relatively low temperatures by using continuous DPCD treatment systems that are shown to be more efficient than batch systems. For instance, Ishikawa and others (1997) achieved 6 log reduction in *B. polymyxa*, *B.cereus*, and *B. subtilis* spores at 45°C, 50°C and 55°C, respectively, by using a continuous micro-bubble system. Micro-bubbling by the use of a filter improved the inactivation of spores by 3 log cycles. There was only 1 log reduction of spores without micro-bubbling and 4 log reduction with micro-bubbling under the same treatment conditions.

The mechanism of inactivation of spores by DPCD is not known. Watanabe and others (2003a) compared the killing effect of DPCD with heat and high hydrostatic pressure (HHP) treatments. DPCD had more lethality than HHP treatment or heat treatment alone, showing that CO_2 had a unique role in inactivation. They suggested that inactivation mechanisms of bacterial spores by DPCD and heat were different, since inactivation of Bacillus spores by heat treatment occurred in a single step whereas inactivation by DPCD occurred in two steps. Ballestra and Cuq (1998) also observed two steps in the inactivation of *B.subtilis* spores at 5 MPa CO_2 and 80°C. They suggested that the first step of inactivation could represent penetration of CO_2 into the cells that is associated with heat activation of the dormant spores. Heat activation can make spores more sensitive to the antimicrobial effects of CO_2. However, there may be another explanation for spore inactivation by DPCD based on the study of Furukawa and others

(2004). This study believes that DPCD is able to germinate bacterial spores even at relatively low treatment temperatures. Approximately, 40% of *B. coagulans* and 70% of *B. licheniformis* spores were germinated by DPCD at 6.5 MPa and 35°C. Therefore, DPCD could be the reason for germination of spores making the resulting vegetative cells more sensitive to heat inactivation. The study of Watanabe and others (2003a) shows that inactivation of *B. coagulans* and *B. licheniformis* spores by heat treatment only is much lower than inactivation obtained when a combination of DPCD and the same heat treatment is applied. In the combined treatment, DPCD is applied first and heat is applied afterwards. Their study suggests that DPCD may decrease heat tolerance of bacterial spores. The calculated z values with and without DPCD were the same. However, the D values with DPCD were much smaller, indicating an upward shift in the log inactivation vs. time curve with DPCD. The role of DPCD and heat treatments in spore inactivation needs to be investigated more explicitly.

Table 2-7. Summary of studies on spore inactivation by DPCD

Solution	Microorganism	Pressure (MPa)	Time	Temp (°C)	System	Log redn.	Reference
Sterile water	*B.subtilis*	20	2 h	35	Batch	0.3	Kamihira and others 1987
Growth medium	*P.roqueforti*	5.52	4 h	45	Batch	>6	Haas and others 1989
Sterile distilled water	*B. megaterium*	5.8	30 h	60	Batch	7	Enomoto and others 1997
[a]PS	*B. polymyxa*	30	60min	45	Micro-bubble	6	Ishikawa and others 1997
	B.cereus	30	60min	50		6	
	B.subtilis	30	60min	55		6	
Sterile Ringer solution	*B.subtilis*	5	1 h	80	Batch	3.5	Ballestra and Cuq 1998
	B. fulva ascospores	5	1 h	80	Batch	0.7	
Sterile water	*B.stearothermophilus*	20	2 h	35	Batch	0	

Table 2-7 Continued

Solution	Microorganism	Pressure (MPa)	Time	Temp (°C)	System	Log redn.	Reference
Orange juice	*S.cerevisiae* ascospores	15	<10min	45	Continuous Membrane filter	>6	Sims and Estigarribia 2002
	Alicyclobacillus acidoterretis spores	7.5	<10min	45		>6	
	G.stearothermophilus	30	2 h	95	Batch	5	Watanabe and others 2003b

[a]PS: Physiological saline

Inactivation of Enzymes by DPCD

Inactivation of certain enzymes that affect the quality of foods by DPCD has been shown by several researchers (Balaban and others 1991a&b, Chen and others 1992&1993, Tedjo and others 2000, Park and others 2002). A summary of the literature including the enzymes treated with DPCD, the amount of activity loss achieved in these treatments and DPCD treatment conditions is given in Table 2-8. DPCD can inactivate certain enzymes at temperatures where thermal inactivation is not effective (Balaban and others 1991a). Among these enzymes, pectinesterase (PE) causes cloud loss in some fruit juices; polyphenol oxidase (PPO) causes undesirable browning in fruits, vegetables, juices and some seafood; lipoxygenase (LOX) causes chlorophyll destruction and off-flavor development in frozen vegetables; and peroxidase (POD) has an important role in discoloration of foods and is used as an index for efficacy of heat treatment in processing fruits and vegetables. The PE, PPO, POD and LOX from various foods were shown to be effectively inactivated by DPCD. Although the number of studies on enzyme inactivation by DPCD is limited, the studies conducted so far point to the potential of DPCD technology in especially fruit and vegetable juice processing, where these enzymes cause quality deterioration if not inactivated.

Studies suggest that enzyme inactivation by DPCD could be due to several causes such as pH lowering, conformational changes of the enzyme, and inhibitory effect of molecular CO_2 on the enzyme.

Balaban and others (1991a) studied the inactivation of PE in orange juice by DPCD. The pH of orange juice must be lowered to 2.4 for substantial PE inactivation. However, by DPCD treatment pH of orange juice was lowered only to 3.1. Therefore, pH-lowering alone was not sufficient to explain enzyme inactivation by DPCD. The results of Chen and others (1992) support their approach. They used a pH control and measured the activity of lobster PPO that was kept under pH of 5.3, which is the same as the pH of samples achieved by DPCD treatment. Although the pH control sample retained 35% of its original activity at 35^{o}C after 30 min, DPCD treated enzyme lost its activity after 1 min at the same temperature.

CO_2 was suggested to have a unique role in the inactivation of enzymes. Balaban and others (1993) applied the following treatments to orange juice and observed the decrease of PE activity. Untreated control had a decrease in PE activity of 8% after 20 days storage. Supercritical CO_2 treatment (31 MPa, 40^{o}C, 45 min) showed 31% reduction; juice acidified with HCl to pH=3.1 and pressurized with N_2 (24 MPa, 40^{o}C, 45 min) had a 36% reduction; juice buffered to pH=3.8 with citrate buffer, then treated with supercritical CO_2 (31 MPa, 40^{o}C, 45 min) reduced PE by 23%; juice pressurized with N_2 (20.6 MPa, 55^{o}C, 1 h) showed an increase in PE activity. These results suggest that the buffered juice PE activity decreased only by the molecular effect of CO_2, while the unbuffered CO_2 combined the effects of pH lowering and CO_2 effects. Pressurized N_2 did

not lower activity. Similarly, Chen and others (1993) have shown that N_2 treatment under the same conditions as CO_2 treatment did not cause any inactivation of PPO.

Table 2-8. Summary of studies on inactivation of enzymes by DPCD

Enzyme	Source of enzyme	Pressure (MPa)	Time	Temp (^oC)	System	Loss of activity (%)	Reference
Lipase	Commercial (62-68% water)	20	2 h	35	Batch	12-22	1
α-amylase	Commercial (62-68% water)	20	2 h	35		0	
Gluco-amylase	Commercial (5-7% water)	20	1 h	35	Batch	0	2
Catalase	Commercial (5-7% water)	20	1 h	35		10	
Lipase	Commercial (5-7% water)	20	1 h	35		0	
Glucose isomerase	Commercial (5-7% water)	20	1 h	35		0	
PE[a]	*Orange juice*	26.9	145 min	56	Batch	100	3
PPO[b]	*Spiny lobster*	5.8	1 min	43	Batch	98	4
PPO	Brown shrimp	5.8	1 min	43		78	
PPO	Potato	5.8	30 min	43		91	
PPO	Spiny lobster	0.1	30 min	33	Batch	98.5	5
LOX[c]	Soybean	10.3	15 min	50	Batch	100	6
POD[d]	Horseradish	62.1	15 min	55		100	
LOX	Soybean	62.1	15 min	35		95	
PPO	Carrot juice	4.9	10 min	5	Batch	61	7
LOX	Carrot juice	2.94	10 min	5		>70	
PPO	Muscadine grape juice	27.6	6.25 min	30	Continuous flow	75	8

[a]PE: Pectinesterase, [b]PPO: Polyphenol oxidase, [c]LOX: Lipoxygenase, [d]POD: Peroxidase
[1]Kamihira and others 1987, [2] Taniguchi and others 1987,[3] Balaban and others 1991b, [4]Chen and others 1992, [5] Chen and others 1993, [6] Tedjo and others 2000, [7] Park and others 2002, [8]Del Pozo-Insfran and others 2006

DPCD can change isoelectric profiles and protein patterns of PPO (Chen and others 1992). However, these changes were not caused by CO_2 under atmospheric pressure (Chen and others 1993). Chen and others (1992) obtained Circular Dichroism spectra of untreated and treated lobster, brown shrimp and potato PPOs. Their results showed that DPCD caused conformational changes in the secondary structures (α-helix, β-sheet, β-turn and random coil) of the enzymes. High pressure is also reported to cause

conformational changes in protein and enzyme molecules (Suzuki and Taniguchi 1972). On the other hand, Hendrickx and others (1998) reported that pressures around 310 MPa can cause irreversible damage to the secondary structure of proteins, but pressures below it cause no change or changes that are reversible upon depressurization. DPCD pressures are very much lower than 310 MPa, therefore, the conformational changes occurring by DPCD may not be caused by a high pressure effect. This needs to be confirmed by further research.

Extent of enzyme inactivation by DPCD is affected by the type and source of the enzyme, DPCD treatment conditions such as pressure, temperature and time, and treatment medium properties. Balaban and others (1991a) observed that higher temperatures and pressures of DPCD treatment results in higher %PE inactivation. An enzyme isolated from different sources has different resistance to DPCD treatment, as is the case with heat inactivation. For example, potato PPO is more resistant to inactivation by DPCD compared to spiny lobster and shrimp PPOs (Chen and others 1992). The presence of other soluble compounds in the treatment medium may have a protective effect against DPCD treatment. Tedjo and others (2000) showed that %LOX and %POD activity increased by increasing sucrose concentration up to 40%. This could be explained by decrease in the solubility of CO_2 as sucrose concentration increases.

DPCD treatment is reported to be more effective than heat treatment in enzyme inactivation and can inactivate enzymes at much lower pressures compared to High Hydrostatic Pressure, an alternative non-thermal processing method. Significant amounts of inactivation of PE, PPO, LOX and POD are possible by DPCD at temperatures lower than 55^{o}C. Park and others (2002) achieved significant inactivation of LOX and PPO in

carrot juice at a temperature as low as 5^{o}C. Heating at 55^{o}C for 30 min results in only about 18% and 13% inactivation of LOX and POD, respectively, while DPCD results in total inactivation of these enzymes after 15 min treatment at the same temperature.

DPCD Treatment Systems

Several batch, semi-continuous and continuous treatment systems have been developed since the first DPCD applications. In a batch system, CO_2 and treatment solution are stationary in a container for a certain period of time during treatment. A semi-continuous system allows a continuous flow of CO_2 through the treatment chamber, while a continuous system allows continuous flow of both CO_2 and the treatment solution through the system.

Most of earlier studies have been performed using batch systems. A typical batch system consists of a CO_2 gas cylinder, a pressure regulator, a pressure vessel, a water bath or heater, a CO_2 release valve, and a data logger (Figure 2-6) (Hong and Pyun 1999). At the beginning of the operation, the sample solution is placed into the pressure vessel and temperature is set to the desired value. Next, CO_2 is introduced into the vessel until the sample in the vessel is saturated at the desired pressure and temperature. The sample solution is left in the vessel for a certain amount of time and then the CO_2 outlet valve is opened to release the gas. Some systems contain an agitator that decreases the time to saturate the sample solution with CO_2.

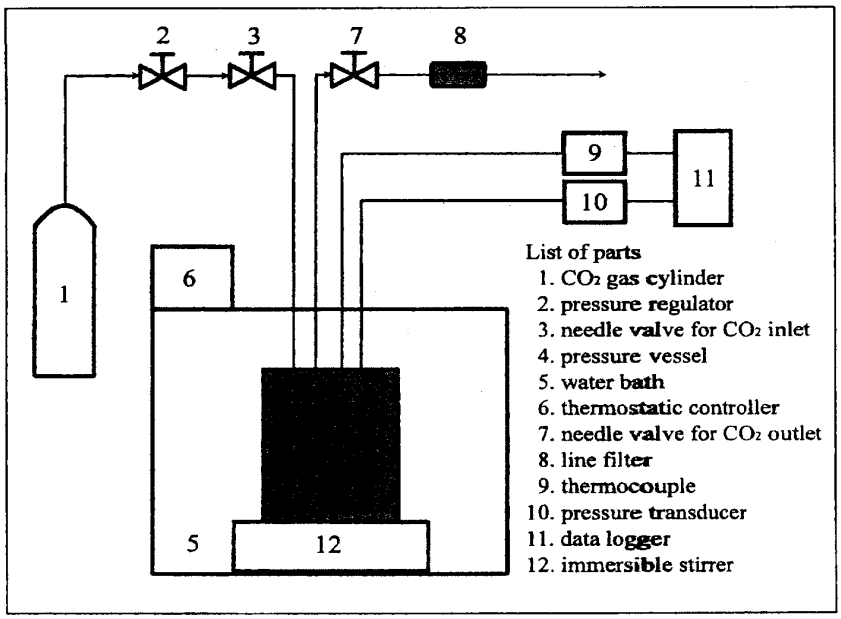


Figure 2-6. A typical batch DPCD system

In 1995, Ishikawa and others (1995a) developed a semi-continuous micro-bubbling system that uses a cylindrical filter to micro-bubble CO_2 entering into the pressure vessel. They showed that the use of the filter significantly increased the efficiency of the system. They could achieve three times more inactivation of enzymes using a micropore filter than without it. They also showed that using a filter increased the concentration of dissolved CO_2 in the sample from 0.4 to 0.92 mol/L at 25 MPa and 35^{o}C. In 1998, Shimoda and others developed a continuous micro-bubble system that was very effective in the inactivation of microorganisms (Figure 2-7). In this system, liquid CO_2 and a saline solution were pumped through a CO_2 dissolving vessel at certain flow rates. Liquid CO_2 was changed to gaseous state using an evaporator and then dispersed into the saline solution from a stainless steel mesh filter with 10 μm pore size. The micro-bubbles of CO_2 moved upwards while dissolving CO_2 into the saline solution. Then, the saline solution saturated with CO_2 was passed through a heater to reach the desired temperature

and a suspension of microorganisms was pumped into it at this point. Another coil with a heater was used to adjust the residence time (Shimoda and others 2001).

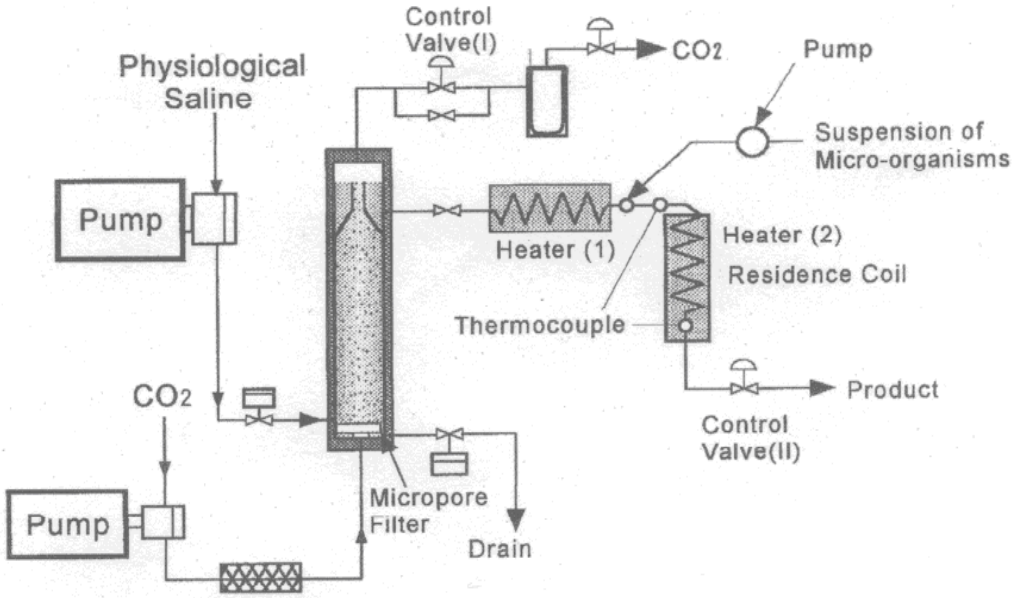


Figure 2-7. A continuous micro-bubble DPCD system

A continuous membrane contact CO_2 system was developed by Sims in 2001 (Figure 2-8) (Sims and Estigarribia 2002). This system consists of four in series hollow polypropylene membrane modules. Each tubular module has 15 parallel fibers of 1.8 mm ID, 39 cm length and 83 cm^2 active surface area. A CO_2 pump is used to pressurize the system, and the test liquid is pumped continuously into the system with a HPLC pump. This setup is very efficient in saturating the liquid with CO_2 since it provides a large contact area between CO_2 and the test liquid by the use of the membranes. In the membrane contactor, CO_2 is not mixed with the test liquid but instead diffuses into it at saturation levels instantaneously. CO_2 is recycled back and re-used.

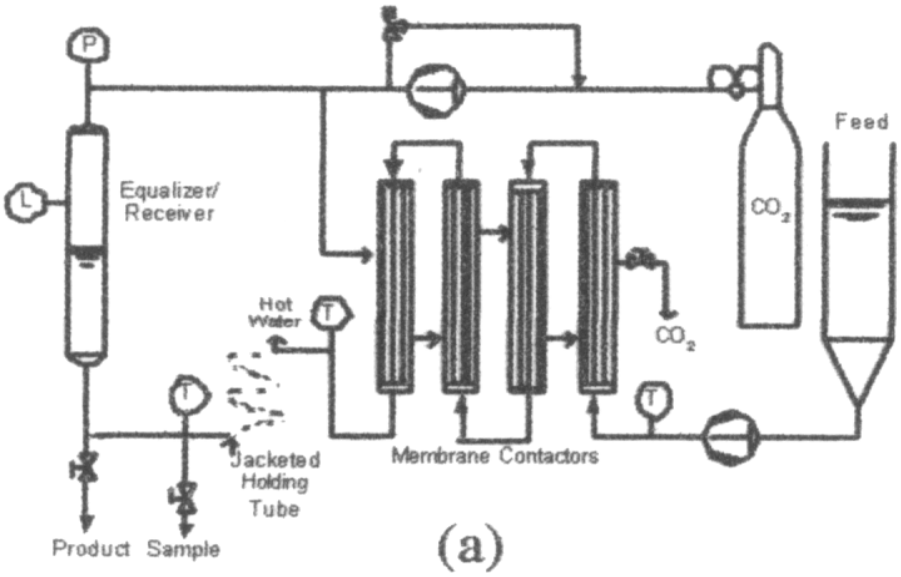


Figure 2-8. A continuous CO_2 membrane contactor system

In 1999, Praxair (Chicago, IL) developed a continuous flow DPCD system (Figure 2-9). This system consists of CO_2 tanks and a CO_2 pump, a product tank and product pump, a high pressure pump, holding coils, decompression valve and a vacuum tank. CO_2 and the product are pumped through the system and mixed before passing through the high pressure pump. This pump increases the pressure to the processing levels, and the product temperature is brought to the desired level in holding coils. Residence time is adjusted by setting the flow rate of the product passing through holding coils. At the end of the process, an expansion valve is used to release CO_2 from the mixture. It is possible to pull out the remaining CO_2 in the product by a vacuum tank. This system has been shown to be very effective in killing pathogens and spoilage bacteria for short periods of time (Folkes 2004, Damar and Balaban 2005, Kincal and others 2005, Lecky 2005, Lim and others 2006).

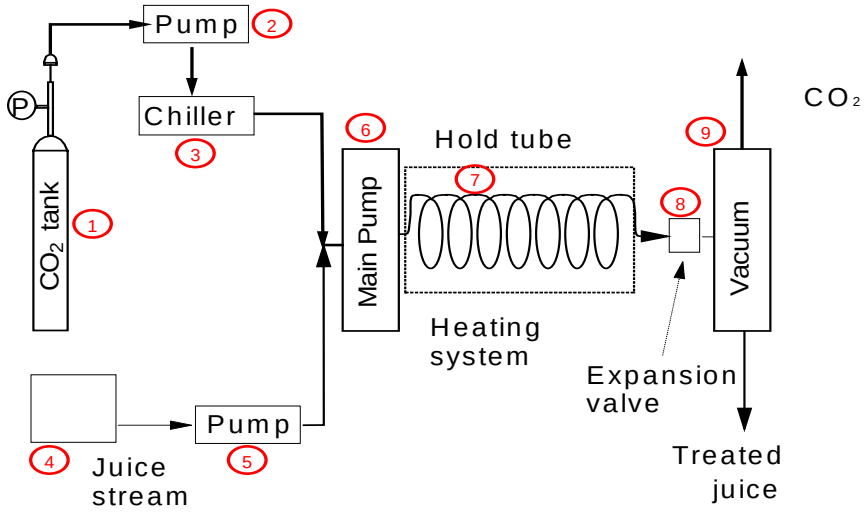


Figure 2-9. A continuous flow DPCD system

DPCD Food Applications and Quality Effects

DPCD has been applied mostly to liquid food products, particularly to fruit juices. To date, there is no commercial food product processed by DPCD. There are a limited number of published studies in the literature regarding the effect of DPCD on the quality of foods including a few test results published by companies offering commercial systems.

Among the first food applications of DPCD is treatment of whole fruits such as strawberry, honeydew melon, and cucumber for inhibition of mold growth. Haas and others (1989) demonstrated that although mold inhibition is possible by DPCD treatment of fruits, DPCD may cause severe tissue damage in some fruits even at low pressures.

Studies with orange juice shows that DPCD treatment can improve some physical and nutritional quality attributes such as cloud formation and stability, color and ascorbic acid retention. Arreola and others (1991a) treated fresh orange juice with DPCD in a batch system from 7 to 34 MPa, 35 to 60^{o}C and for 15 to 180 min time periods. They also

had temperature controls that were kept under the same temperatures for the same amount of time without DPCD treatment. Ascorbic acid retention of DPCD treated orange juice was between 71 to 98%. Ascorbic acid retention levels of DPCD treated samples were significantly higher than that of temperature controls. Higher ascorbic acid retention by DPCD was explained by the exclusion of O_2 from the system and lower pH of orange juice by DPCD. Ascorbic acid has higher stability under low pH and oxidizes easily when oxygen is present in the environment. On the other hand, cloud of orange juice was enhanced by 1.3 to 4.0 times after DPCD treatment compared to original untreated orange juice. Cloud stability of orange juice treated by DPCD at 29 MPa and 50^{o}C for 4 h was retained after 66 days of refrigerated storage. However, temperature controls (50^{o}C for 4 h) and room temperature controls (25^{o}C for 4 h) lost cloud completely during refrigerated storage. In the same study, instrumentally measured color scores showed that DPCD treated juice was brighter than untreated juice. Sensory evaluation of DPCD treated and untreated juices indicated that there was no significant difference in flavor, aroma and overall acceptability of these samples. The color and cloudiness of DPCD treated juice were preferred over those of untreated juice.

Park and others (2002) treated carrot juice with a combined effect of 4.9 MPa DPCD and 600 MPa ultra-high pressure. They observed reduction of pectin methylesterase (PME) activity by 65%, and a cloud loss of 47%. This suggests that the cloud loss in different food systems, even with the same enzyme (PME), could follow different mechanisms, and cloud retention in e.g. orange juice does not necessarily imply cloud retention in carrot juice.

Later studies using continuous systems also show nutritional and sensory quality retention and improvements in the physical attributes of orange juice treated with DPCD. Kincal and others (2006) obtained up to 846% cloud increase in orange juice by DPCD treatment (38 MPa, room temperature, 10 min). There were no significant changes in pH and ^{o}Brix of treated samples. Small, but statistically insignificant increase in L* and a* values of color occurred by DPCD. Sensory evaluations of DPCD treated and untreated orange juice were not significantly different. Ho (2003) used the continuous flow system of Praxair (Chicago, IL) and reported that there were no significant differences between physical attributes (pH, ^{o}Brix and titratable acidity), nutritional content (vitamin C and folic acid) and aroma profile for untreated and DPCD treated orange juice.

Folkes (2004) used continuous DPCD technology for pasteurization of beer and compared physical and sensory quality attributes of DPCD treated beer with that of fresh (untreated) and heat pasteurized beer. The aroma and flavor of DPCD treated beer was not significantly different from fresh beer even after 1 month storage at 1.67^{o}C, but heat treated beer was found significantly different than others in taste and aroma at the end of storage (α=0.1). DPCD treated beer had significantly less foam capacity and stability compared to heat pasteurized beer, but not at levels detrimental to the finished product quality. On the other hand, beer haze was significantly reduced by DPCD.

Lim and others (2006) treated mandarin juice with DPCD using the continuous flow system by Praxair and measured the pH, ^{o}Brix, titratable acidity, cloud and color after DPCD treatment at 13.8-41.4 MPa, 25-45^{o}C and 7-9 min. DPCD treatment enhanced the cloud up to 38.4%, increased lightness and yellowness, and decreased

redness of mandarin juice. DPCD treated samples had higher titratable acidity than untreated samples. The pH and ^{o}Brix did not change after DPCD treatment (α=0.05).

It is important to conduct studies regarding the consumer likeability of food products that are processed by DPCD since the consumer is the target in commercialization of this technology.

Objectives of the Study

The objectives of this study were:

i. To quantify microbial reduction in coconut water as a function of treatment conditions such as pressure, temperature, time and CO_2 level

ii. To evaluate quality of DPCD treated coconut water during storage

iii. To compare untreated fresh, DPCD- and heat-treated coconut water by sensory evaluation

iv. To identify flavor compounds in coconut water and compare the flavor profile of untreated, DPCD- and heat-treated coconut water

CHAPTER 3
MATERIALS AND METHODS

Preliminary Experiments with Coconuts

Juice Extraction and Initial Quality Tests

Eight immature green coconuts (Malaysian Dwarf) were obtained from Homestead, FL. A ½ in Makita Drill (Buford, GA) was used to drill two holes on opposite sides of coconuts and the water was poured into 1L glass bottles. Each bottle was numbered from 1 to 8 and stored in a refrigerator (4^{o}C). Weight of coconuts ranged between 1.85 to 2.40 kg and coconut water extracted from these ranged between 435 to 490 g. ^{o}Brix was between 6.3 and 6.6, while pH ranged between 5.35 and 5.50. Total aerobic plate counts (APC) were between zero count and >190 cfu/mL, and there was no yeast and mold (YM) growth initially (Table A-1). APC and YM counts were repeated at day 9 for selected bottles and increase in counts were observed (Table A-2). Presence of PPO and POX enzyme activity in coconut water was confirmed by following the method described by Campos and others (1996).

Pinking of Coconut Water

Coconut water from the eight coconuts in each bottle changed color during refrigerated storage. The pictures of the coconut water in each bottle at day 0 and day 9 were given in Figure A-1. Some of the bottles showed browning of coconut water at day zero. This could be due to enzymatic browning that was accelerated by introducing phenolic compounds from the outer surface of the green shell, as well as heating and metal contact during drilling of the coconuts.

Preliminary tests were conducted to understand the mechanisms causing or accelerating pinking in coconut water.

In Test 1, coconut water extracted from one immature green coconut was divided into two. One part was placed into 20 mL glass test tubes and divided into five treatment groups (three tubes/group). Treatments were control (no treatment)(1), frozen and thawed at 4^{o}C the next day(2), N_2 bubbling for 15 min (3), heating at 80^{o}C for 5 min (4) and heating at 80^{o}C for 5 min while exposed to the air (5). All tubes were stored under refrigeration (4^{o}C). Tubes were observed for color at days 0, 4, 7, 9 and 12. On day 7, one of the three tubes from control, frozen/thawed and heating (closed caps) groups were removed from that group and bubbled with air for 15 min. Color observation results are given in Table A-3. Two out of three N_2 bubbled tubes and all open heated tubes turned pink earlier than others on day 4. On day 12, one tube of N_2 bubbled and two unaerated controls, frozen/thawed and heated (closed cap) tubes were still colorless whereas all aerated tubes, open heated tubes and two N_2 bubbled tubes were pink. Although it is hard to draw a clear conclusion on the effect of heating or N_2 bubbling based on this test, aeration seems to accelerate pinking.

In Test 2, the second part of the coconut water was placed into 50 mL opaque plastic cups and were exposed to different treatments such as ascorbic acid (100 ppm)(1), potassium metabisulfite (40 ppm)(2) or 0.1N HCl addition to lower the pH to 4.0(3) and 3.0(4). Two cups were untreated and used as control (pH=4.8). The color observations were done every day until day 12 and also 3 months later (Table A-4). Control cups turned pink on day 12 whereas others were still colorless. At the end of 3 months, all

cups other than ascorbic acid added and potassium metabisulfite added cups turned pink. Ascorbic acid and potassium metabisulfite seem to stabilize the color of coconut water.

Test 3 was conducted to observe the effect of aeration and heating on pinking of coconut water. Coconut water extracted from a coconut was divided into 20 mL glass tubes, three tubes in each treatment. Treatments were control (1), heated at 85^{o}C for 5 min (2), boiled for 5 min (3), and air bubbled for 15 min (4). Color observations on day 6 and day 10 showed that all heated and aerated tubes eventually turned pink, whereas control tubes were still clear at the end of 10 days refrigerated storage (Table A-5). These results suggested that aeration and heating might accelerate pinking. It is unlikely that pinking is due to microorganisms since boiling did not prevent it.

Tests with Commercial Coconut Water Drinks

In order to understand some properties of commercially available coconut water, six different brands of coconut water drinks were obtained from the market and their sensory evaluation was made by an informal tasting. The measured pH and ^{o}Brix values and the contents of these products are given in Table A-6. Four of these products were in aluminum cans while two others were in Tetrapak boxes. The pH of these drinks changed between 4.12 and 5.16 while the ^{o}Brix was in the range of 5.6 to 10.8. Cooked, metallic, soapy and artificial coconut flavors were recognized by some of the panelists. Products with lower ^{o}Brix were mostly found to have a bland or no taste whereas the ones with higher ^{o}Brix were usually found to be too sweet.

Extraction of Coconut Water from Coconuts

About 1,140 immature green coconuts (*Cocos nucifera*, Malaysian Dwarf) were obtained from growers (El Salvador Farm) in Homestead, Florida. Coconuts were left in a commercially available bleach solution (1.0% (v/v)) and rinsed with water before

cutting. Washed coconuts were passed through a band saw and cut horizontally in one end. The liquid inside was taken to a clear glass bottle by the use of a peristaltic pump, and checked for color, smell and taste. Any turbid, or abnormally colored liquid was discarded. Clear liquid was placed in 3 gallon plastic pail containers that were kept in ice. Once each pail was full, the juice was frozen at –20^{o}C immediately in order to prevent any microbial or enzymatic activity. This procedure was used to mix juices from many coconuts and make the sample homogeneous as much as possible. Although all the coconut water could not be mixed into one batch, the liquid was a representative of a broad number of coconuts. During experiments whenever needed, the pails were taken randomly into 4^{o}C cold room and thawed. Pictures in Figure A-2 show steps used in extraction of coconut water.

Formulation of Coconut Water Beverage

Preliminary tests were performed to determine the necessity for acidification, sweetening and carbonation of coconut water. Safety considerations against *C. botulinum* required acidification. Food grade citric acid (Presque Isle, North East, PA), malic acid (Presque Isle, North East, PA) and pHase (Jones-Hamilton, Walbridge, OH) were compared by preliminary tastings for their suitability to sweeten coconut water. Malic acid was chosen as the most suitable acid and added to coconut water to lower the pH to 4.30. Malic acid is naturally present in coconut water and was preferred over citric acid and pHase by the panelists. Preliminary tasting showed that a sweetener was needed to compensate for the sourness caused by acidification. Splenda (McNeil-PPC, Fort Washington, PA), which is basically a chemically modified form of sucrose, was used as the sweetener and the amount was determined by informal tastings. Splenda has no caloric value and was preferred over other artificial sweetners because it gives relatively

higher sweetness (600 times that of sucrose) and lack of strong aftertaste. ^{o}Brix of coconut water did not change after Splenda addition. Finally, carbonated coconut water was compared to non-carbonated for likeability by informal tastings. Carbonation was done at 4^{o}C and 1.82 atm CO_2 pressure. It was decided to carbonate coconut water after acidification and sweetening because carbonated samples were preferred over non-carbonated by panelists.

DPCD Processing Equipment

Continuous-flow DPCD System

A continuous high pressure CO_2 machine of 55.16 MPa pressure and about 0.8 liters/min flow rate capacity (Praxair Co., Chicago, IL) was used for pasteurization of coconut juice. The components of the system and their functions were described in section "DPCD treatment systems" of Chapter 2.

The system was run at a juice flow rate of 417 g/min in order to obtain 6 min residence time in the holding tube (79.2 m length and 0.635 cm ID). Sterile water was run through the system until the desired levels of pressure, temperature and CO_2 level were reached. Coconut water was then poured in the juice tank and the first 3.5 L of coconut water were discarded. Approximately 1 L of treated coconut water was collected into a sterile 1 L glass bottle at the exit valve. Processed coconut water was cooled down immediately at 4^{o}C until further use. Whenever the treatment parameters were changed, sterile water was run through the system until the desired levels were reached. The equipment was cleaned after each use as described below.

Cleaning of the Equipment

Oxonia and Principal solutions (Ecolab, St. Paul, MN) were the chemicals used to sanitize the equipment. Concentrations of solutions were determined as 0.38% Principal

and 0.44% Oxonia solutions (v/v) with the help of an Ecolab representative. The equipment was first cleaned with 26.5 L of Principal solution and then with 22.7 L of Oxonia solution the day before the experiment. On the day of the experiment, 24 L of sterile distilled water was passed through the equipment. At the end of the experimental run, the same sanitization procedure was followed. Previous cleanability studies on DPCD equipment shows that a concentration of 0.5% Principal solution and 0.28% Oxonia solution were sufficient to confirm that the equipment was sanitized (Lecky 2005).

Heat Pasteurization Equipment

Heat pasteurization equipment consisted of a water bath (Precision Scientific Group, Chicago, IL) that was set to the pasteurization temperature (74^{o}C), two 5.4 m stainless steel tubing (0.476 cm ID) and a peristaltic pump (Figure 3.1). Coconut water was pumped by the peristaltic pump at a flow rate of 385 mL/min through the first stainless steel tubing (placed in the water bath) in order to be heated to 74^{o}C and then passed through a second stainless steel tubing at 74^{o}C for 15 s. D value of *L.monocytogenes* at 74^{o}C is 0.72 s and its z value is 5.56^{o}C (Freier 2001). Treatment for 15 s gives 20 log cycles reduction in this microorganism. Coconut water exiting the second tubing was immediately cooled to approximately 10^{o}C by passing through 3.2 m of stainless steel tubing (0.476 cm ID) that was placed in ice slush. Heat treated coconut water was collected in sterile glass containers (6 L) and placed in the cold room and at 4^{o}C.

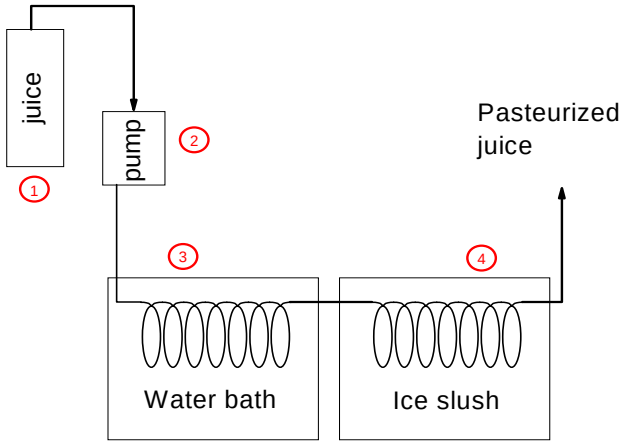


Figure 3.1. Schematic drawing of heat pasteurization equipment

Carbonation Equipment

Untreated, DPCD and heat pasteurized coconut water samples were carbonated by using a Zahm & Nagel Pilot Plant Carbonator (Zahm & Nagel Co., Buffalo, NY) with a capacity of around 7.5 liters. Carbonator was cleaned by soap, distilled water and alcohol before each use. Coconut water at 4^{o}C was placed in the carbonator unit that was kept in ice throughout carbonation in order to keep the temperature of the juice at about 4^{o}C. CO_2 gas was sent from the gas tank (BOC Group, NJ) to the carbonator and the air remaining in the carbonator was replaced by CO_2 gas. Next, the CO_2 pressure was brought to 1.82 atm and CO_2 was bubbled through the juice until all juice inside the carbonator was collected. Carbonated coconut water was immediately filled into glass champagne bottles of 750 mL capacity each and capped with metal caps. All carbonated water bottles were stored at 4^{o}C.

Optimization of DPCD Treatment Conditions for Microbial Reduction

Aging of Coconut Water

Aging of coconut water was necessary to bring the initial microbial load of coconut water to 10^7 colony forming units (cfu/mL). Frozen coconut water kept in plastic pails at –20^{o}C, was thawed for 1 week at 4^{o}C and then formulated by the addition of malic acid to lower the pH to 4.3 and 0.7% (w/w) Splenda with a final ^{o}Brix of 6.0. Then the coconut water was aged at room temperature (24^{o}C) for about 46 h in order to increase microbial load to $>10^7$ cfu/mL.

Experimental Design

Response surface methodology (RSM) was used for the design and optimization of DPCD treatment conditions for microbial reduction. DPCD process variables were pressure, temperature, CO_2 to juice ratio (w/w) and residence time. Experimental conditions were determined by a 3-factor, 3-level Box-Behnken design, which is one of the Response surface designs. Residence time was decided to be 6 min, and kept constant throughout the treatments since long times would not be economically feasible.

Independent variables were pressure (13.8, 24.1, 34.5 MPa), temperature (20, 30, 40^{o}C) and CO_2 to juice ratio (7, 10, 13 g CO_2/ 100 g juice). The maximum pressure level was chosen as 34.5 MPa because this pressure can be achieved safely considering the limitations of the system, where 55.16 MPa is the maximum. The minimum pressure level was chosen as 13.8 MPa since below that pressure level a significant microbial reduction was not expected based on previous studies. Minimum temperature was determined by the limitations of the equipment and had to be chosen as the room temperature at the time of the experiment. Middle temperature value was 30^{o}C that was a close to the critical temperature for CO_2 (31^{o}C). Maximum temperature (40^{o}C) was in

supercritical range, and higher temperatures than this could affect the quality of the juice. Dependent variable was log reduction in aerobic microbial load (cfu/mL) of juice after treatment. Microbial log reduction was calculated for each experimental run as; log[(initial number of cfu /mL)([number of cfu/mL after treatment)]. Fifteen experimental runs were determined by applying Box-Behnken coded design. The codes and conditions for each variable are shown in Table 3-1. The following equations give the relation between the codes (X1, X2, X3) and the variables (T, P and % CO_2 level):

$X1 = 0.10 * T(^{o}C) - 3.0$

$X2 = 0.097 * P(MPa) - 2.33$

$X3 = 0.333 * \%\ CO_2(g\ CO_2/\ 100\ g\ juice) - 3.33$

Table 3-1. Three factor-3 level Box-Behnken experimental run coded variables and conditions

RUN#	Coded T : X1	Coded P : X2	Coded CO_2/juice ratio: X3	Temperature (^{o}C)	Pressure (MPa)	% CO_2 Level (w/w)
1	-1	-1	0	20	13.8	10
2	1	-1	0	40	13.8	10
3	-1	1	0	20	34.5	10
4	1	1	0	40	34.5	10
5	-1	0	-1	20	24.1	7
6	1	0	-1	40	24.1	7
7	-1	0	1	20	24.1	13
8	1	0	1	40	24.1	13
9	0	-1	-1	30	13.8	7
10	0	1	-1	30	34.5	7
11	0	-1	1	30	13.8	13
12	0	1	1	30	34.5	13
13	0	0	0	30	24.1	10
14	0	0	0	30	24.1	10
15	0	0	0	30	24.1	10

X1: Code for Temperature, X2: Code for Pressure, X3: Code for % CO_2 level

Storage Study

A storage study was conducted for 9 weeks and samples were taken at weeks 0, 2, 3, 5 and 9 in order to evaluate microbial, physical (pH, color, titratable acidity, ^{o}Brix) and sensory attributes of untreated (fresh control), DPCD and heat pasteurized coconut water beverage samples. Flavor profiles of stored samples were also analyzed instrumentally. Storage study was ended at the 9th week since the microbial load for untreated coconut water exceeded 10^{5}cfu/mL and the flavor was undesirable.

At the beginning of the storage study, frozen coconut water was thawed at 4^{o}C and formulated by malic acid and Splenda addition. DPCD treated samples were treated at previously determined optimum conditions (25^{o}C, 34.5 MPa, 13% CO_2), and heat treated samples were pasteurized at 74^{o}C for 15 s. All samples were then carbonated and capped in 750 mL champagne bottles, and stored at 4^{o}C until further needed. These steps are shown in a schematic drawing (Figure 3-2). Untreated control samples were prepared fresh as described above for each week of sensory panels, whereas the DPCD and heat pasteurized samples were stored samples. DPCD and heat pasteurized samples were analyzed for microbial load prior to the taste panels in order to ensure the safety of these samples.

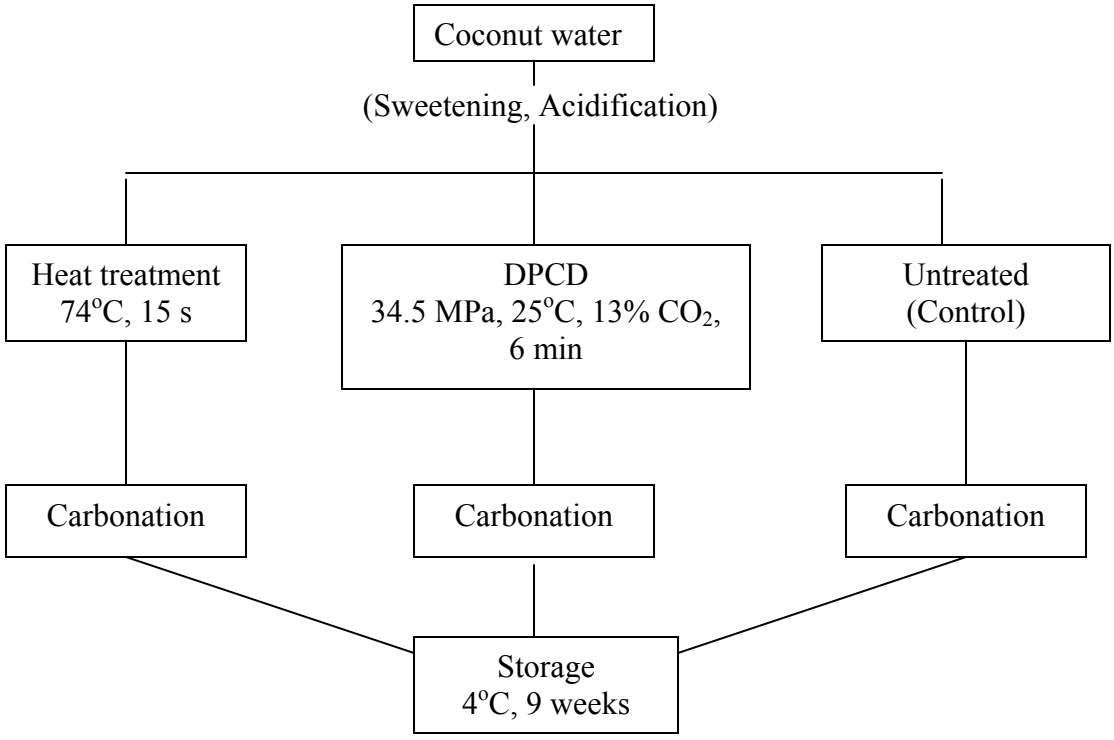


Figure 3-2. Schematic drawing of steps followed in preparation of storage study samples

Microbial Tests

Aerobic plate count (APC), and yeast and mold count (YM) of untreated, DPCD and heat pasteurized samples were determined by using 3M Petrifilms (3M Microbiology, St.Paul, MN). The pH of coconut water was first adjusted to around 7.0 with 1N NaOH and then 10-fold serial dilutions were prepared by adding 10 mL of coconut water into 90 mL of Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria,CA). Two replicates of each dilution were prepared and each was plated on two replicates of 3M Petrifilms. Aerobic plate petrifilms were incubated at 35°C for 48 hr, while yeast and mold petrifilms were incubated at 25°C for 5 days before counting. The petrifilms with the cfu's between 20 and 200 were taken into consideration and the average cfu's corresponding to the dilution was calculated.

pH

Orion (EA 920) pH meter (Boston, MA) was used for pH measurements. The pH meter was calibrated using pH 4 and pH 7 standard solutions (Fisher Scientific, NJ) on each test day. The pH measurements were done in triplicate.

Titratable Acidity (%TA)

A Brinkmann Instrument (Brinkmann Instruments Co., Westbury, NY) consisting of Metrohm 655 Disomat, Metrohm 614 Impulsomat and Metrohm 632 pH-meter was used for titration of coconut water samples. Samples were placed in a vacuum oven at room temperature (22^{o}C) and 0.75 atm vacuum for 1 hr in order to remove CO_2 gas before titrating. 20 mL of coconut water sample was titrated to an end point of pH 8.2 by using standardized 0.1 N NaOH and the amount of NaOH used for titration was recorded. Percent titratable acidity (w/v) was expressed as % malic acid and calculated by the following equation:

%TA= (mL of NaOH used) (Normality of NaOH) (meq of malic acid = 0.067) (100)/ (mL of sample)

%TA measurements were done in triplicate for each sample.

^{o}Brix

A Fisherbrand hand held refractometer with a 0^{o} to 18^{o} Brix scale (Fisher Scientific, Pittsburg, PA) was used for ^{o}Brix measurements. 2-3 drops of coconut water were placed onto the prism and the reading was recorded. Measurements were done in duplicate.

Color

Color of coconut water samples was measured in a CIE L* (Lightness) a* (Redness) b*(Yellowness) color scale by using the Colorgard 14 system (BYK-Gardner

Inc., Columbia, MD). Quartz halogen lamp (2845 K) was used as the light source, and allowed to warm up for 10 min prior to measurements. The system was calibrated using black (Zero reference) and white standard (L, a*, b*: 94.31, -0.92, -0.50) tiles. A standard measurement was done by placing a glass cup filled with 50 mL of distilled water and the white tile placed on top of the cup in a facedown position. The same procedure was followed with the coconut water samples. The cup was rinsed with distilled water and wiped with Kimwipes between samples. Measurements were done in triplicates.

Sensory Evaluation

Sensory panels were conducted during storage at weeks 0, 2, 3, 5 and 9 in order to evaluate overall likeability, aroma, taste and off flavor of untreated, DPCD and heat pasteurized samples. University of Florida FSHN Dept.'s taste panel facility (University of Florida, Gainesville, FL) consisting of 10 private booths with computers was used to conduct sensory panels. Samples were stored capped in champagne bottles in an ice bath before being poured into 60 mL plastic cups, in order to prevent carbonation loss. Each sample was assigned with a randomly selected three-digit code, and placed in cups on a tray in all possible combinations of order. Red light was used in the panelist booths in order to prevent bias on samples due to pinking of some samples. Panelists were asked to answer some demographic questions at the beginning, and then were offered with an untreated (fresh control) reference, and three samples (fresh control, DPCD treated, heat treated). Panelists were asked to rate aroma and taste difference of each sample from the given reference (continuous 15 cm line scale with values from 0 to 15) using difference-from-control test. In addition, overall likeability (9 point hedonic scale), off flavor (6 point scale) and their purchase intent for each sample were asked. Panelists took a bit of

cracker and a sip of water to rinse their mouth between the samples. Fifty untrained panelists evaluated the samples at each storage week. Compusense 5 software (Compusense Inc., Ontario, CA) was used to design and conduct the test, and to collect and analyze the data. Sample ballots that were used in sensory panels are given in Table E-10.

Flavor Analysis

Solid phase micro-extraction (SPME) was used to extract aroma compounds from coconut water. The SPME fiber was a 1 cm StableFlex PDMS/CAR/DVB fiber (Supelco, St.Louis, MO) which is a bipolar phase fiber suitable to extract high and low volatile compounds. 10 mL of coconut water was placed into 40 mL glass vials and brought to 42-45^{o}C in a water bath. SPME fiber was inserted into the headspace of the vial and extraction was held under continuous stirring at 42-45^{o}C for 45 min using a magnetic stir bar. SPME fiber was inserted into the GC injection port and exposed for 5 min for desorption of aroma compounds. GC/O (HP 5890 Series II) equipment with a FID detector was used to separate and analyze the aroma compounds. Two different columns were used in GC/O; a non-polar DB-5 column (Zebron, 30 m x 0.32 mm ID x 0.50 μm FT) and a polar Carbowax column (Restek, 30 m x 0.32 mm ID x 0.5 μm df). Temperature programming conditions for GC/O using each column are given in Table 3-2. With each column, two persons sniffed twice each SPME extract. Sniffers used a continuous scale slide marked as low, medium and high to rate the intensity of the sniffed compound and also indicated aroma descriptors of each sniffed compound at the corresponding retention time. The chromatograms for both the FID and sniff port were recorded and saved. C5-C20 alkane standards were run at each experiment day and their

retention times were recorded. Their literature linear retention indices (LRI's) were plotted against their retention times and the equation relating the LRI's as a function of retention times was obtained by using the Excel graph options. The same equation was used to calculate LRI's of the sniffed compounds at the corresponding retention times. Examples of LRI calculations of standard alkanes and the formulas relating LRI's to the retention times are given in Table C-1 and Figure C-1, respectively, for the Carbowax column, and in Table C-2 and Figure C-2, respectively, for the DB-5 column. An aromagram was constructed by plotting average sniff intensity (average of sniff port peak areas) against the calculated LRI's.

A GC/MS (Perkin Elmer; Wellesley, MA) equipment with quadrupole-ionization detector was used for identification of flavor compounds in coconut water. This equipment used TurboMass 5.01 (Wellesley, MA) software for the integration and analysis, and a NIST (MS Research 2.0) database as the library of the compounds for the identification. The SPME extracts were injected and exposed through the injection port for 5 min. GC-MS temperature programming conditions were; 40^{o}C (initial) to 240^{o}C (final) at a 7^{o}C/min ramp rate and with a 9.5 min holding time. Each peak on GC/MS chromatogram was first integrated and then searched through the NIST database for the identification by using the software. The software gave a list of compound names, that matched the peak with the degree of match for each listed compound over 1000. An example of this identification procedure including the chromatogram and the NIST identification sheet is shown in Figure C-3. C5-C20 alkane standards were used to obtain an equation relating retention times of compounds to the LRI's.

Table 3-2. Temperature programming conditions used for GC/O runs with DB-5 and Carbowax columns.

Column Type	Initial Oven Temp. (^oC)	Final Oven Temp. (^oC)	Ramp rate (^oC/min)	Final holding time (min)	Detector A Temp. (^oC)	Detector B Temp. (^oC)	Injector Temp. (^oC)
DB-5	40	265	7	5	270	110	220
Carbowax	40	240	7	5	250	110	220

Data Analysis

Response surface regression analysis of Box-Behnken experimental data was performed using SAS 9.1 software program (Cary, NC). A 3-D Response surface plot was obtained using STATISTICA 6.0 (Tulsa, OK). The optimal conditions for pressure, temperature and CO_2 level were determined by considering the statistical significance ($p < 0.10$) of each variable on microbial reduction.

The significance of difference between treatment means for the storage study data (pH, %TA, ^{o}Brix, color (L*, a*, b*), sensory attributes) was determined by analysis of variance (ANOVA) using SAS 9.1 software (Cary, NC) at a significance level of $\alpha=0.05$. The means for each treatment were compared using Duncan's multiple comparison test ($\alpha=0.05$) to determine statistically different samples. Effects of storage time and interaction effects were also included in the ANOVA analysis.

CHAPTER 4
RESULTS AND DISCUSSION

Formulation of Coconut Water Beverage

Regulatory and consumer likeability aspects were considered in the formulation of the coconut water based beverage. FDA regulations regarding low acid foods require that action be taken to inhibit the growth of *C.botulinum*. Coconut water had a pH of around 5.0, and therefore, it must be lowered to below 4.6. Informal taste panels were conducted to decide on the suitability of different organic acids and commercially available pH lowering compounds. Malic acid was liked the most and was used to lower the pH to 4.3. Splenda (McNeil-PPC, Fort Washington, PA) was also added as a sweetener at about 0.7 % (w/w) to compensate for the resulting sourness. Preliminary tasting studies also showed that carbonated coconut water was preferred over non-carbonated. Therefore, coconut water beverage was formulated as a carbonated, acidified and sweetened beverage with a pH of 4.3 and ^{o}Brix of 6.0.

Objective 1: Quantification of Microbial Reduction in Coconut Water as a Function of Treatment Conditions

To quantify microbial reduction in coconut water as a function of DPCD treatment conditions, response surface methodology (RSM) was used. The number of experimental runs and the treatment conditions at each run were determined by using a 3-factor 3-level Box-Behnken experimental design. This design is one of the response surface designs that allows fitting of a quadratic model and has the advantage of requiring fewer number of runs compared to other response surface designs when three factors are used. The Box-

Behnken design suggests a sphere in the cubic process space where the surface of the sphere is tangential to the midpoints of the each edge of the cubic space (Figure 4-1). Center point experiments were replicated three times.

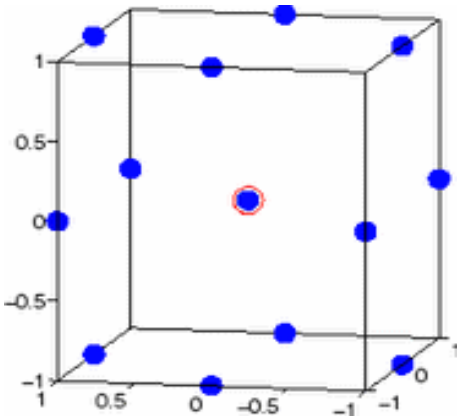


Figure 4-1. Geometry of the 3-factor 3-level Box-Behnken design

Three factors of this design that represented independent variables in the RSM model were X_1:Temperature (coded), X_2:Pressure (coded) and X_3: CO_2 level (coded). The dependent variable was Y: log microbial reduction. Coconut water that was thawed and formulated by acidification and sweetening was aged at room temperature (24^{o}C) to reach an initial load of 10^7cfu/mL. Next, 15 experimental runs that were determined by Box-Behnken design were conducted at the three levels of temperature, pressure and CO_2 levels (Table 3-1). Table 4.1 shows the experimental conditions of each run and the measured log reduction in total numbers of aerobic bacteria. The log reductions were calculated by subtracting final log numbers of bacteria from initial log numbers. Initial and final numbers of bacteria were determined by taking average cfu/mL counts on petrifilms with the cfu's less than 200 cfu/mL. The average initial and final aerobic plate counts (APC) ± standard deviations at each experimental condition are given in Table B-1.

Table 4-1. Log microbial reductions at each experimental point determined by Box-Behnken design

RUN#	X1	X2	X3	T (^oC)	P (MPa)	CO_2 level (g/100g juice)	Log microbial reduction experimental (A)	Log microbial reduction predicted (B)	Residual: (A-B)
1	-1	-1	0	20	13.8	10	4.92	4.90	0.02
2	1	-1	0	40	13.8	10	5.03	5.15	-0.12
3	-1	1	0	20	34.5	10	4.90	4.90	0.00
4	1	1	0	40	34.5	10	5.61	5.15	0.46
5	-1	0	-1	20	24.1	7	4.47	4.25	0.22
6	1	0	-1	40	24.1	7	5.40	5.34	0.06
7	-1	0	1	20	24.1	13	5.42	5.66	-0.24
8	1	0	1	40	24.1	13	4.66	5.06	-0.40
9	0	-1	-1	30	13.8	7	5.30	5.15	0.15
10	0	1	-1	30	34.5	7	4.71	5.15	0.56
11	0	-1	1	30	13.8	13	5.90	5.72	0.18
12	0	1	1	30	34.5	13	6.18	5.72	0.46
13	0	0	0	30	24.1	10	5.58	5.38	0.20
14	0	0	0	30	24.1	10	4.99	5.38	-0.39
15	0	0	0	30	24.1	10	5.22	5.38	-0.16

X1: Coded variable for Temperature (T); X2: Coded variable for Pressure (P); X3: Coded variable for CO_2 level

The RSM analysis of data was done using SAS 9.1 statistical software program (Cary, NC). First, the following quadratic model that included three variables X1, X2 and X3 was used and the RSM regression was conducted on the data:

$$Y= a+ b*X1 + c*X2 + d*X3 + e*X1*X1 + f*X2*X1+ g*X2*X2 + h*X3*X1 + i*X3*X2 + j*X3*X3$$

where Y: log microbial reduction, X1: Temperature (coded), X2: Pressure (coded), X3: CO_2 level (coded) and the letters from a to j represent corresponding coefficients for each parameter of this model. The SAS code and output of the analysis are given in Table B-2 and B-3, respectively. The regression coefficient R^2 was 0.76 for this model. Significance of each parameter was decided at α=0.1 level and the parameters with p value > 0.1 were

excluded from the model. Results showed that only the parameters X3 and X3*X1 were significant, therefore any parameter with X2 (Pressure) variable were excluded from the model. Similarly, Sims and Estigarribia (2002) reported that increasing CO_2 pressure from 7.5 to 15 MPa did not significantly increase microbial reduction.

Next, another RSM regression analysis was performed by using the modified model that involves only the parameters with variables X1 and X3:

$Y= a + b*X1 + c*X3 + d*X1*X3 + e*X1*X1 + f* X3*X3$

The SAS output of this analysis is given in Table B-4. The regression coefficient R^2 was 0.63 for the model. The model with the estimated coefficients gives the prediction of log microbial reduction (log red) as a function of temperature (coded) and CO_2 level (coded): log reduction = $5.381 + 0.124*Temp + 0.284*CO_2 – 0.355*Temp^2 - 0.423*CO_2*Temp + 0.05*CO_2^2$

Coefficients were determined for the coded values of each variable. The log reductions predicted at fifteen experimental runs using this equation are close to the experimental log reductions (Table 4-1). Three-dimensional plots of the response surface for this equation are given in Figure 4-2.

Apparently, there is not an optimum point on the surface plot at which ∂(log reduction) /∂(Temp)= 0 and ∂(log reduction/$\partial(CO_2)$=0 gives the highest microbial reduction. The surface plot shows that at lower- and mid-temperatures, microbial reduction increases as CO_2 level increases. However, at higher-temperatures this behavior changes, and either CO_2 level is not effective or causes a decrease in microbial reduction. The amount of dissolved CO_2 has a primary role in microbial reduction. CO_2 solubility is affected by temperature change and decreases as temperature increases (Dodds and others

1956). Therefore, increased CO_2 does not cause increased microbial reduction at higher temperatures due to its limited solubility. On the other hand, highest microbial reductions were achieved at temperatures close to middle temperature (i.e. temperatures around 25-30^oC) and highest CO_2 level (i.e. CO_2 levels around 13%). Therefore, the optimal conditions of DPCD treatment for microbial reduction in coconut water were selected to be 25^oC and 13% (g CO_2/100 g juice). Predicted log microbial reduction at these conditions is 5.77. Predicted log microbial reductions at different levels of temperature and CO_2 using the model can be found in CD file: "predicted log reductions.doc".

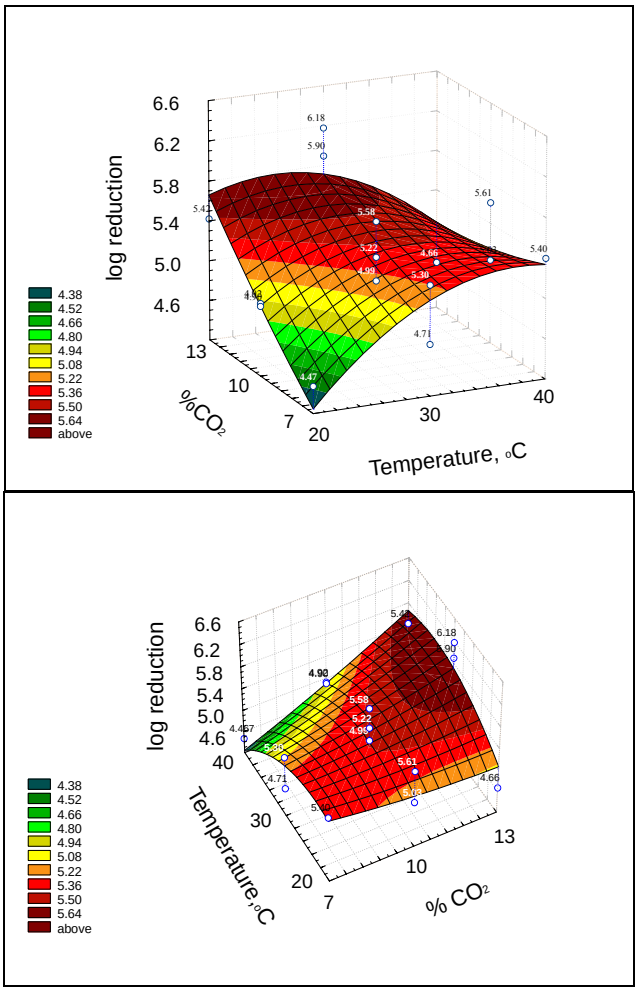


Figure 4-2. Plots of the response surface for the quadratic model with the variables X1: Temperature (coded) and X3: %CO_2 level (coded)

Objective 2: Evaluation of Physical, Chemical and Microbial Quality of DPCD Treated Coconut Water Beverage during Storage

The storage study was conducted at 4^{o}C for 9 weeks for "untreated", DPCD treated and heat treated coconut water beverage. Untreated samples were obtained by thawing the fresh frozen coconut water and formulating it by acidification, sweetening and carbonation. Heat- treated samples were pasteurized at 74^{o}C for 15 s after sweetening and acidification. DPCD treated coconut water was processed at the previously determined optimum conditions (Temp=25^{o}C, CO_2 level=13%) for microbial reduction after sweetening and acidification. The pressure was 34.5 MPa and treatment time was 6 min. Heat and DPCD treated samples were carbonated after treatments. Samples were tested for microbial growth, pH, titratable acidity, ^{o}Brix and color throughout storage.

Microbial quality of coconut water beverages was evaluated by measuring total aerobic bacteria (APC) and yeast and mold (YM) counts. The plot of APC results for each treatment during storage time are shown in Figure 4-3 and the data (cfu/mL) is given in Table D-1. One tail t-tests (α=0.05) were used to determine whether there was significant difference in APC and YM counts of each treatment between week 0 and week 9 (Table D-2). Data showed that number of aerobic bacteria in untreated coconut water stayed almost unchanged during the first 6 weeks but showed significant increase after week 6 and reached > 10^5 cfu/mL at the end of 9 weeks. There is only one data point after week 6 to show that increase, therefore further study would be useful to understand the extent of this increase between weeks 6 and 9. In addition, the comparison of carbonated coconut water with non-carbonated coconut water for microbial counts would help to understand if carbonation was the reason for no microbial increase during the first 6 weeks.

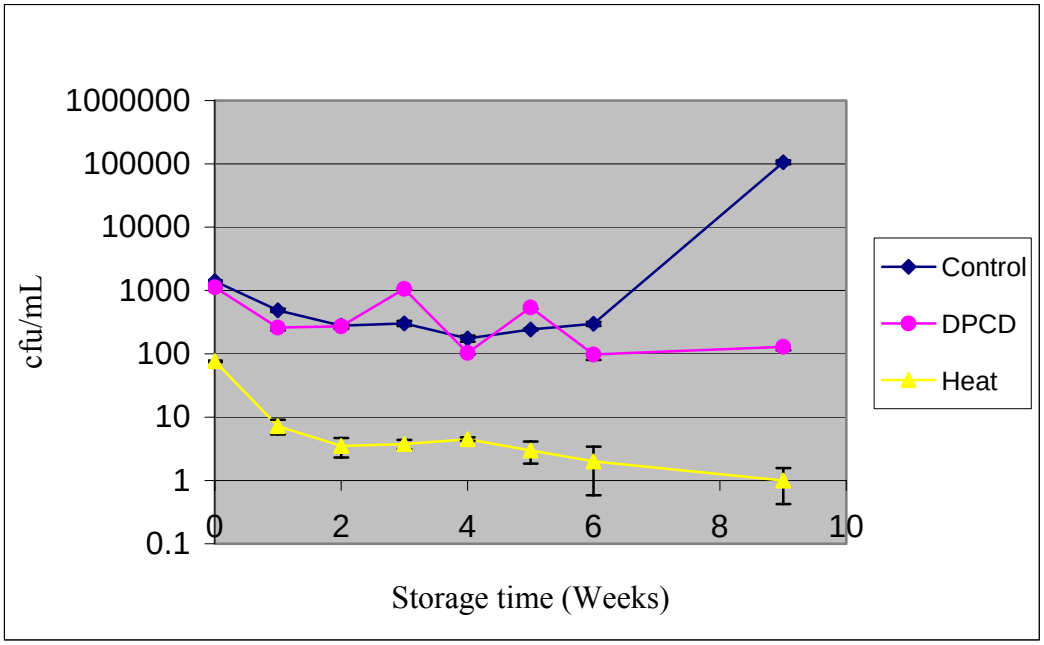


Figure 4-3. Total aerobic plate counts (APC) of untreated control, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa,13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

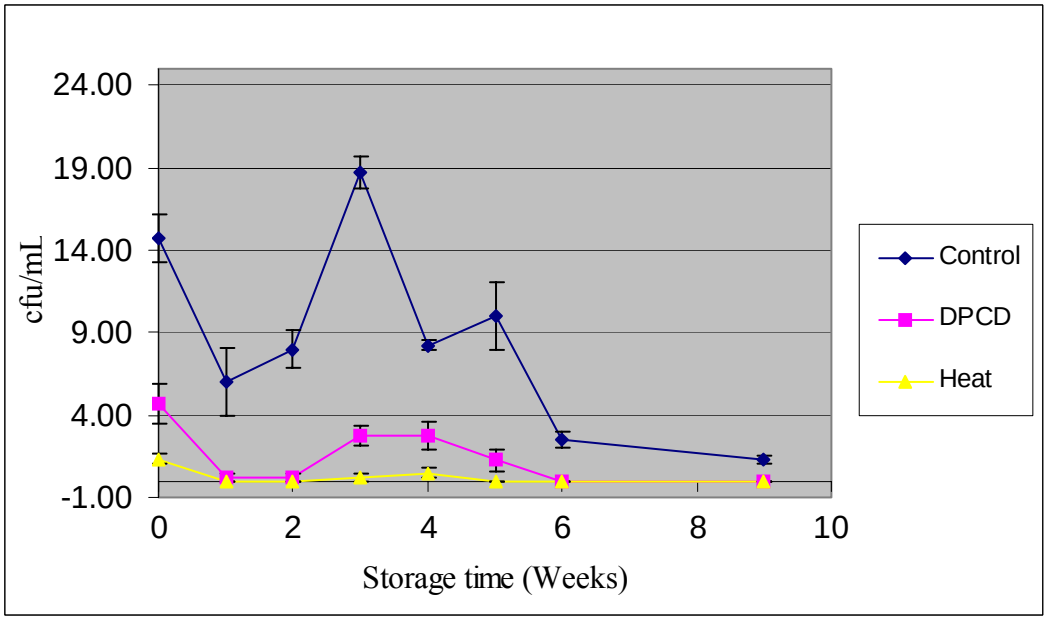


Figure 4-4. Yeast counts of untreated control, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

Numbers of aerobic bacteria decreased significantly in DPCD and heat treated samples. The lack of oxygen in the bottles caused by carbonation might have caused the

decrease in the microbial growth. It is important to note that untreated coconut water had initial microbial loads of around 3 logs, but DPCD or heat treatments unexpectedly did not cause total inactivation that must be achieved by pasteurization. In order to understand the real cause for the presence of microorganisms after treatments, every step of the process was reevaluated for the possibility of contamination. The carbonation process was a possible cause since this step is conducted in non-aseptic conditions. The carbonation process was repeated by using sterile water under similar conditions to coconut water, and the initial and final microbial counts of sterile distilled water showed that carbonation might cause contamination by up to 3 logs. The APC counts for distilled water before and after carbonation are given in Table D-3. Heat treated samples were apparently less contaminated than DPCD treated samples. The decrease in the aerobic bacteria growth from week 0 to 9 was 1 log in DPCD treated samples and approximately 2 logs in heat treated samples.

Yeast counts of all treatments were low throughout storage. There was no detectable mold growth while yeast counts were only around 1 log initially and decreased to no growth by the end of storage (Figure 4-4). Yeast counts for each treatment are given in Table D-4.

Measured pH values of untreated, DPCD treated and heat treated coconut water are given in Table D-5 and the plot of pH during storage is shown in Figure 4-5. Statistical analysis of pH data by analysis of variance (ANOVA) suggests a significant storage time and treatment interaction (Table D-6). DPCD treated samples had significantly lower pH than other treatments (α=0.05). However, the pH means of treatments are 4.199, 4.197 and 4.190 for heat treated, control and DPCD treated samples, respectively. Although

these values are statistically significantly different, they are exactly the same values for two significant figures, i.e. 4.20. It is suggested that the high accuracy of the pH meter in the triplicate measurements lowers the sum of squares for errors and causes this result. The pH of the samples did not change much during storage and was fluctuating around 4.20. Theoretically, a pH change was not expected during storage except for microbial problems. However, microbial data do not support such a decrease. The slight fluctuations in pH for the samples could be explained by sample-to-sample differences.

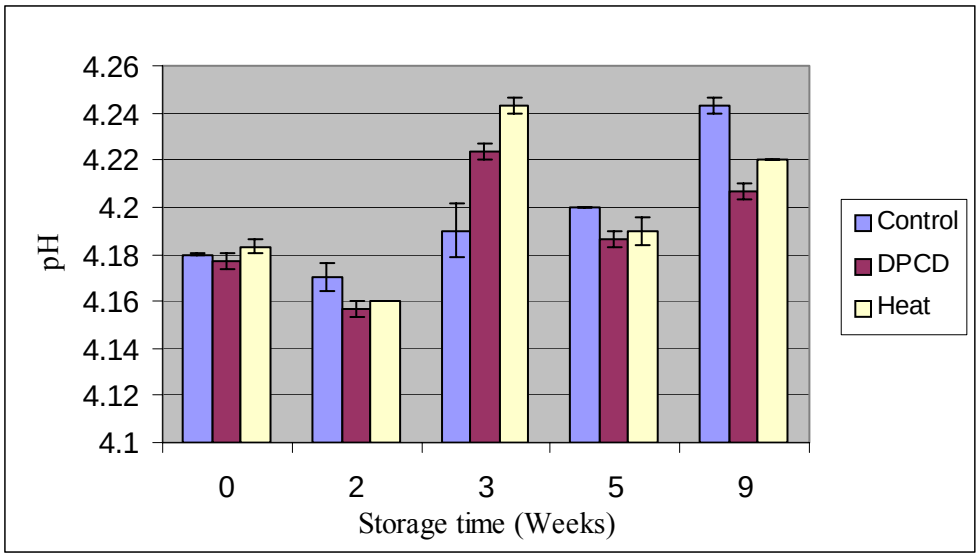


Figure 4-5. The pH of untreated, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

°Brix values for treatments during storage were close, and the maximum change in °Brix was 0.15 units. This could possibly be due to sample-to-sample variation. Mean °Brix values of treatments were 6.04, 6.0 and 6.0 for control, DPCD and heat treated coconut water, respectively. Theoretically, a change in °Brix of samples was not expected during storage unless there is evaporation or fermentation of the samples. Samples were tightly capped in glass champagne bottles and microbial data or pH data do not support such changes. Standard errors for °Brix measurements are zero, which indicates the high

repeatability of the measurements. Statistical analysis of data showed significant weekly changes and treatment differences since sum of squares of the error term is too low as a result of high repeatability in the measurements. The data is given in Table D-7 and the plot of the data during storage is given in Figure 4-6. The SAS output of ANOVA for ^{o}Brix data is shown in Table D-8.

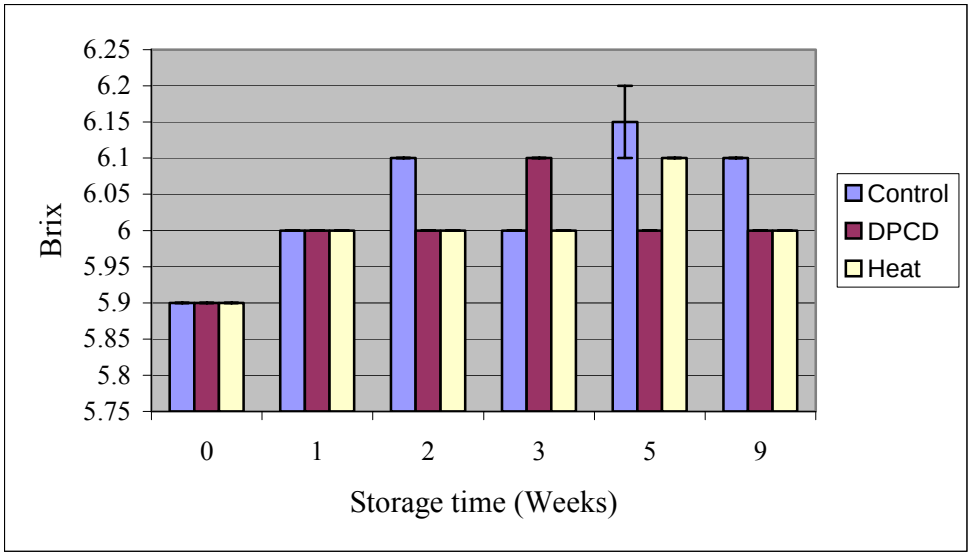


Figure 4-6. The ^{o}Brix of untreated, DPCD and heat treated coconut water during storage (DPCD treatment at 25^{o}C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74^{o}C for 15 s)

Titratable acidity for untreated, DPCD and heat treated coconut water were measured during storage and expressed as % malic acid (w/v) equivalents (Table D-9). Statistical analysis of data by ANOVA showed that DPCD treated samples had significantly higher titratable acidity (mean = 0.282 g malic acid / 100 mL coconut water) whereas untreated and heat treated samples had mean values of 0.259 and 0.266 g malic acid / 100 mL coconut water, respectively (Table D-10). The weekly mean % titratable acidity values for each treatment are given in Figure 4-7. The reason for higher overall titratable acidity of DPCD treated samples may be insufficient removal of CO_2 during vacuum treatment. DPCD treated samples were expected to have higher amounst of CO_2

due to the residual dissolved CO_2 after DPCD application. Increase in titratable acidity of juices by DPCD was observed also by studies of Kincal D. (2000) for orange juice and Lim and others (2006) for tangerine juice.

During storage, titratable acidity may change due to acid generation by microbial growth. However, there was no increasing trend in % TA for the samples during storage. The only treatment that shows a significant increase in microbial growth was the untreated control sample, but it did not show an increase in titratable acidity during the last week of storage. Although heat treated samples did not show a microbial increase, titratable acidity showed some fluctuations during storage. These could be due to the bottle-to-bottle variations during carbonation.

Color of coconut water samples was measured in CIE color scale as L*, a* and b* values and the data is shown in Table D-11. Data from week 0 were omitted because of measurement errors. The plots of L*, a* and b* values against storage weeks are presented in Figure 4-8, 4-9 and 4-10, respectively. The data shows slight changes in L*,a*,b* values for treatments during storage. L* values of the samples, representing lightness, decreased from week 0 to 5 and then increased slightly at week 9. The a* value, which represents redness on the positive scale and greenness on the negative scale, increased from week 2 to 5, then decreased for heat treated sample, and increased up to week 9 for the untreated control sample; whereas it increased from week 2 to 3 and then decreased for the DPCD treated sample. These results need to be considered with caution because some of the samples started pinking from the first day of storage. Color measurements were done on randomly selected bottles at each storage week. Therefore, there was large variation in redness for even the same treatment sample from one bottle

to another, depending on the initiation of pinking. From the preliminary experiments, it was known that once the coconut water in a bottle starts pinking, the intensity of pinking increased during storage. Normally, one would expect an increase in a* value for all treatments because independent of the treatment, all bottles eventually showed pinking during storage. The changes in L*, a* and b* values of the treatments could be due to bottle to bottle variations and it is not possible to make a clear conclusion based on this data.

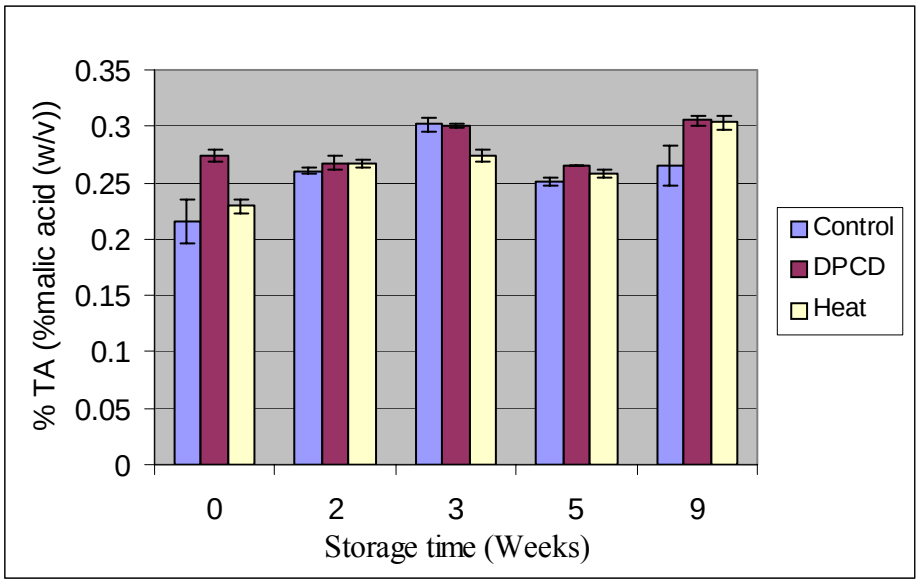


Figure 4-7. Titratable acidity (as % malic acid (w/v)) of untreated, DPCD treated and heat pasteurized samples during storage (DPCD treatment at 25^{o}C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74^{o}C for 15 s)

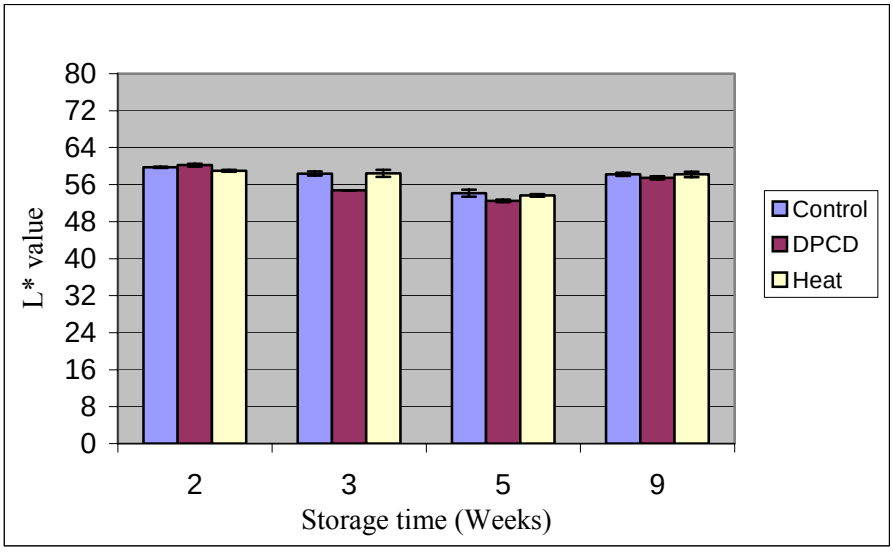


Figure 4-8. Mean L* values of untreated control, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

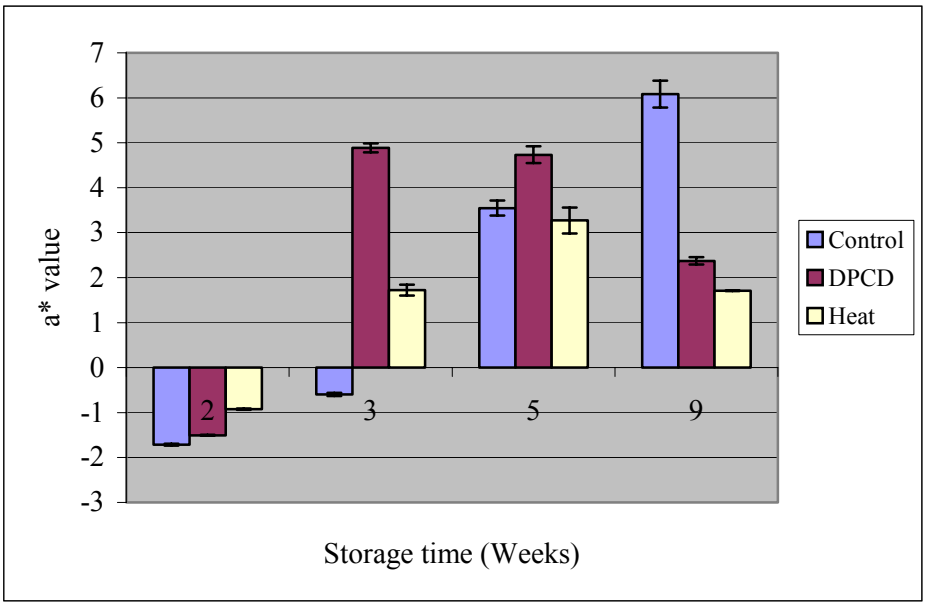


Figure 4-9. Mean a* values of untreated control, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

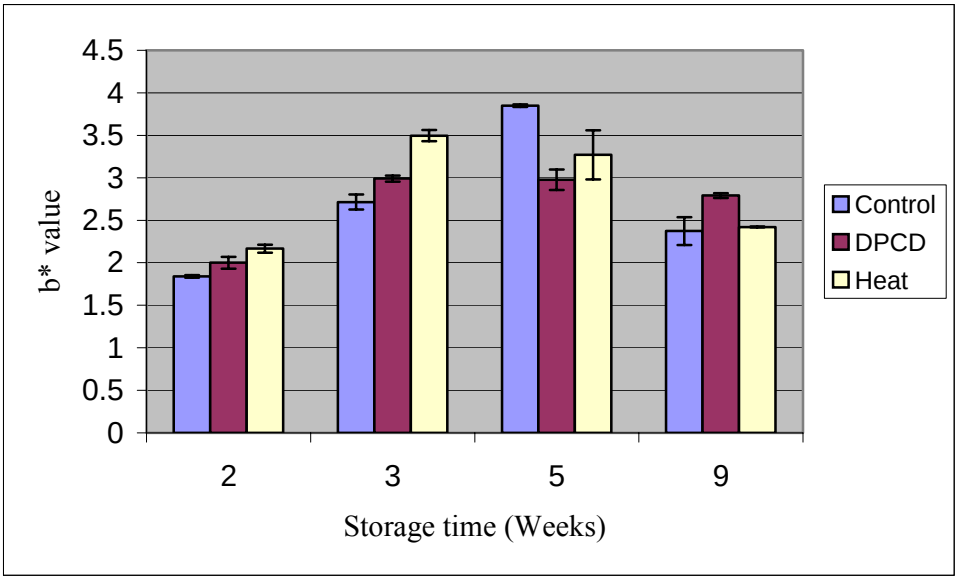


Figure 4-10. Mean b* values of untreated control, DPCD and heat treated coconut water during storage (DPCD treatment at 25°C, 34.5 MPa, 13% CO_2 for 6 min; Heat treatment at 74°C for 15 s)

Objective 3: Comparison of Untreated Control, DPCD and Heat Treated Coconut Water by Sensory Evaluation

Consumer panels of 50 untrained panelists were used to evaluate overall likeability, aroma, taste and off flavor of untreated (control), DPCD and heat treated coconut water beverage during storage at 4°C in glass champagne bottles. Panels were conducted at weeks 0, 2, 3, 5 and 9. The taste panel data during storage is presented in Table E-1.

Overall likeability of samples was rated on a 9 point scale where the score 1= dislike extremely, and 9= like extremely. ANOVA was conducted to see if there were significant differences in overall likeability of samples due to treatment or storage time effects. The SAS output of ANOVA is shown in Table E-2. The means of overall likeability scores for each treatment for the overall storage time shows that untreated control (mean=5.03^{a}) and DPCD treated sample (mean=4.95^{a}) were liked the most and heat treated sample (4.58^{b}) was liked significantly less than the other samples. Results showed that there was significant storage time-treatment interaction at α=0.05, therefore,

overall likeability of treatments was changing at different rates during storage time. For this reason, overall likeability of different treatments was compared separately at each storage time by ANOVA. The mean overall likeability scores and standard errors are given for treatments at each storage week in Table E-3. Figure 4-11 shows the comparison of each treatment for overall likeability scores at different storage weeks. Initially, DPCD treated and untreated samples were liked significantly more than heat treated sample. However, starting from the 2nd week, overall likeability of samples moved close to each other and this difference became insignificant. It is hard to explain the reason for this change. There could be some flavor and aroma change in the samples to cause a change in overall likeability scores. Since the samples were carbonated and stored in glass bottles, flavor change due to oxidization is not expected. From the previous studies, a change in the overall likeability due to storage time could be possible since microbial growth during storage could affect flavor and aroma. Kincal and others (2005) reported that microbial load increased in DPCD treated samples during storage. However, the results of microbial tests showed that there was no increase in the microbial counts of DPCD or heat treated samples. Therefore, the change in overall likeability should not be due to microbial changes.

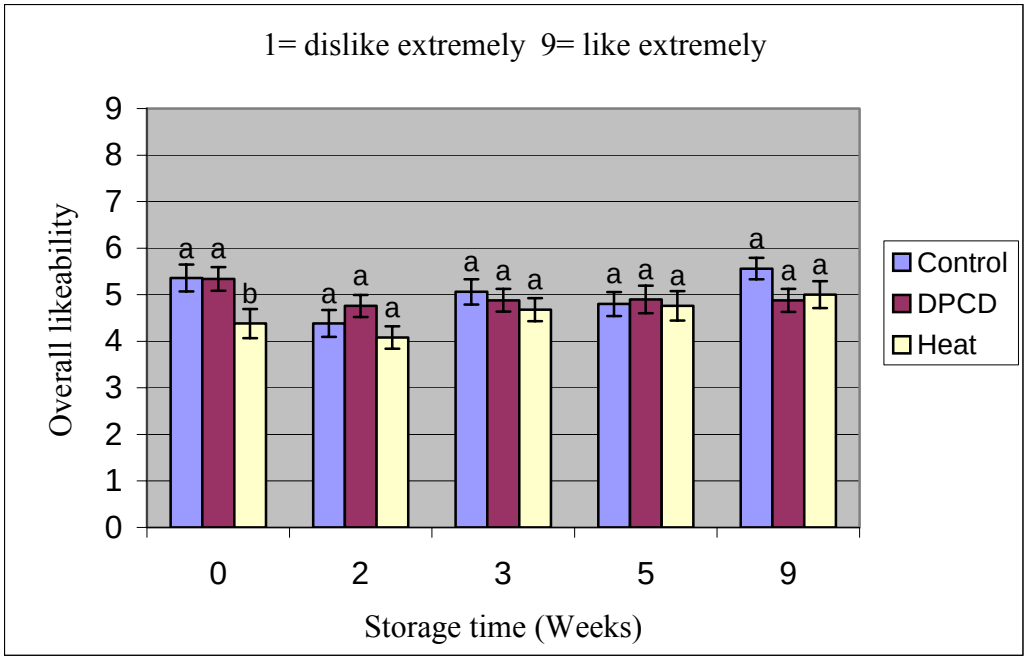


Figure 4-11. Comparison of overall likeability of each treatment during storage

A difference from control test was conducted to evaluate the taste and aroma of heat and DPCD treated samples. Fresh untreated coconut water was given to the panelists as a reference control every week, and panelists were asked to rate the difference in taste and aroma of three samples from this reference control. One of the samples was the same as the reference control. Ideally, the difference from reference control for the control sample should be rated as zero by the panelists since they are the same. Although most of the panelists rated this as close to zero, some panelists rated this difference as high as 10. The taste and aroma difference data for control samples were sorted from lowest to highest scores at each week and the frequency of each score was plotted as histograms. Figure 4-12 and Figure 4-13 show the histograms of aroma difference and taste difference scores, respectively, for the control samples. Scores greater than 6 for taste difference and greater than 5 for aroma difference from control were excluded from the

data, and the ANOVA was conducted on this new 'corrected' data. This correction was needed to exclude the effect of outlying panelists from the statistical analysis results.

Statistical analysis of corrected aroma difference from control data by ANOVA showed that DPCD and heat treated coconut water treatments were not significantly different in the overall mean aroma scores (Table E-4). Storage was not significantly affecting the aroma scores of the samples (α=0.05). The overall mean scores for aroma difference were 2.12^{a}, 1.92^{a} and 1.15^{b} for DPCD-treated, heat-treated and untreated control samples. The weekly comparison of treatments for the aroma scores shows that DPCD and heat treated samples were not rated significantly different for aroma (Figure 4-14). The mean aroma difference from control scores of the panelists at each week are given in Table E-5.

The SAS output of the ANOVA of the taste difference-from control data is in Table E-6. The overall mean values for taste difference scores were 2.08^{a}, 3.67^{b} and 4.17^{c} for the control, DPCD and heat treated coconut water, respectively. Treatments were significantly different for the taste scores. On the other hand, weekly ANOVA results showed that DPCD and heat treated samples were rated significantly different at week 0 only, and this difference was insignificant starting at week 2 until the end of storage (Figure 4-15). The mean taste difference-from control scores for panelists at each week are given in Table E-7. These results confirm the overall likeability of the samples throughout storage. Heat treated samples were rated significantly higher for the taste difference from control at week 0 and liked the least. The low intensity levels of flavor and aroma in coconut water may cause larger relative errors where comparing differently

treated samples, and mask the protective effects of DPCD compared to thermal treatments.

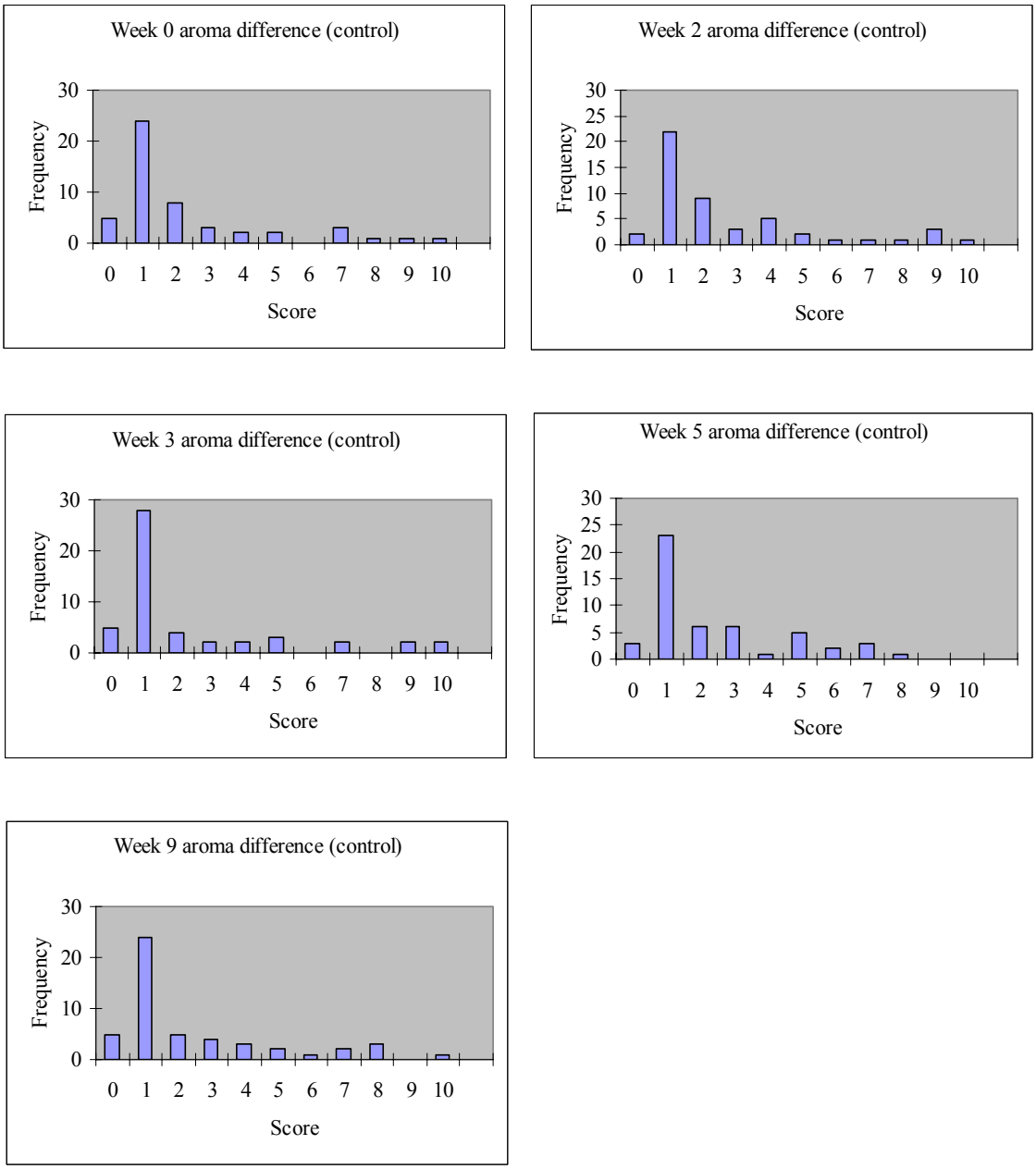


Figure 4-12.The frequency histograms of storage study aroma difference from control scores of untreated (control) samples

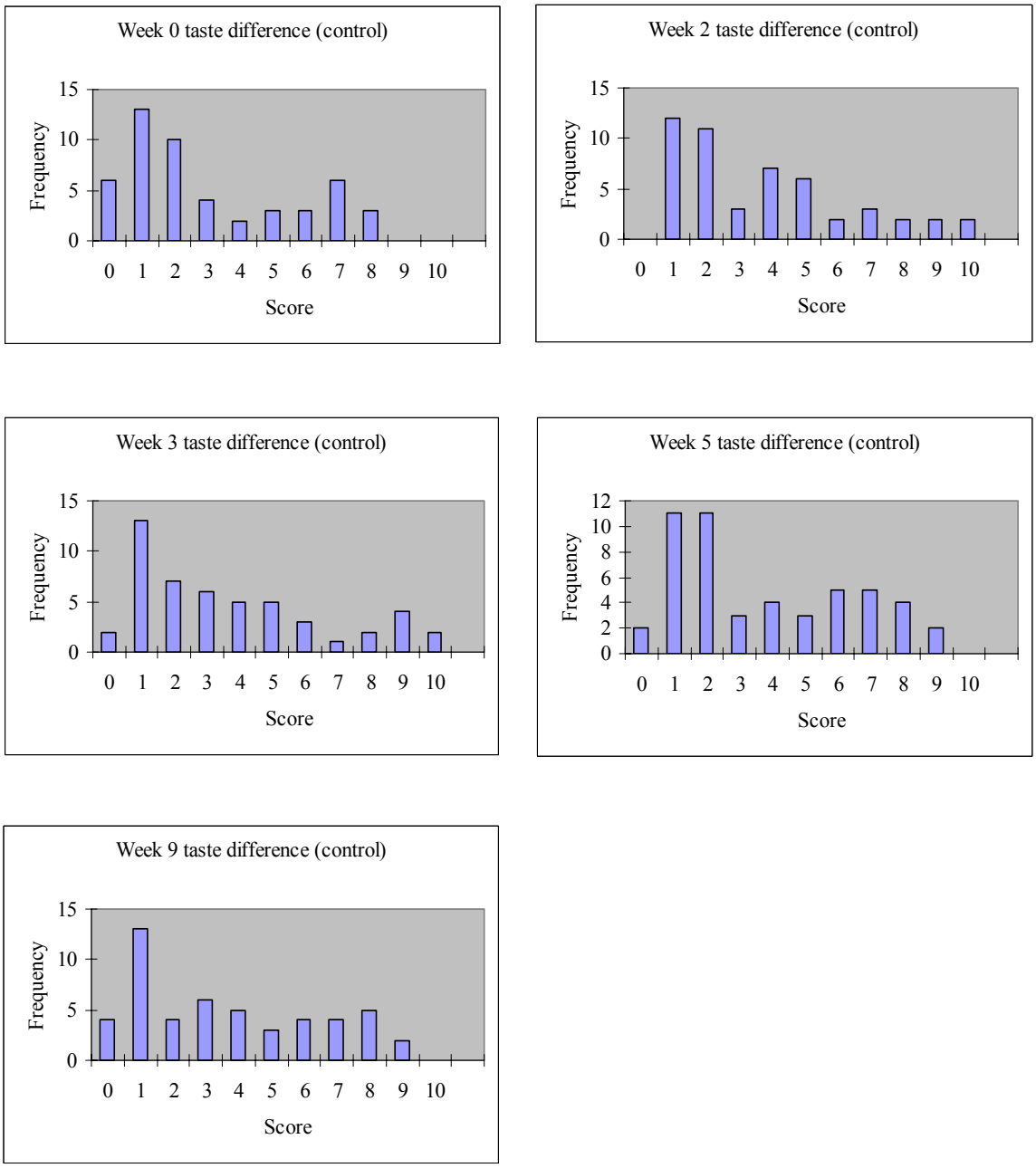


Figure 4-13.The frequency histograms of storage study taste difference from control scores of untreated (control) samples

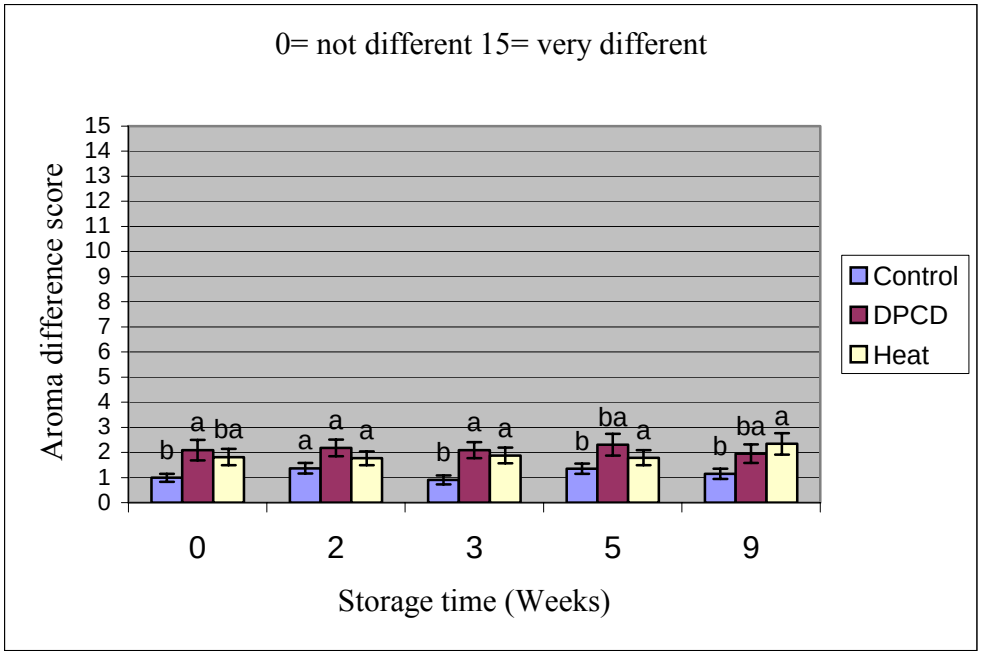


Figure 4-14. Comparison of treatments for aroma difference from control scores during storage

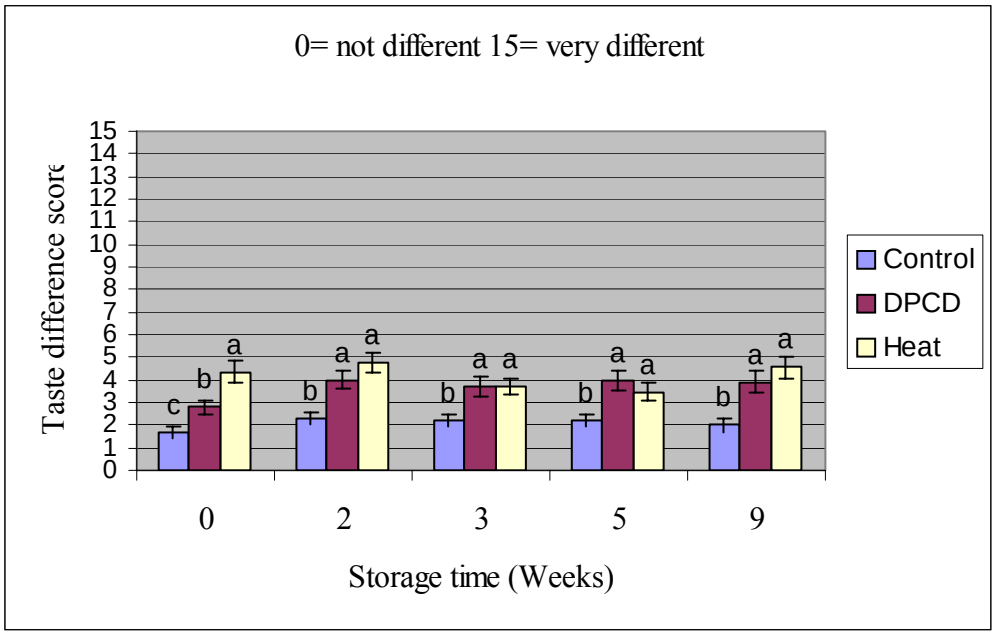


Figure 4-15. Comparison of treatments for taste difference from control scores during storage

Panelists were also asked to rate off flavor in the samples on a 6-point scale. The ANOVA of the data suggested that heat treated samples had significantly higher overall mean off-flavor scores (mean=2.99^{b}) than untreated (mean=2.68^{a}) and DPCD treated

(mean=2.66[a]) coconut water (Table E-8). Weekly comparison of the treatments for off flavor formation showed that significant difference between treatments was only occurring at weeks 0 and 2, and became insignificant starting from week 3 (Figure 4-16). The weekly mean off-flavor scores are in Table E-9. These results also confirm the overall likeability and taste scores for treatments. These results suggest that heat treated samples had some off flavor at the beginning of storage which caused a significantly higher rating for taste difference from untreated control and lowest rating for likeability of heated samples initially. However, in later weeks either this off flavor was masked by other flavors, or DPCD treated samples also developed off flavors and overall likeability or taste difference scores for treatments became closer.

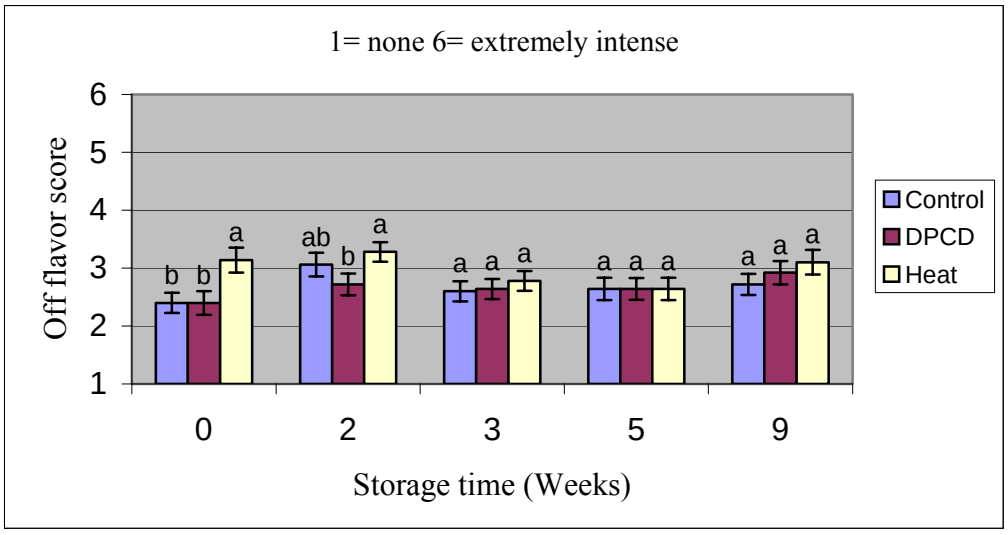


Figure 4-16. Comparison of treatments for off flavor scores during storage

Overall mean values and comparison of means for overall likeability, taste and aroma differences from control and off flavor scores for each treatment are summarized in Table 4-2.

Table 4-2. Comparison of overall mean values for sensory attributes from different treatments (α=0.05).

α=0.05	Overall Likeability*	Aroma difference from control*	Taste difference from control*	Off flavor*
Untreated	5.03[a]	1.15[a]	2.08[a]	2.68[a]
DPCD	4.95[a]	1.92[b]	3.67[b]	2.66[a]
Heat	4.58[b]	2.12[b]	4.17[c]	2.99[b]

*Different letters in a column mean no significant difference between means at □=0.05. (Mean values are averages of all weeks)

To evaluate purchasing potential of the DPCD treated coconut water beverage, panelists were asked if they would buy the product. The percentages of panelists answering "yes" to that question are given in Table 4-3 for each treatment at each storage week. The overall percentages of panelists who would purchase the products were 32.8% for untreated control, 34.8% for DPCD treated and 28% for heat pasteurized samples. Panelists who answered "no" were asked if they would buy that product if they knew about its rehydrating properties. Table 4-4 gives the percentages of the panelists who were still saying "no" to purchasing the products. Panelists who answered "no" to the first question and still answering "no" to the second question were 72.4% for control, 72.0% for DPCD and 76.4% for heat pasteurized coconut water. It seems that informing the panelists about the health benefits of coconut water could only slightly change their purchase intent.

Table 4-3. The percentages of panelists answering "yes" to the question: Would you buy that product?

Week	Control (% of panelists)	DPCD (% of panelists)	Heat (% of panelists)
0	34	38	22
2	32	30	16
3	38	36	32
5	26	40	34
9	34	30	36

Table 4-4. The percentages of panelists answering "no" the first purchase intent question and answering still "no" the second purchase intent question: Would you buy this product if you knew coconut water had rehydrating properties?

Week	Control (% of panelists)	DPCD (% of panelists)	Heat (% of panelists)
0	76	68	85
2	91	69	76
3	71	72	76
5	57	80	70
9	67	71	75

Objective 4: To Identify Flavor Compounds in Coconut Water and Compare Flavor Profile of DPCD and Heat Treated Coconut Water

Literature studies on coconut flavors are limited to fresh coconut meat, milk, or roasted and grated coconut meat (Lin and Wilkens 1970, Jayalekshmy and others 1991, Jirovetz and others 2003). Since there was no information on flavor compounds of young green coconut water in the literature, it was necessary to identify flavor compounds in untreated coconut water before comparison of flavor profiles in DPCD and heat treated samples. Flavor compounds were identified in untreated coconut water using GC/MS with the National Institute of Standards and Technology (NIST) library database and also some compounds were tentatively identified by matching LRI's obtained from GC/O Carbowax and DB-5 columns with those obtained from the literature databases. The flavor compounds that were identified in coconut water by GC/MS match and also by tentative match using GC/O are listed in Table 4-5. Studies with coconut show that δ-lactones give the characteristic coconut aroma, and also some esters, aldehydes and alcohols are among the flavor compounds in coconut meat or milk (Lin and Wilkens 1970, Jirovetz and others 2003). Although none of the δ-lactones were identified in this study from coconut water, some esters and aldehydes were identified. In order to have a better confirmation, standard chemicals of some of these suspected compounds were obtained and run in GC/MS. Corresponding GC/MS chromatograms are given in Figure

C-4. Each of the corresponding peaks were integrated and identified by the software, and their retention times, GC/MS degree of match values and calculated LRI's were recorded, and are given in Table C-3. GC/MS identification outputs that were obtained by NIST library match of each peak can be found in: CD file "feb2nd GCMS Standards 4 groups.xls". Coconut water samples were run by GC/MS at the same conditions as standards, and some flavor compounds were positively confirmed using a similar peak integration and identification procedure. The compounds that were positively confirmed in coconut water are shown in red color in Table 4-5 with the calculated LRI's for those observed in fresh coconut water (LRI Wax observed) and calculated LRI's of the standard chemicals (LRI Wax standard). Some of GC/MS chromatograms that were obtained by running fresh coconut water samples are given in Figure C-5, and the peak identification outputs obtained using NIST library matches can be found in: CD files "March 9th GCMS CW identification of peaks.xls" and "Feb1st GCMS CW identification of peaks.xls". Some of the flavor compounds were tentatively determined in fresh coconut water (Table 4-5). Two sniffers recorded the retention times and gave the aroma descriptors for sniffed compounds using the olfactory port of GC/O. This procedure was repeated twice in GC/O with Carbowax and DB-5 columns. LRI's of the sniffed compounds were calculated for each column. Literature flavor databases (Acree and Arn 2005, CREC 2005) provide LRI's of the chemical compounds in various GC/O columns with aroma descriptors. 1-Octene-3-one and 2,6-nonadienal had LRI's close to literature values in both columns and expected aroma descriptors by the sniffers (Table 4-5). Retention times, calculated LRI's and aroma descriptors given by sniffers in GC/O runs are given in Table C-4 for each column used. The raw data of FID and olfactory port

responses can be found in CD folders: “feb7th DB5 CW” and “feb 8th CW wax”. Methional is tentatively identified in coconut water by matching its observed LRI in Carbowax column with the literature LRI (Table 4-5), and because its boiled/cooked potato aroma described by sniffers (Table 4-6) is typical of that compound.

Table 4-5. The list of flavor compounds that were identified in untreated fresh coconut water

Compound	LRI Wax observed GC/MS / GC/O	LRI Wax standard/ literature	LRI DB-5 observed	LRI DB-5 literature	GC/MS degree of match (over 1000)	Tentative match
Ethyl butanoate	1049/1055	1048	797	800	826	
1-Butanol	1163	1154/1145	-----	675	893	
Octanal	1308/1311	1313/1302	-----	1002	864	
Octene-3-one,1	1316	1315	980	980	----------	Tentative
6-methyl,5-heptene-2-one	1355	1362	------	------	atomic spectrum matched	
Nonanal	1412	1419/1409	------	1107	873	
Ethyl octanoate	1450/1456	1451/1444	1196	1195	917	
Methional	1476	1478	------	913	------	Tentative
2,6-nonadienal	1575	1611	1152	1155	-------	Tentative
Undecanal	1630/1631	1630	------	1306	881	
Methyl dodecanoate	1819	1820	------	1509	873	
Octanoic acid	2077	2077/2047	------	1279	849	

Standard chemicals of GC/MS identified compounds were run in GC/O and sniffed by the sniffers in order to understand whether these flavor compounds were aroma active at certain concentrations, and also to be familiar with the possible aroma compounds in coconut water. Tables 4-6 and 4-7 give a list of the standard chemicals that were sniffed through GC/O with DB-5 and Carbowax columns, with the observed LRI’s, retention times and the aroma descriptors given by the sniffers. Some of these compounds were not aroma active at the given concentrations, but sniffers detected most of the standard

chemicals at 10 ppm and 100 ppm concentrations through DB-5 or Carbowax columns. Table 4-7 shows the experiment with standard chemicals that were run through Carbowax column. Coconut water was also run at the same experimental conditions and LRI's were calculated and aroma descriptors were recorded in order to see if any of the standards could be detected. Among these compounds, 6-methyl-5-heptene-2-one and nonanal were aroma active at 100 ppm and 10 ppm concentrations, respectively; however, they were not detected by sniffers. Since these two compounds were detected in coconut water by GC/MS identification, these results suggest that the concentrations of 6-methyl-5-heptene-2-one and nonanal were lower than 100 ppm and 10 ppm, respectively. On the other hand, methyl dodecanoate and octanoic acid were not aroma active at 100 ppm and 10 ppm concentrations, respectively, and were not detected by sniffers. Therefore, although some flavor compounds were detected in coconut water, concentrations were not high enough to be detected by sniffers. Table 4-8 summarizes the list of the detected flavor compounds in coconut water and the aroma descriptors given to them. The raw data with FID and olfactory responses corresponding to the Tables 4-6 and 4-7 can be found in CD folders: "Table 4-6 raw data Jan 25th", "Table 4-7 raw data March 18th".

Aroma profiles of DPCD and heat treated carbonated coconut water beverages were developed by sniffing each sample twice by two sniffers in GC/O olfactory port using a polar Carbowax column. C5-C20 alkane standards were used to calculate LRI values of the sniffed compounds. Aromagrams of the samples were constructed by taking average peak areas of the sniffed compounds in the olfactory port and the corresponding aroma descriptors given by sniffers. Only the compounds that were sniffed at least twice during four sniffs were reported in the aromagrams. The retention times, calculated

LRI's, peak areas of each olfactory response and average peak areas are given with the corresponding aroma descriptors in Table C-5 and C-6 for DPCD (25^{o}C ,34.5 MPa, 13% CO_2, 6 min) and heat (74^{o}C, 15 s) treated samples, respectively. The raw data from GC-O runs can be found in CD file: "March22nd GCO carbonated CW DPCD and heat.xls". Figure 4-17 gives the comparison of the aromagrams for DPCD (25^{o}C, 34.5 MPa, 13%CO_2, 6 min) and heat treated (74^{o}C, 15 s) carbonated coconut water beverages stored at 4^{o}C for 2 weeks. Results showed that most of the aroma compounds were common in DPCD and heat treated coconut water beverages. However, a few more compounds were sniffed in heat treated samples. GC/MS chromatograms of coconut water samples also show more peaks detected in heat treated samples compared to DPCD treated coconut water (Figure C-6). Additional aroma compounds in heat treated samples were described as fruity, green, nutty, rancid, unpleasant, fatty and burnt aromas. These aromas were probably developed by decomposition of compounds due to heating.

Table 4-6. Standard chemicals (10 ppm of each in a mixture) that were run in GC/O with DB-5 column

Compound name	DB-5 Literature LRI	LRI observed (DB-5)	Rt (min) (DB-5)	Aroma descriptor by sniffer	Aroma descriptor from literature
Propanol	536	------	------	Not sniffed	Alcohol, pungent
Ethanol	668	------	------	Not sniffed	Sweet
Butanol	675	------	------	Not sniffed	Medicine, fruit
Octanal	1006	1004	11.30	Soapy, fruity	Fat, soap, lemon, green
Nonanal	1107	1106	13.53	Butter, chemical,soap	Piney, floral, citrusy
Nonanol	1154	------	------	Not sniffed	Fat, green
Ethyl octanoate	1195	1196	15.44	Sweet, rose	Fruity, fat, floral
Nonanoic acid	1275	1271	16.97	Liquid soap	Green, fat
Octanoic acid	1279	------	------	Not sniffed	Sweat, cheese
Undecanal	1291	1295	17.44	Old leather	Oil, pungent, sweet

Table 4-6. Continued

Compound name	DB-5 Literature LRI	LRI observed (DB-5)	Rt (min) (DB-5)	Aroma descriptor by sniffer	Aroma descriptor from literature
Gamma-nonalactone	1366	1358	18.66	Sweet, candy	Coconut, peach
Delta-decalactone	1469	1463	20.62	Fruity, bubble-gum	Peach
Methyl dodecanoate	1509	------	------	Not sniffed	Fat, coconut
Carvacrol	------	------	------	Not sniffed	------
2-ethyl-1-hexanol	------	------	------	Not sniffed	------

Table 4-7. Standard chemicals (100 ppm each in a mixture) that were run in GC/O with Carbowax column

Chemical Name	LRI observed (Carbowax)	LRI literature (Carbowax)	Rt (min)	Aroma descriptor by sniffer
Methyl dodecanoate	1813	1795	20.98	No odor
6-methyl-5-heptene-2-one	1362	-----	13.08	Green, chemical
Octanal	1311	1302	12.12	Fatty, green, rancid
Ethyl butanoate	1058	1048	7.29	Fruity, sweet, bubblegum
Ethyl octanoate	1449	1444	14.73	Sweet
Undecanal	1624		17.88	Green

Table 4-8. The descriptors given by sniffers for the flavor compounds identified in coconut water

Compound	Descriptors from sniffers
Ethyl butanoate	Sweet, apple, candy, fruity
Octanal	Green, fatty, rancid
Octene-3-one,1	Mushroom, dirt
6-methyl, 5-heptene-2-one	Aroma active at 100 ppm concentration, but not sniffed in coconut water
Nonanal	Aroma active at 10 ppm concentration, but not sniffed in coconut water
Ethyl octanoate	Sweet, cotton-candy
Methional	Boiled/ cooked potato
2,6-nonadienal	Green, almond, woody
Undecanal	Woody, rancid, soapy, nutty
Methyl dodecanoate	Not aroma active at 100 ppm concentration, and not sniffed in coconut water
Octanoic acid	Not aroma active at 10 ppm concentration, and not sniffed in coconut water

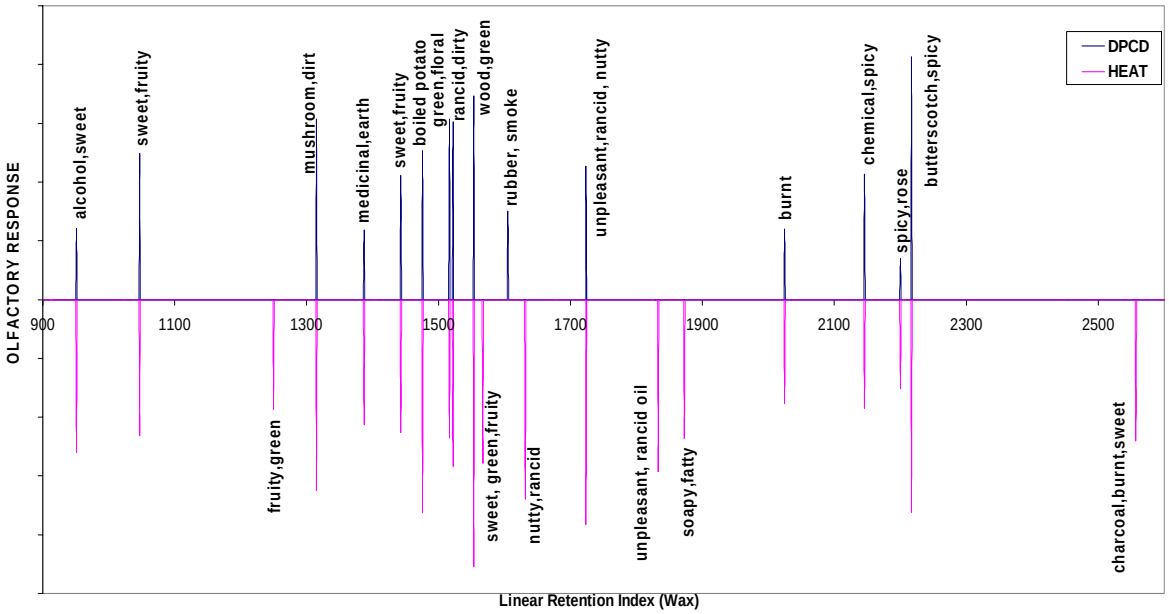


Figure 4-17. Comparison of aromagrams of DPCD (25°C, 34.5 MPa, 13% CO_2, 6 min) and heat (74°C, 15 s) treated carbonated coconut water beverages obtained from olfactory port responses (2 weeks storage at 4°C).

CHAPTER 5
CONCLUSIONS

This study involved the formulation of a coconut water beverage, cold pasteurization of this beverage with dense phase CO_2 (DPCD) technology, evaluation of physical, chemical, microbial and sensory quality of DPCD pasteurized coconut water compared to fresh and heat pasteurized samples, and optimization of DPCD treatment conditions for microbial reduction in coconut water.

By considering regulatory and sensory aspects, coconut water needed to be acidified, sweetened and carbonated. It was acidified with malic acid to a pH around 4.30, sweetened by Splenda (McNeil-PPC, Fort Washington, PA) at a level of 0.7% (w/w), having a ^{o}Brix of 6.0, and carbonated at 4^{o}C and 184 KPa pressure.

The first objective was to quantify microbial reduction in coconut water as a function of treatment conditions. The response surface methodology (RSM) analysis of microbial reduction data showed that pressure did not have a significant effect in microbial reduction and the microbial reduction was predicted as a function of temperature and CO_2 level by the quadratic equation:

log microbial reduction = $5.381 + 0.124*Temp + 0.284*CO_2 – 0.355*Temp^2 - 0.423*CO_2*Temp + 0.05*CO_2^2$ (coefficients were calculated for coded values of CO_2 level (CO_2) and temperature (Temp)).

The response surface did not give an optimum point where the ∂(log reduction)/∂(Temp)= 0 and ∂(log reduction/∂(CO_2)=0 gives the highest microbial reduction. The response surface plot suggested higher microbial reductions at mid-

temperatures and higher CO_2 levels. Therefore, the optimum conditions of DPCD treatment for microbial reduction were determined as 25^{o}C, 34.5 MPa and 13% CO_2 (g CO_2/100 g juice) with a 6 min treatment time, which causes 5.77 log reduction in total aerobic bacterial count.

The second objective was to evaluate physical, chemical and microbial quality of DPCD treated coconut water during storage. The quality attributes such as pH, ^{o}Brix, titratable acidity, color, aerobic bacteria and yeast counts for DPCD treated coconut water were measured during 9 weeks of refrigerated storage and compared to those of untreated control and heat pasteurized samples. Aerobic bacteria and yeast counts for untreated coconut water increased significantly at the end of 9 weeks, and the aerobic bacteria count reached above 10^5cfu/mL, became cloudy, and developed off odors indicating end of shelf-life. On the other hand, the aerobic bacteria counts and yeast counts for DPCD and heat treated coconut water decreased significantly at the end of 9 weeks. Carbonation process was shown to be a possible cause for contamination in DPCD and heat pasteurized samples. The pH and ^{o}Brix of all samples stayed around 4.20 and 6.0, respectively, throughout storage. Titratable acidity of DPCD treated samples was significantly higher than fresh and heat pasteurized samples, possibly because of the dissolved CO_2 remaining in coconut water from DPCD treatment. All samples eventually turned pink during refrigerated storage, independent of the type of treatment. The preliminary studies on pinking suggested that heating and aeration might accelerate pinking. Further studies are needed to elaborate the cause of pinking.

The third objective was to compare untreated control, DPCD and heat treated coconut water by sensory evaluation. Untrained panelists evaluated coconut water

samples at weeks 0, 2, 3, 5 and 9 for overall likeability, taste, aroma, off flavor and purchase intent. DPCD treated and fresh coconut water samples were liked similarly whereas heat pasteurized coconut water was significantly less liked at the beginning of storage. DPCD and heat pasteurized samples were not significantly different for aroma difference from control scores. On the other hand, taste difference from control scores for DPCD and heat pasteurized samples were significantly different initially and became similar beginning from 2nd week. Heat pasteurized samples had significantly higher off flavor scores than DPCD treated samples during the first two weeks.

The fourth objective was to identify flavor compounds in coconut water and compare flavor profiles of DPCD and heat treated coconut water. Flavor compounds such as esters (ethyl butanoate, ethyl octanoate), aldehydes (octanal, undecanal, 2,6-nonadienal) and others were identified in young green coconut water. The aroma profiles of DPCD and heat treated coconut water beverages showed that heat treated coconut water had more aroma active compounds than the DPCD treated coconut water. These were probably created by thermal decomposition during heat treatment and were described as unpleasant, fatty, green and burnt aroma by sniffers.

This study showed that DPCD treatment extended shelf-life of coconut water beverage that was acidified, sweetened and carbonated, and the sensory quality of DPCD treated coconut water was better than heat pasteurized coconut water during the first two weeks.

As a recommendation for future studies, it would be useful to investigate the mechanisms and causes of pinking in coconut water so that the means of prevention could be elaborated. Further studies on sensory evaluation with trained panelists are

recommended for better description of the aroma differences between different treatments. Further studies on instrumental analysis of flavors are also recommended for more detailed identification of the aroma active compounds.

APPENDIX A
RESULTS OF PRELIMINARY TESTS WITH COCONUT WATER

Table A-1. Initial aerobic plate count (APC) and yeast and mold (YM) counts for coconut water from eight immature green coconuts

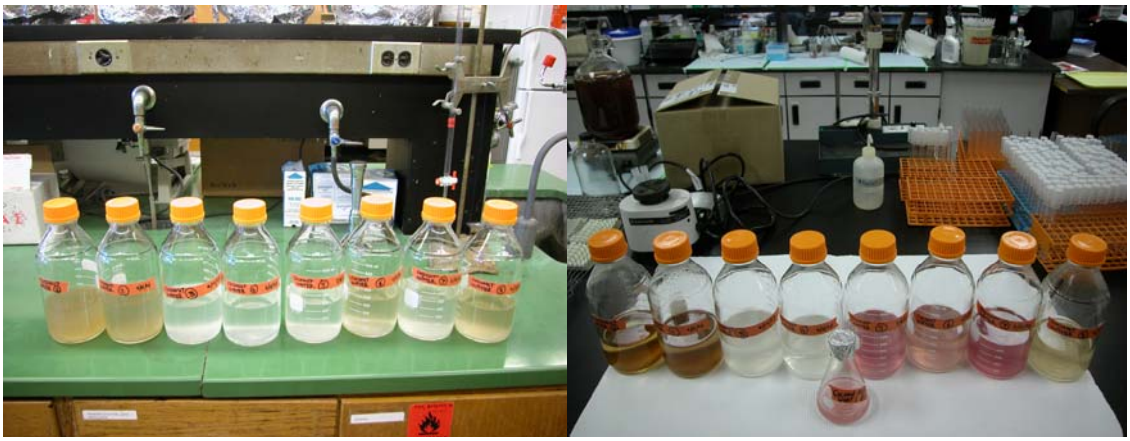
Coconut #	1	2	3	4	5	6	7	8
APC	0-4	51-63	TNTC[a] TNTC	TNTC TNTC	170-184	182-179	139-137	181-197
YM[c]	NG[b]	NG	NG	NG	NG	NG	NG	NG

[a] too numerous to count ; [b] no growth; [c] numbers in red color indicate mold growth (if there is any)

Table A-2. Day 9 aerobic plate count (APC) and yeast and mold (YM) counts for coconut water from selected coconuts of eight immature green coconuts

	Dilution #	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Coconut # 1	APC	74-60 69-65	7	0-2 1-1	0-0 0-0	0-0 0-1	0-0 0-1
	YM[a]	9-8 2-10	1-0 3-3	0-0 0-0	0-0 0-0	0-0 0-0	
Coconut # 3	APC		TNTC[b] TNTC	TNTC TNTC	135-TNTC	46-56 58-59	1-3 5-0
	YM	146-164 172-173	39-38 28-21	2-1 1-2	0-0 0-0	0-0 0-0	
Coconut # 5	APC	TNTC TNTC	186-175 245-175	20-27 27-47	4-5 2-1	0-0 0-0	0-0 0-0
	YM	69-91	4-0 12-0	3-2 0-0	0-0 0-0	0-0 0-0	

[a] numbers in red color indicate mold growth (if there is any); [b]too numerous to count



(Day 0) (Day 9)

Figure A-1. Pictures of coconut water from eight immature green coconuts at day 0 (left) and day 9 (right)

Table A-3. Preliminary pinking test 1: Visual observation of the color of coconut water after different treatments during storage at 4°C in glass tubes

Observation Time:	Control (1)**			Frozen/ Thawed (2)**			Heated (open air) (85°C; 5 min) (3)**			Heated (closed) (85°C; 5 min) (4)**			N_2 bubbled for 15 min (5)**		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Day 0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Day 4	C	C	C	C	C	C	P	P	P	C	C	C	P	P	C
Day 7	*			*						*					
Day 9	P	C	C	P	C	C	P	P	P	P	C	C	P	P	C
Day 12	P	C	C	P	C	C	P	P	P	P	C	C	P	P	C

*On day 7, marked tubes were aerated for 15 min; **numbers in parentheses imply treatment numbers referring to the text; T1,T2,T3 indicates three tube replicates; C: Clear color; P: Pink color.

Table A-4. Preliminary pinking test 2: Visual observation of the color of coconut water after different treatments during storage at 4°C in opaque plastic cups

Observation Time:	Control (1)*		Ascorbic acid added (100ppm) (2)*		Potassium metabisulfite added(40ppm) (3)*		pH=4.0 (by 0.1N HCl) (4)*		pH=3.0 (by 0.1N HCl) (5)*	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
Day 0 to 11	C	C	C	C	C	C	C	C	C	C
Day 12	P	P	C	C	C	C	C	C	C	C
3 months	P	P	C	C	C	C	P	P	P	P

C1: Cup 1; C2: Cup 2; C: Clear color; P: Pink color; *numbers in parentheses imply treatment numbers referring to the text

Table A-5. Preliminary pinking test 3: Visual color observation of untreated, heat treated or aerated coconut water during storage in glass tubes at 4°C.

Observation Time:	Control (1)*			Heated at 85°C for 5 min (2)*			Boiled for 5 min (3)*			Aerated for 15 min (4)*		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Day 0	C	C	C	C	C	C	C	C	C	C	C	C
Day 6	C	C	C	P	C	C	P	P	P	P	P	C
Day 10	C	C	C	P	P	P	P	P	P	P	P	P

*Numbers in parentheses imply treatment numbers referring to the text; T1, T2, T3 indicates three tube replicates; C: Clear color; P: Pink color

Table A-6. The pH, °Brix and ingredients of commercially available coconut water beverages

Brand Name	pH	°Brix	Ingredients (from Label)
Conchita	5.15	9.5	Immature coconut juice, sugar, coconut meat, potassium metabisulfite
La Fe	5.16	8.9	80% immature coconut juice, water, coconut pulp, sugar, citric acid, sodium metabisulfite
Coco Rico	4.12	10.8	Carbonated water, HFCS, coconut extract, sodium benzoate
Goya	5.07	8.9	Immature coconut juice, sugar, coconut pulp, water,citric acid, potassium metabisulfite
KeroCoco	4.89	5.6	Natural coconut water, preservative INS 223 (Sodium metabisulfite)
Grace	4.85	5.9	Coconut water

Figure A-2. Pictures showing the steps of extraction of coconut water from coconuts

APPENDIX B
BOX-BEHNKEN EXPERIMENTAL DESIGN, DATA AND ANALYSIS

Table B-1. The average initial and final aerobic plate counts (APC) ± standard deviations at 15 experimental runs from 3-factor, 3-level Box-Behnken experimental design

Temperature (^oC)	Pressure (MPa)	CO_2 level (g CO_2 / 100 g juice)	Initial APC (cfu/mL)*	Final APC (cfu/mL)*
20	13.8	10	(1.26E+07)± (5.06E+05)	151±17
40	13.8	10	(1.83E+07)± (9.95E+05)	170±10
20	34.5	10	(1.26E+07)± (5.06E+05)	163±24
40	34.5	10	(1.83E+07)± (9.95E+05)	45±10
20	24.1	7	(1.26E+07)± (5.06E+05)	430±16
40	24.1	7	(1.83E+07)± (9.95E+05)	73±7
20	24.1	13	(1.26E+07)± (5.06E+05)	48±12
40	24.1	13	(1.83E+07)± (9.95E+05)	403±93
30	13.8	7	(4.80E+07)± (8.16E+06)	231±51
30	34.5	7	(4.80E+07)± (8.16E+06)	933±115
30	13.8	13	(4.80E+07)± (8.16E+06)	62±19
30	34.5	13	(4.80E+07)± (8.16E+06)	32±4
30	24.1	10	(4.80E+07)± (8.16E+06)	127±4
30	24.1	10	(1.26E+07)± (5.06E+05)	130±23
30	24.1	10	(1.83E+07)± (9.95E+05)	111±13

*Averages of the plates with APC counts lower than 200 colony forming units (cfu's)

Table B-2. SAS software code used for the response surface methodology (RSM) analysis of 15 experimental runs determined by Box-Behnken experimental design

```
data coconut;
input RUN X1 X2 X3 TEMP PRESSURE PCTCO2 LOGRED;
datalines;
1     -1    -1    0     20    2000  10    4.924279286
2     1     -1    0     40    2000  10    5.032002168
3     -1    1     0     20    5000  10    4.896250562
4     1     1     0     40    5000  10    5.609238576
5     -1    0     -1    20    3500  7     4.46690209
6     1     0     -1    40    3500  7     5.39912823
7     -1    0     1     20    3500  13    5.419129308
8     1     0     1     40    3500  13    4.660391098
9     0     -1    -1    30    2000  7     5.297425871
10    0     1     -1    30    5000  7     4.712758289
11    0     -1    1     30    2000  13    5.895911402
12    0     1     1     30    5000  13    6.176091259
13    0     0     0     30    3500  10    5.577437516
14    0     0     0     30    3500  10    4.986427193
15    0     0     0     30    3500  10    5.221058405
;
proc print data=coconut;
run;
proc rsreg data=coconut;
model logred = x1 x2 x3;
run;
```

Table B-3. SAS software output of the response surface methodology (RSM) regression analysis of 15 experimental-run data determined by Box-Behnken experimental design including variables X1 (coded variable for Temperature), X2 (coded variable for Pressure) and X3 (coded variable for $\%CO_2$ level)

```
                          The RSREG Procedure
              Coding Coefficients for the Independent Variables
                   Factor    Subtracted off      Divided by
                   X1                     0        1.000000
                   X2                     0        1.000000
                   X3                     0        1.000000

                     Response Surface for Variable LOGRED
                   Response Mean                  5.218295
                   Root MSE                       0.386683
                   R-Square                         0.7625
                   Coefficient of Variation         7.4101
                              Type I Sum
      Regression          DF      of Squares    R-Square    F Value    Pr > F
      Linear               3        0.778169      0.2472       1.73    0.2752
      Quadratic            3        0.628499      0.1997       1.40    0.3452
      Crossproduct         3        0.993417      0.3156       2.21    0.2045
      Total Model          9        2.400085      0.7625       1.78    0.2716
                                         Sum of
               Residual           DF         Squares     Mean Square
               Total Error         5        0.747618        0.149524
```

Table B-3 Continued

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|	Parameter estimate
Intercept	1	5.261641	0.223251	23.57	<.0001	5.261641
X1	1	0.124275	0.136713	0.91	0.4050	0.124275
X2	1	0.030590	0.136713	0.22	0.8318	0.030590
X3	1	0.284414	0.136713	2.08	0.0920	0.284414
X1*X1	1	-0.340179	0.201236	-1.69	0.1517	-0.340179
X2*X1	1	0.151316	0.193341	0.78	0.4693	0.151316
X2*X2	1	0.193980	0.201236	0.96	0.3793	0.193980
X3*X1	1	-0.422741	0.193341	-2.19	0.0805	-0.422741
X3*X2	1	0.216212	0.193341	1.12	0.3143	0.216212
X3*X3	1	0.064925	0.201236	0.32	0.7600	0.064925

Canonical Analysis of Response Surface Based on Coded Data

Critical Value		
Factor	Coded	Uncoded
X1	0.517443	0.517443
X2	0.016346	0.016346
X3	-0.532945	-0.532945

Predicted value at stationary point: 5.218255

	Eigenvectors		
Eigenvalues	X1	X2	X3
0.258999	-0.093844	0.822796	0.560535
0.109837	0.430314	0.541235	-0.722423
-0.450109	0.897788	-0.173411	0.404852

Stationary point is a saddle point.

Table B-4. SAS software output of the response surface methodology (RSM) regression analysis of 15 experimental-run data determined by Box-Behnken experimental design including variables X1 (coded variable for Temperature) and X3 (coded variable for $\%CO_2$ level)

The RSREG Procedure

Coding Coefficients for the Independent Variables

Factor	Subtracted off	Divided by
X1	0	1.000000
X3	0	1.000000

Response Surface for Variable LOGRED

Response Mean	5.218295
Root MSE	0.360958
R-Square	0.6275
Coefficient of Variation	6.9172

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	2	0.770683	0.2448	2.96	0.1030
Quadratic	2	0.489564	0.1555	1.88	0.2080
Crossproduct	1	0.714840	0.2271	5.49	0.0439
Total Model	5	1.975087	0.6275	3.03	0.0707

Residual	DF	Sum of Squares	Mean Square
Total Error	9	1.172617	0.130291

Table B-4 Continued

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|	parameter
Intercept	1	5.381014	0.173399	31.03	<.0001	5.381014
X1	1	0.124275	0.127618	0.97	0.3556	0.124275
X3	1	0.284414	0.127618	2.23	0.0528	0.284414
X1*X1	1	-0.355100	0.187292	-1.90	0.0905	-0.355100
X3*X1	1	-0.422741	0.180479	-2.34	0.0439	-0.422741
X3*X3	1	0.050004	0.187292	0.27	0.7955	0.050004

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
X1	3	1.306751	0.435584	3.34	0.0696
X3	3	1.371256	0.457085	3.51	0.0625

The RSREG Procedure

Canonical Analysis of Response Surface Based on Coded Data

Factor	Critical Value Coded	Uncoded
X1	0.531209	0.531209
X3	-0.598452	-0.598452

Predicted value at stationary point: 5.328918

Eigenvalues	Eigenvectors X1	X3
0.140206	-0.392502	0.919751
-0.445302	0.919751	0.392502

Stationary point is a saddle point.

APPENDIX C
GC/O AND GC/MS FLAVOR ANALYSIS DATA AND RESULTS

Table C-1. Excel output of alkane standards' linear retention index (LRI) calculations in GC/O with a Carbowax column

Standard alkane	Retention time (min)	LRI literature	LRI calculated by formula
C10	8.57	1000	1008
C11	10.05	1100	1089
C12	12.14	1200	1198
C13	14.17	1300	1302
C14	16.19	1400	1405
C15	18.03	1500	1502
C16	19.8	1600	1599
C17	21.49	1700	1697
C18	23.09	1800	1797
C19	24.61	1900	1899
C20	26.04	2000	2002

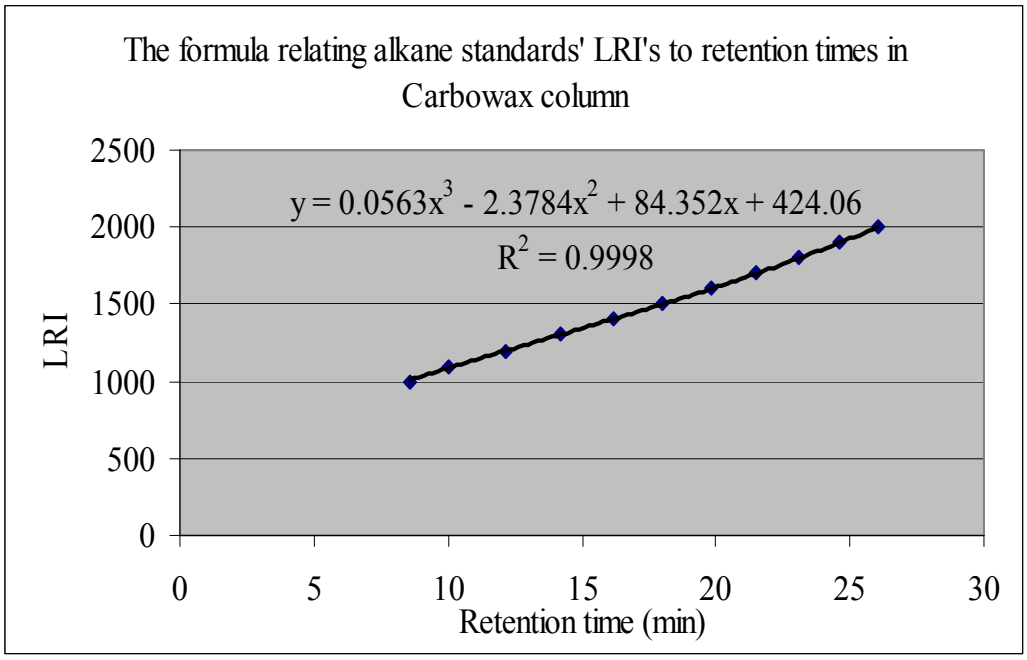


Figure C-1.Plot of the formula relating the LRI's to the retention times for aroma compounds in GC/O with a Carbowax column

Table C-2. Excel output of alkane standards' linear retention index (LRI) calculations in GC/O with a DB-5 column

Standard alkane	Retention time(min)	LRI literature	LRI's calculated by the formula
C7	4.78	700	706
C8	6.68	800	794
C9	8.9	900	895
C10	11.27	1000	1002
C11	13.44	1100	1102
C12	15.57	1200	1203
C13	17.57	1300	1302
C14	19.47	1400	1400
C15	21.26	1500	1499
C16	22.96	1600	1598
C17	24.57	1700	1698
C18	26.09	1800	1798
C19	27.54	1900	1900
C20	28.91	2000	2002

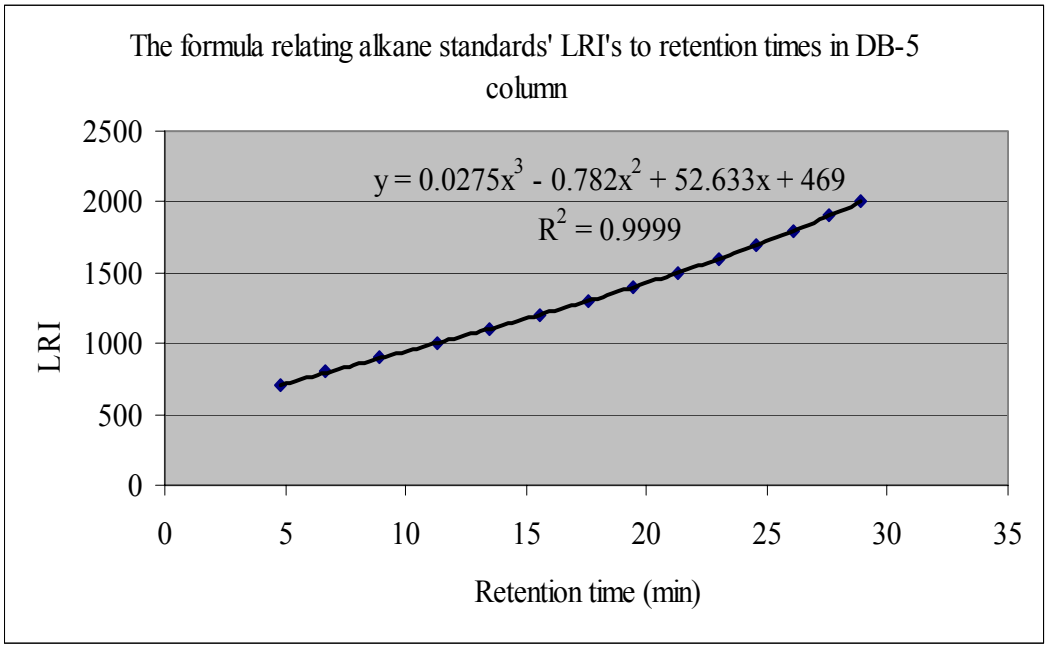


Figure C-2. Plot of the formula relating the LRI's to the retention times for aroma compounds in GC/O with a DB-5 column

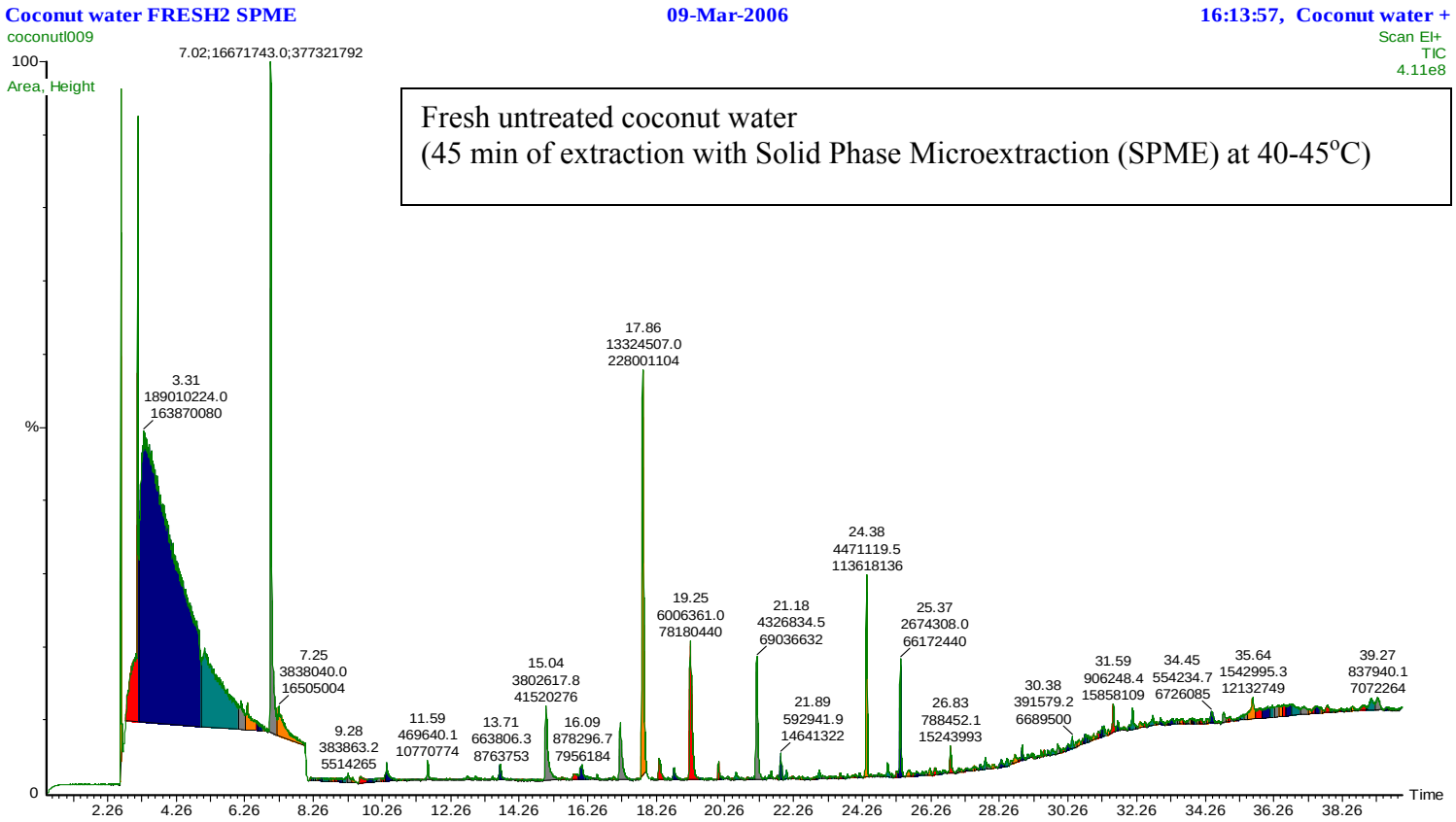
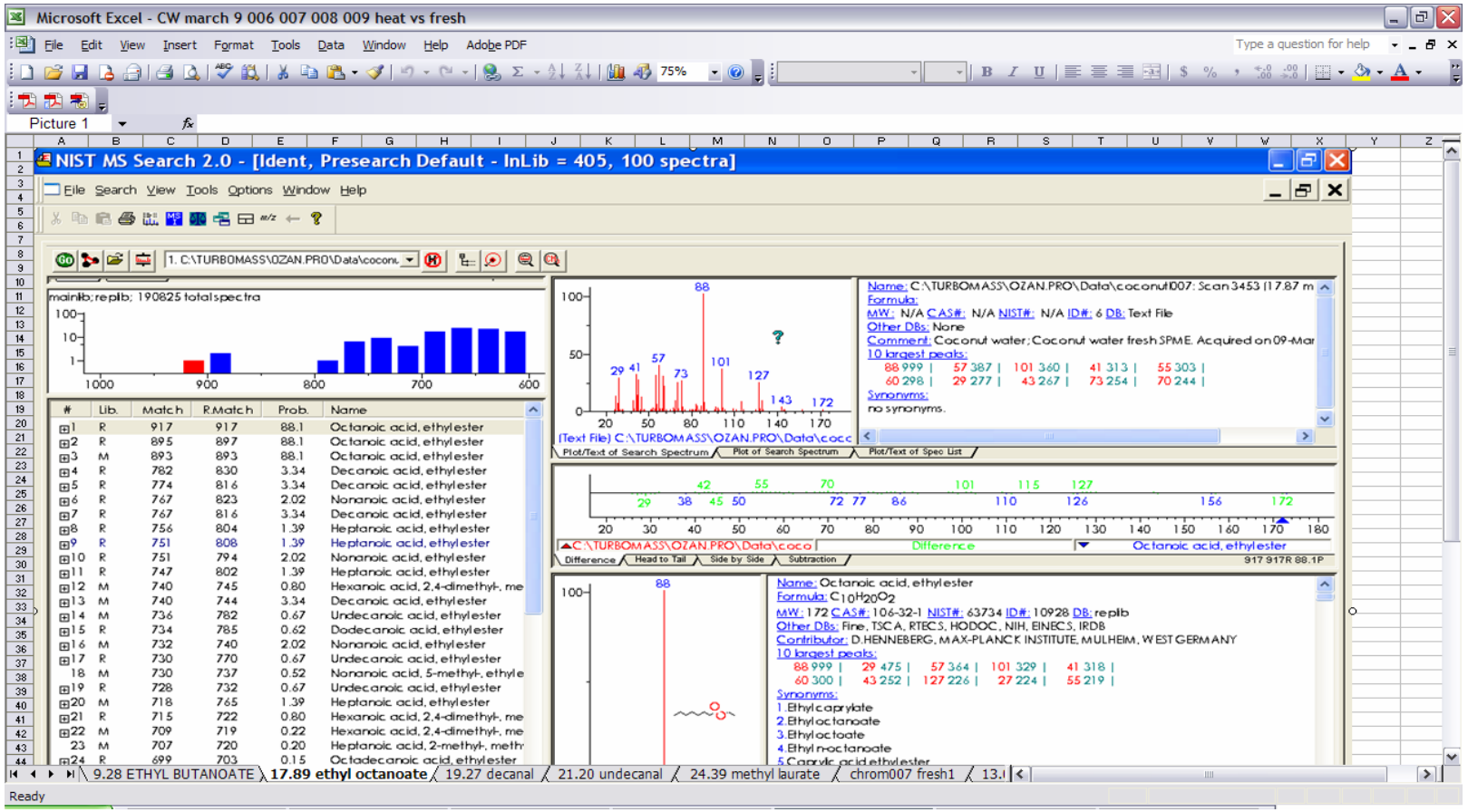


Figure C-3. An example of GC/MS peak identification using National Institute of Science and Technology (NIST) library database

Step 1. Integration of peaks on GC/MS chromatogram (Numbers above each peak represents retention time in minutes, peak height and peak area from top to bottom, respectively)

(Figure C-3 Continued)



Step 2. Identification of a selected peak (the peak with Rt= 17.86 min) using National Institute of Science and Technology (NIST) library database

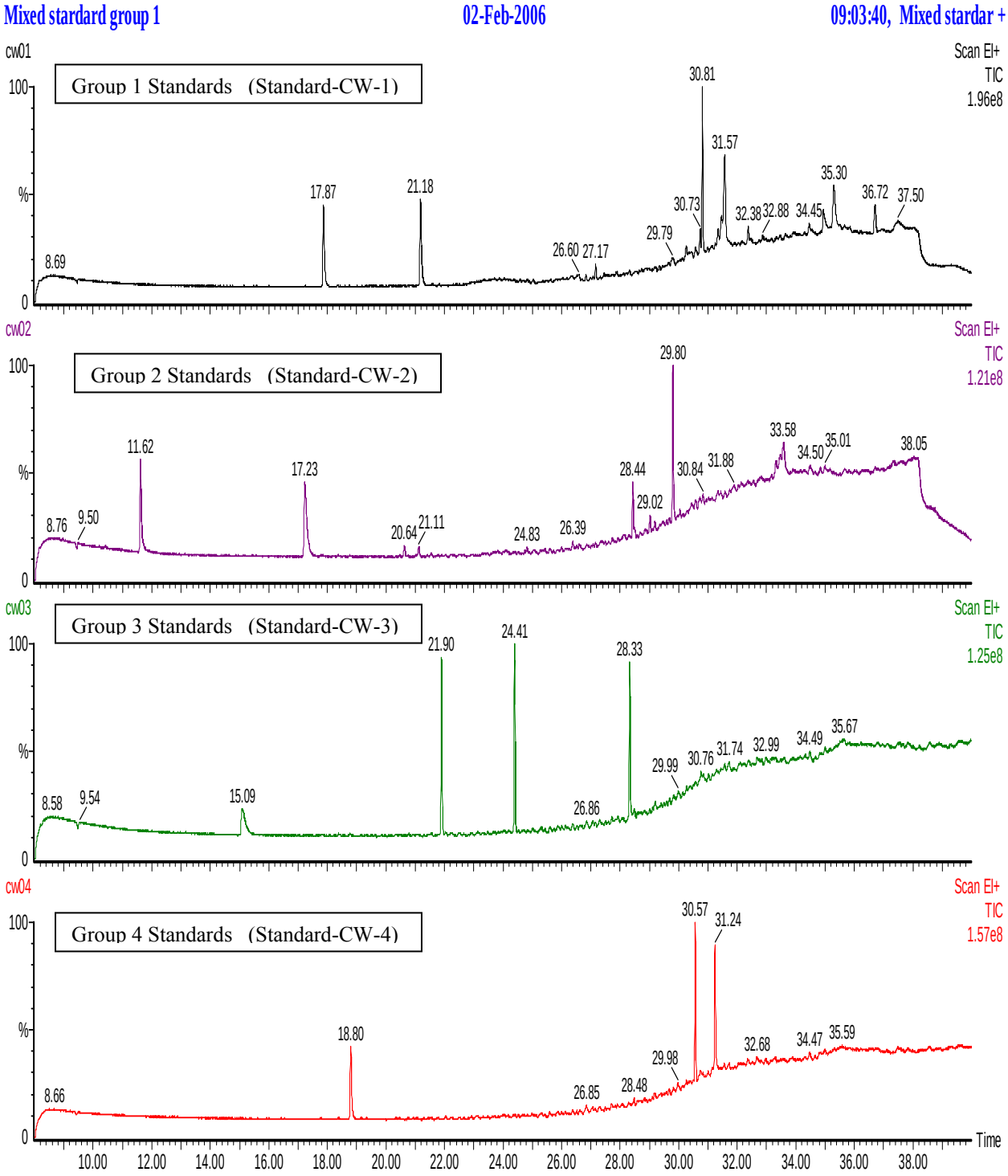


Figure C-4. GC/MS chromatograms of the four mixed groups of standard chemicals that were run in GC/MS for a possible confirmation

(The list of standards in each mixed group are given in C-5).

Table C-3. Retention times (RT), linear retention indices (Wax LRI) and GC/MS degree of match values of four mixed group of standard chemicals that were run in GC/MS for possible confirmation

Standard - CW-1 (Group 1 standards)	RT (min)	Wax LRI	GC/MS identified/degree of match
Ethyl caprylate (ethyl octanoate)	17.87	1451	890
Undecanal	21.18	1630	947
δ-Decalactone	30.81	2248	900
Standard - CW-2 (Group 2 standards)			
1-Butanol	11.62	1154	906
Nonanal	17.23	1419	890
γ-Nonalactone	28.44	2084	883
Nonanoic acid	29.80	2178	934
Standard - CW-3 (Group 3 standards)			
Octanal	15.09	1313	883
Nonanol	21.90	1671	904
Methyl laurate (Methyl dodecanoate)	24.41	1820	905
Octanoic Acid	28.33	2077	912
Standard - CW-4 (Group 4 standards)			
2-Ethyl-1-hexanol	18.80	1500	929
Carvacrol	30.57	2232	905
Decanoic acid	31.24	2278	889

GC/MS running conditions:
Mass Spec Method
Wax column: 40min run
(solvent delay of 8 min)

GC Method
Wax column:40min run
(40^{o}C-240^{o}C at ramp rate of 7^{o}C- 9.5min hold)
Injector temp - 240°C
Injection volume - 0.5ul

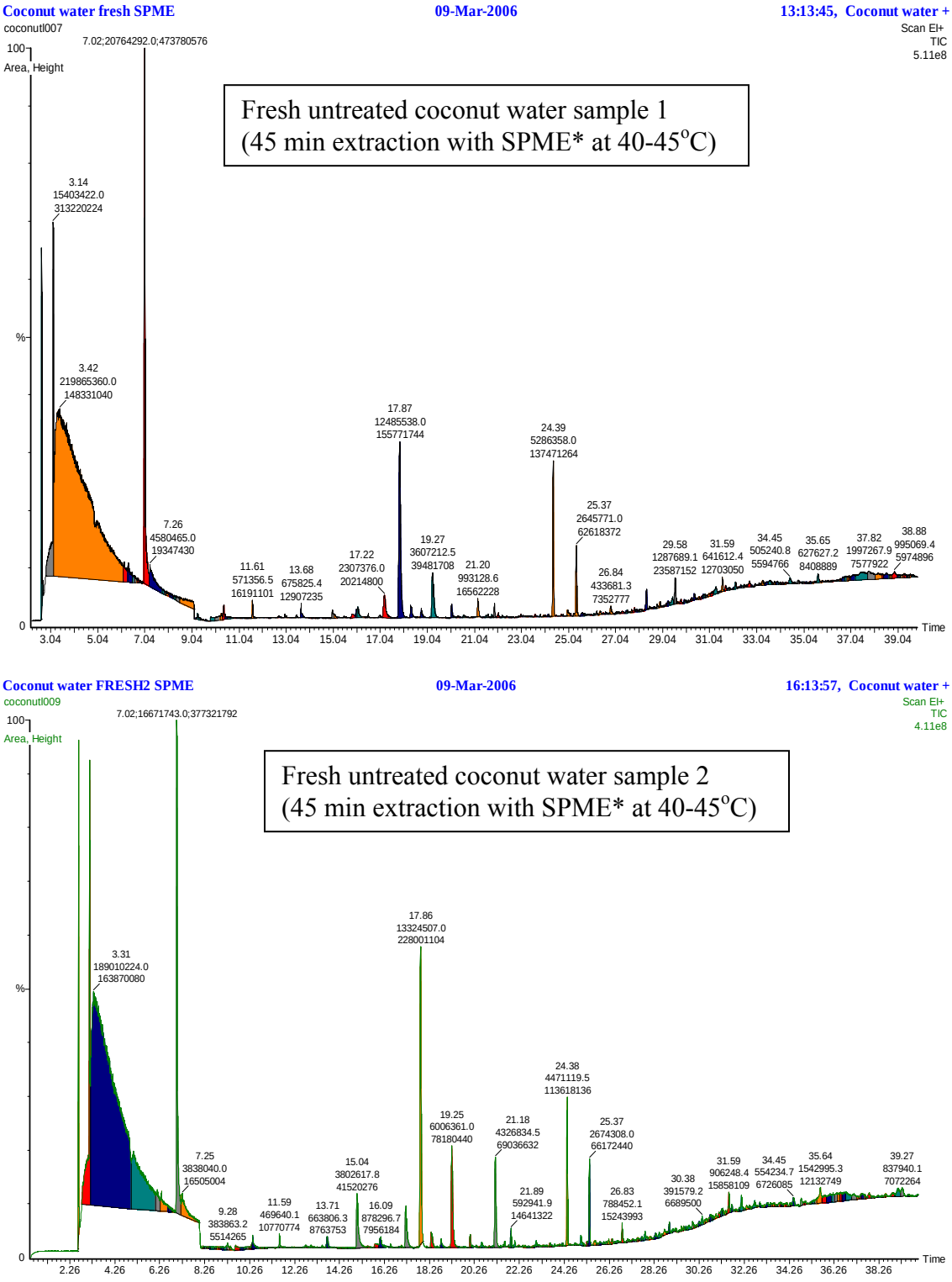


Figure C-5. Sample GC/MS chromatograms obtained by running fresh coconut water samples.

*SPME: Solid phase microextraction

Table C-4. Flavor compounds identified in coconut water through GC/O runs: Retention times, calculated Linear Retention Indices (LRI's) and aroma descriptors given by sniffers in GC/O runs with DB-5 and Carbowax columns

DB-5 Column			Carbowax Column		
Retention time (min)	Linear Retention Index(LRI)	Aroma descriptors by sniffers	Retention time (min)	Linear Retention Index(LRI)	Aroma descriptor by sniffers
6.77	797	Fruity,apple,sweet	9.43	1055	Sweet,fruity, candy,flower
7.82	845	Sweet,fruity,candy	9.74	1072	Sweet, candy
10.81	980	Mushroom,medicinal, earth	13.34	1260	Green, fruity
11.23	999	Fruity, sweet	14.83	1336	Mushroom, dirt
13.8	1117	Medicinal, chemical,pencil	17.17	1456	Sweet, coconut,candy
14.57	1152	Rancid, green, dirt	19.37	1575	Almond,green, rancid,wood, pencil
15.46	1196	Sweet,candy, rose,flower	24.25	1875	Old leather
15.87	1216	Green, fruity			
16.95	1269	Pencil, wood			
18.48	1347	Old leather			

(The same colored letters corresponds to the literature matched compounds based on the LRI's at DB-5 and Carbowax columns : Ethyl butanoate, 1-octene-3-one, ethyl octanoate, 2,6-nonadienal).

Table C-5. Peak areas of the sniffed compounds (olfactory port responses) and the aroma descriptors given by sniffers for DPCD treated (25°C, 34.5 MPa, 13% CO_2, 6 min) and carbonated coconut water samples in GC/O with Carbowax column

DPCD treated and Carbonated Coconut Water							
RT[a] (min)	LRI[b]	AROMA DESCRIPTOR	DPCD1[c]	DPCD2	DPCD3	DPCD4	DPCD AVE[d]
2.05	951	ALCOHOL,SWEET, FRUTY	0	339374	624020	0	240849
3.05	1047	SWEET,FRUITY, ROSE	792069	206632	0	985164	495966
6.2	1250		0	0	0	0	0
7.45	1315	MUSHROOM,NUTTY, DIRT,RANCID	672647	413901	670617	694377	612886
8.87	1387	ALCOHOL,MEDICINAL, EARTH	708085	234793	0	0	235720
9.96	1443	SWEET,FRUITY, COTTONCANDY	971195	0	711411	0	420652
10.57	1476	BOILED POTATO	750623	0	735610	539867	506525
11.32	1516	GREEN,CHEMICAL, MEDICINAL,FLORAL	929356	0	803886	723693	614234
11.43	1522	BURNT, DIRTY, RANCID OIL,WOOD	882670	391418	671714	469414	603804
11.99	1553	PENCIL,WOOD, ALMOND,GREEN,NUTTY	792753	409736	774259	793723	692618
12.23	1567		0	0	0	0	0
12.9	1605	RUBBER,SMOKEY, BURNT,ROSE	339245	0	351643	513251	301035
13.36	1631		0	0	0	0	0
14.94	1724	DIRT,NUTTY,RANCID, BOILED NUT,EARTH	956838	0	437109	422308	454064
16.76	1833		0	0	0	0	0
17.4	1873		0	0	0		0
19.78	2025	ALCOHOL, BURNT	0	538833	0	421482	240079
21.54	2146	CHEMICAL,GLUE, MEDICINAL,SPICY	869299	0	834402	0	425925
22.3	2200	ROSE,OLD SPICE	294379	0	265553	0	139983
22.52	2217	BUTTERSCOTCH,SPICY, BURNT CARAMEL	1046072	575606	983842	698169	825922

[a] Retention time; [b]Linear retention index; [c] Peak areas of each of four replicates of DPCD treated samples; [d] Average peak areas of four replicates

Table C-6. Peak areas of the sniffed compounds (olfactory port responses) and the aroma descriptors given by sniffers for heat treated (74°C, 15 s) and carbonated coconut water in GC/O with Carbowax column

Heat treated and Carbonated Coconut Water							
RT[a]	LRI[b]	AROMA DESCRIPTOR	HEAT1[c]	HEAT2	HEAT3	HEAT4	HEAT AVE[d]
2.05	951	ALCOHOL,SWEET, FRUTY	585587	467181	799533	0	463075
3.05	1047	SWEET,FRUITY,ROSE	658491	338353	720327	0	429293
6.2	1250	FRUITY,GREEN	797552	0	718493	0	379011
7.45	1315	MUSHROOM,NUTTY, DIRT, RANCID	863051	441346	686111	782807	693329
8.87	1387	ALCOHOL, MEDICINAL, EARTH	835683	0	535560	640610	502963
9.96	1443	SWEET, FRUITY, COTTONCANDY	705454	0	840408	327752	468404
10.57	1476	BOILED POTATO	731560	449456	1E+06	414806	686963
11.32	1516	GREEN,CHEMICAL, MEDICINAL, FLORAL	959563	0	624267	613652	549371
11.43	1522	WOODY, PENCIL	446908	731984	747628	0	481630
11.99	1553	PAPER, WOOD, ALMOND,GREEN, FLORAL	538844	979147	1E+06	697627	835177
12.23	1567	SWEET,FLORAL, FRUITY,CANDY, GREEN,BANANA	553982	587634	545965	517102	551171
12.9	1605		0	0	0	0	0
13.36	1631	WOODY,DIRT, RANCID,SOAPY, ALMOND, NUTTY	871909	566836	725192	610807	693686
14.94	1724	DIRT,NUTTY,RANCID, BOILED NUT,EARTH	666693	836192	778033	693411	743582
16.76	1833	NUTTY,RANCID,BAD, RANCID OIL	639297	739951	694090	0	518335
17.4	1873	SOAPY,FAT,ROSY, FLORAL	934488	0	698580	503850	534230
19.78	2025	METAL,COOKED, RANCID, BURNT	429039	0	485639	721339	409004
21.54	2146		0	0	0	0	0
22.3	2200	SPICY,FLORAL, SOAP	628453	0	792350	0	355201
22.52	2217	ROSE,OLD SPICE	688938	0	563572	0	313128
27.00	2557	BUTTERSCOTCH, SPICY, BURNT	970501	478469	1E+06	0	663632

[a] Retention time; [b]Linear retention index; [c] Peak areas of each of four replicates of heat treated samples; [d] Average peak areas of four replicates

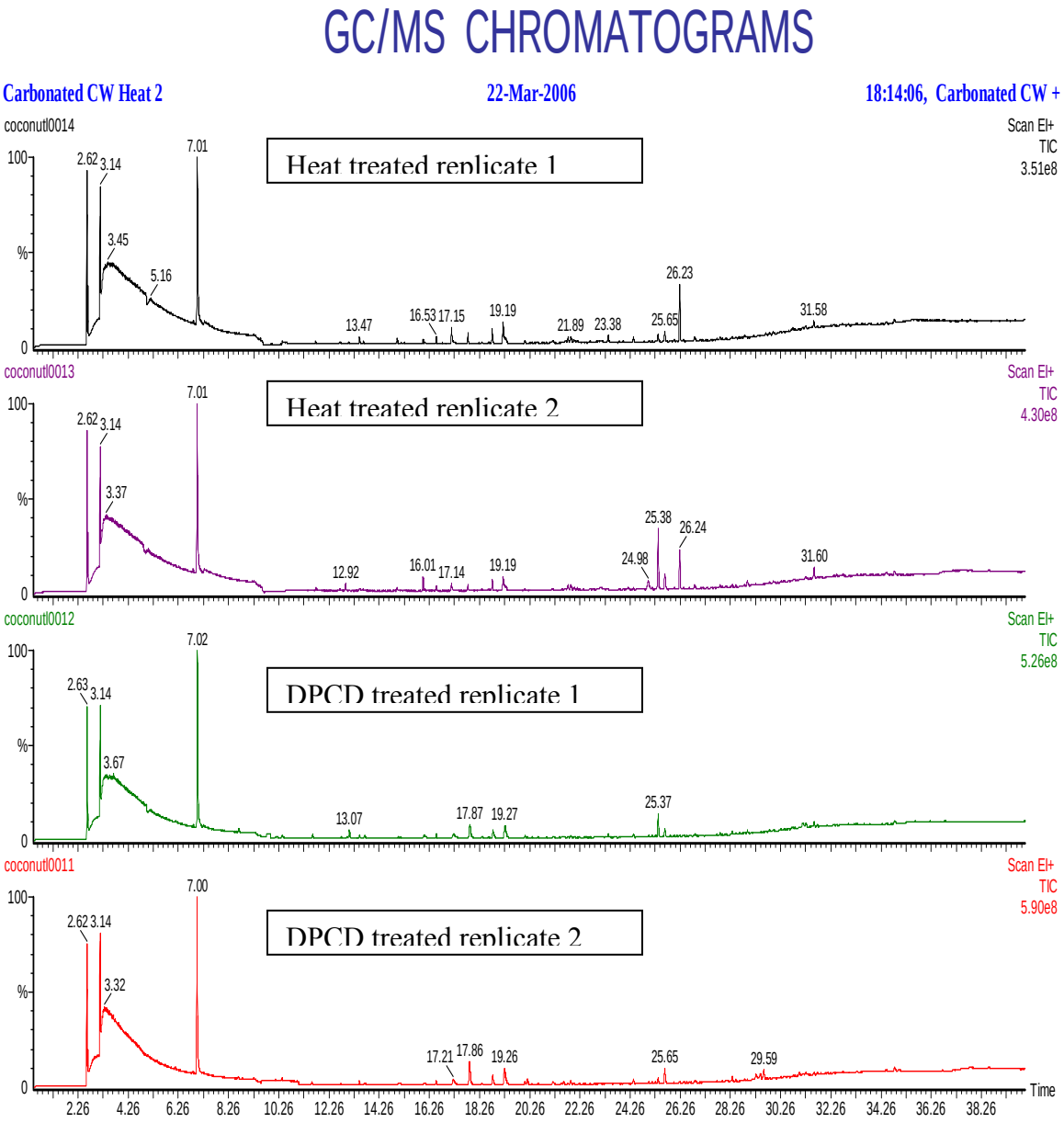


Figure C-6. GC/MS chromatograms of DPCD treated (coconut0011 and coconut0013) and heat treated (coconut0013 and coconut0014) coconut water beverages (carbonated)

(Samples were extracted for 45 min by solid phase microextraction (SPME) at 40-45^{o}C).

APPENDIX D
STORAGE STUDY: MICROBIAL, CHEMICAL AND PHYSICAL QUALITY DATA

Table D-1. Total aerobic plate counts (APC) of untreated, DPCD treated (34.5 MPa, 25°C, 13% CO_2, 6 min) and heat treated (74°C, 15 s) coconut water during storage (4°C)

Storage time (Week)	Control (Untreated)*	DPCD treated*	Heat pasteurized*
0	1410 ± 46	1130 ± 43	76.33 ± 2
1	485 ± 28	260 ± 27	7.25 ± 2
2	278 ± 19	272 ± 23	3.50 ± 1
3	303 ± 28	1060 ± 25	3.75 ± 0
4	175 ± 18	103 ± 6	4.50± 0
5	242 ± 10	540 ± 28	3.0 ± 1
6	298 ± 22	98 ± 18	2.0 ± 1
9	107000 ± 7150	130 ± 16	1.0 ± 1

*Mean of number of colony forming units (cfu's) on petrifilms with counts less than 200 ± Std Error (number of replicate petrifilms at each dilution is four)

Table D-2. Excel outputs of one-tail t tests conducted for comparison of mean aerobic plate counts (APC) and yeast and mold (YM) counts for week 0 and week 9 samples.

Control (untreated) coconut water samples' APC: week0 vs week9 comparison

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1[a]	*Variable 2*[b]
Mean	1407.5	106500
Variance	8425	2.04E+08
Observations	4	4
Hypothesized Mean Difference	0	
df	3	
t Stat	-14.70	
P(T<=t) one-tail	0.0003	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.0007	
t Critical two-tail	3.18	

t-Test: Two-Sample Assuming Unequal Variances

Table D-2 Continued

DPCD treated coconut water samples' APC: week0 vs week9 comparison		
	Variable 1[a]	*Variable 2*[b]
Mean	1125	129.625
Variance	7500	1991.411
Observations	4	8
Hypothesized Mean Difference	0	
df	4	
t Stat	21.60	
P(T<=t) one-tail	1.36E-05	
t Critical one-tail	2.13	
P(T<=t) two-tail	2.72E-05	
t Critical two-tail	2.78	
Heat treated coconut water samples' APC: week0 vs week9 comparison		
t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1[a]	*Variable 2*[b]
Mean	76.33	1
Variance	16.33	1.33
Observations	3	4
Hypothesized Mean Difference	0	
df	2	
t Stat	31.34	
P(T<=t) one-tail	0.0005	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.001	
t Critical two-tail	4.30	
Control (untreated) coconut water samples' YM: week0 vs week9 comparison		
t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1[a]	*Variable 2*[b]
Mean	14.67	1.25
Variance	6.33	0.25
Observations	3	4
Hypothesized Mean Difference	0	
df	2	
t Stat	9.10	
P(T<=t) one-tail	0.006	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.012	
t Critical two-tail	4.30	

Table D-2 Continued

DPCD treated coconut water samples' YM: week0 vs week9 comparison		
t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1[a]	*Variable 2*[b]
Mean	4.666667	0
Variance	4.333333	0
Observations	3	4
Hypothesized Mean Difference	0	
df	2	
t Stat	3.882901	
P(T<=t) one-tail	0.030191	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.060382	
t Critical two-tail	4.302656	

Heat treated coconut water samples' YM: week0 vs week9 comparison

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1[a]	*Variable 2*[b]
Mean	1.333333	0
Variance	0.333333	0
Observations	3	4
Hypothesized Mean Difference	0	
df	2	
t Stat	4	
P(T<=t) one-tail	0.028595	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.057191	
t Critical two-tail	4.302656	

[a](Variable 1) corresponds to Week 0; [b](Variable 2) corresponds to Week 9

Table D-3. Aerobic plate counts (APC) and yeast and mold (YM) counts of sterile distilled water before and after carbonation with the Zalhm carbonator

	APC counts			[b]YM counts*	
Dilution:	0	10^{-1}	10^{-2}	0	10^{-1}
Initial counts:	0-0 0-0	0-0 0-0	0-0 0-0	0-0 0	0-0 0-0
Final counts:	[a]TNTC- TNTC	114-124 122-141	------	231-230 186-179	26-20 17-24

[a] too numerous to count ; [b] numbers in red color indicate mold growth (if there is any)
* Numbers in red color indicate mold growth (if there is any)

Table D-4. Yeast and mold (YM) counts of untreated, heat treated (74°C, 15 s) and DPCD treated (34.5 MPa, 25°C, 13% CO_2, 6 min) coconut water beverages during storage

Storage time (Week)	Control (Untreated)**	DPCD treated**	Heat pasteurized**
0	14.67± 1	4.67 ± 1	1.33 ± 0
1	6.00 ± 2	0.25 ± 0	0 ± 0
2	8.00 ± 1	0.25 ± 0	0 ± 0
3	18.75 ± 1	2.75 ± 0	0.25 ± 0
4	8.25 ± 0	2.75 ± 1	0.50± 0
5	10.00 ± 2	1.25± 1	0 ± 0
6	2.50 ± 0	0 ± 0	0 ± 0
9	1.25 ± 0.25	0 ± 0	0 ± 0

(Numbers in red color indicate mold growth (if there is any)) ; **Mean of number of colony forming units (cfu's) on petrifilms with counts less than 200 ± Std Error (number of replicate petrifilms at each dilution is four)

Table D-5. The pH of untreated, DPCD treated and heat pasteurized samples during storage

Storage time (Week)	Control (Untreated)*	[a]DPCD treated*	[b]Heat pasteurized*
0	4.18 ± 3.44E-08	4.18 ± 0	4.18 ± 0
2	4.17 ± 0	4.16 ± 0	4.16 ± 0
3	4.19 ± 0	4.22 ± 0	4.24 ± 0
5	4.20 ± 0	4.19 ± 0	4.19 ± 0
9	4.24 ± 0	4.20 ± 0	4.22 ± 0

*Mean of three replicate measurements ± Std Error; Treatment conditions: [a] 34.5 MPa, 25°C, 13% CO_2 ; [b]74°C, 15 s

Table D-6. SAS software output of analysis of variance (ANOVA) for the pH data of different treatments from the storage study

```
                                The ANOVA Procedure
                              Dependent Variable: pH
                                            Sum of
      Source                DF              Squares      Mean Square    F Value    Pr > F
      Model                 14           0.03118667       0.00222762      38.55    <.0001
      Error                 30           0.00173333       0.00005778
      Corrected Total       44           0.03292000

                      R-Square     Coeff Var      Root MSE       pH Mean
                      0.947347      0.181182      0.007601      4.195333

   Source                        DF        Anova SS     Mean Square    F Value    Pr > F
   treat                          2      0.00069333      0.00034667       6.00    0.0064
   week                           4      0.02412000      0.00603000     104.37    <.0001
   week*treat                     8      0.00637333      0.00079667      13.79    <.0001
                          Duncan's Multiple Range Test for pH
  NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise
                                        error rate.
                             Alpha                          0.05
                             Error Degrees of Freedom         30
                             Error Mean Square          0.000058
                           Number of Means              2            3
                           Critical Range         .005668      .005957
                  Means with the same letter are not significantly different
Duncan Grouping           Mean        N    treat
       A               4.199333     15    heat
       A               4.196667     15    control
       B               4.190000     15    CO2
Duncan Grouping           Mean        N    week
       A               4.223333      9     9
       A               4.218889      9     3
       B               4.192222      9     5
       C               4.180000      9     0
       D               4.162222      9     2
```

Table D-7. The ^{o}Brix of untreated, DPCD treated and heat pasteurized samples during storage

Storage time (Week)	Control (Untreated)*	[a]DPCD treated*	[b]Heat pasteurized*
0	5.9 ± 0	5.9 ± 0	5.9 ± 0
1	6.0 ± 0	6.0 ± 0	6.0 ± 0
2	6.1 ± 0	6.0 ± 0	6.0 ± 0
3	6.0 ± 0	6.1 ± 0	6.0 ± 0
5	6.15 ± 0.05	6.0 ± 0	6.1 ± 0
9	6.1± 0	6.0 ± 0	6.0 ± 0

*Mean of two replicate measurements ± Std Error; Treatment conditions: [a] 34.5 MPa, 25^{o}C, 13% CO_2, 6 min; [b]74^{o}C, 15 s

Table D-8. SAS software output of analysis of variance (ANOVA)for °Brix data of different treatments from the storage study

The ANOVA Procedure
Dependent Variable: Brix

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	0.17805556	0.01047386	37.71	<.0001
Error	18	0.00500000	0.00027778		
Corrected Total	35	0.18305556			

R-Square	Coeff Var	Root MSE	Brix Mean
0.972686	0.277136	0.016667	6.013889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	2	0.01388889	0.00694444	25.00	<.0001
week	5	0.11472222	0.02294444	82.60	<.0001
week*treat	10	0.04944444	0.00494444	17.80	<.0001

Duncan's Multiple Range Test for Brix
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	0.000278
Number of Means	2 3
Critical Range	.01429 .01500

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	6.041667	12	control
B	6.000000	12	CO2
B	6.000000	12	heat

The ANOVA Procedure
Duncan's Multiple Range Test for Brix
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05				
Error Degrees of Freedom	18				
Error Mean Square	0.000278				
Number of Means	2	3	4	5	6
Critical Range	.02022	.02121	.02184	.02227	.02259

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	week
A	6.083333	6	5
B	6.033333	6	9
B	6.033333	6	2
B	6.033333	6	3
C	6.000000	6	1
D	5.900000	6	0

Table D-9. Titratable acidity (as % malic acid (w/v)) of untreated, DPCD treated and heat pasteurized coconut water beverages during storage

Storage time (Week)	Control (Untreated)*	[a]DPCD treated*	[b]Heat pasteurized*
0	0.2156 ± 0.0192	0.2738 ± 0.0058	0.2291 ± 0.0057
2	0.2604 ± 0.0024	0.2674 ± 0.0065	0.2664 ± 0.0034
3	0.302 ± 0.0062	0.3002 ± 0.0016	0.2738 ± 0.0059
5	0.2512 ± 0.0029	0.2654 ± 0.0001	0.258 ± 0.0039
9	0.2656 ± 0.0176	0.3052 ± 0.0039	0.3033 ± 0.0055

*Mean of three replicate measurements ± Std Error; Treatment conditions: [a] 34.5 MPa, 25°C, 13% CO_2, 6 min ; [b]74°C, 15 s

Table D-10. SAS software output of analysis of variance (ANOVA) for % titratable acidity data of different treatments from storage study

The ANOVA Procedure
Dependent Variable: %TA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	0.02876390	0.00205456	10.81	<.0001
Error	30	0.00570096	0.00019003		
Corrected Total	44	0.03446486			

R-Square	Coeff Var	Root MSE	%TA Mean
0.834586	5.121316	0.013785	0.269173

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	2	0.00433421	0.00216710	11.40	0.0002
week	4	0.01831396	0.00457849	24.09	<.0001
week*treat	8	0.00611573	0.00076447	4.02	0.0024

Duncan Grouping	Mean	N	treat
A	0.282426	15	CO2
B	0.266119	15	heat
B	0.258976	15	control

Duncan Grouping	Mean	N	week
A	0.292022	9	3
A	0.291379	9	9
B	0.264763	9	2
B	0.258188	9	5
C	0.239515	9	0

Table D-11. The mean L*, a*, b* values of untreated, DPCD (34.5 MPa, 25°C, 13% CO_2, 6 min) and heat treated (74°C, 15 s) coconut water beverages during storage

L* values Week:	*Control	*DPCD treated	*Heat treated
2	59.74±0.17	60.21±0.31	58.97±0.23
3	58.38±0.44	54.75±0.02	58.44±0.75
5	54.15±0.78	52.50±0.28	53.67±0.28
9	58.21±0.35	57.48±0.34	58.21±0.60
a* values Week:	*Control	*DPCD treated	*Heat treated
2	-1.71±0.02	-1.50±0.01	-0.92±0.02
3	-0.60±0.04	4.90±0.10	1.72±0.12
5	3.55±0.17	4.73±0.19	3.27±0.29
9	6.08±0.30	2.37±0.08	1.71±0.01
b* values Week:	*Control	*DPCD treated	*Heat treated
2	1.84±0.02	2.00±0.07	2.17±0.05
3	2.71±0.09	2.99±0.03	3.50±0.07
5	3.85±0.02	2.98±0.12	3.27±0.29
9	2.37±0.16	2.79±0.03	2.42±0.01

*Mean of three replicate measurements ± Std Error;

APPENDIX E
STORAGE STUDY TASTE PANELS: DATA AND ANALYSIS

Table E-1. Taste panel data output obtained by Compusense software: Sensory evaluation scores of treatments during the storage study (Evaluation score scales: Overall likeability: 9 point scale; Aroma difference and taste difference from control: 15 cm line scale; Off flavor: 6 point scale; Purchase intent and ask again: 1=Yes and 2=No)

Storage time (Weeks)	Judges	*Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase[a] Intent	Ask [b] again
0	1	control	6	1	5	2	1	
0	1	DPCD	8	0.1	5.4	1	1	
0	1	heat	4	2.8	7.1	2	2	2
0	2	control	4	1	2	3	2	2
0	2	heat	1	2.7	5	5	2	2
0	2	DPCD	6	1.1	0.8	2	2	1
0	3	DPCD	6	0.6	3	3	2	2
0	3	control	7	5	1.5	3	2	2
0	3	heat	7	2	4	3	2	2
0	4	DPCD	2	6.6	2.5	5	2	2
0	4	heat	3	0.2	0.5	4	2	2
0	4	control	4	0.1	2.8	3	2	2
0	5	heat	3	0	1.6	3	2	2
0	5	control	7	0	0	1	1	
0	5	DPCD	7	0	0	1	1	
0	6	heat	9	0.2	0.1	3	1	
0	6	DPCD	8	1.3	1.5	2	1	
0	6	control	9	0.1	0.1	3	1	
0	7	control	3	1.8	1.1	4	2	1
0	7	DPCD	6	0	0	4	1	
0	7	heat	5	0	6.1	4	2	1
0	8	control	5	0.6	2.2	2	2	2
0	8	heat	6	0.1	0.8	1	2	2
0	8	DPCD	5	1.5	0.9	2	2	2
0	9	DPCD	3	1.5	2.7	2	2	2
0	9	control	3	1.2	0.2	2	2	2
0	9	heat	3	0.2	2.6	3	2	2
0	10	DPCD	2	0.1	0.5	3	2	2
0	10	heat	2	0	3.3	2	2	2
0	10	control	2	0.2	6.2	4	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
0	11	heat	5	3	2	3	2	2
0	11	control	5	0.5	4	3	2	2
0	11	DPCD	5	0.5	2	2	2	2
0	12	heat	8	8	2.6	1	1	
0	12	DPCD	8	7.1	5.2	1	1	
0	12	control	8	2.5	1.8	1	1	
0	13	control	9	8.4	6.2	4	1	
0	13	DPCD	8	0	8	4	1	
0	13	heat	7	0	8	4	1	
0	14	control	7	1.2	0.5	2	1	
0	14	heat	4	3.3	6.6	1	2	2
0	14	DPCD	7	1.8	2.5	1	1	
0	15	DPCD	7	9	5.5	1	1	
0	15	control	9	0	0	3	1	
0	15	heat	6	0	2	2	1	
0	16	DPCD	6	3	4.7	5	1	
0	16	heat	7	8.3	4.4	4	1	
0	16	control	4	9.3	1	3	2	1
0	17	heat	5	0.1	0.3	3	2	2
0	17	control	5	0.2	0.3	3	2	2
0	17	DPCD	5	0.5	0.6	3	2	2
0	18	heat	7	0.2	0.2	1	1	
0	18	DPCD	7	0.2	0.3	1	1	
0	18	control	7	0.3	0.3	1	1	
0	19	control	3	0.1	6.3	4	2	2
0	19	DPCD	3	0.1	5.6	4	2	2
0	19	heat	2	0.3	7.7	5	2	2
0	20	control	5	1.4	1.8	1	2	1
0	20	heat	6	3.3	4.3	2	2	1
0	20	DPCD	6	1.1	1.9	2	2	1
0	21	DPCD	2	5.4	4.8	5	2	2
0	21	control	2	6.1	5.6	5	2	2
0	21	heat	1	5.2	5.9	4	2	2
0	22	DPCD	6	8.5	1.5	1	2	2
0	22	heat	6	2.5	2	1	2	2
0	22	control	6	1	1.1	1	2	2
0	23	heat	2	1	4	2	2	2
0	23	control	3	0.1	0.2	1	2	2
0	23	DPCD	2	0.1	0.2	1	2	2
0	24	heat	4	0	2.8	2	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
0	24	DPCD	8	0.3	0.1	1	1	
0	24	control	7	0.6	1	1	1	
0	25	control	6	6.6	0.9	3	2	2
0	25	DPCD	5	9.4	1.9	3	1	
0	25	heat	1	1.5	9.9	6	2	2
0	26	control	3	7.1	2.2	2	2	2
0	26	heat	1	1.9	10	5	2	2
0	26	DPCD	3	1	1	1	2	2
0	27	DPCD	7	1.4	1.5	1	1	
0	27	control	5	1.6	1.6	1	2	2
0	27	heat	5	3	2.2	1	2	2
0	28	DPCD	7	0.6	4.3	2	1	
0	28	heat	8	0.6	6.2	4	1	
0	28	control	6	0.6	4.1	3	1	
0	29	heat	2	2.2	8.7	6	2	2
0	29	control	3	1.6	6.7	5	2	2
0	29	DPCD	3	1.5	6.2	5	2	1
0	30	heat	1	0	8	4	2	2
0	30	DPCD	4	0.2	3.7	3	2	2
0	30	control	7	0.1	1.4	2	1	
0	31	control	2	4.2	7.9	2	2	1
0	31	DPCD	5	2.8	3.5	1	2	1
0	31	heat	3	2.2	8.1	3	2	1
0	32	control	5	0.1	0.1	3	2	1
0	32	heat	3	0.4	4	3	2	2
0	32	DPCD	5	0.7	1.8	2	2	2
0	33	DPCD	3	0.3	3.8	4	2	2
0	33	control	4	3.4	1.4	3	2	2
0	33	heat	5	0.5	1.2	3	2	2
0	34	DPCD	4	4.7	4.8	3	2	2
0	34	heat	5	2.5	4.4	4	2	2
0	34	control	6	1.5	0.2	1	2	2
0	35	heat	3	5.6	8.9	4	2	2
0	35	control	4	6.7	1.5	1	2	2
0	35	DPCD	4	0.9	3.5	1	2	2
0	36	heat	6	0.7	4.8	4	1	
0	36	DPCD	6	0.4	6.9	1	1	
0	36	control	8	0.6	5.2	1	1	
0	37	control	4	1	7.6	2	2	2
0	37	DPCD	6	2.8	8.4	2	2	1
0	37	heat	7	2.5	9.1	1	1	

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
0	38	control	4	0.2	7.4	4	2	2
0	38	heat	6	0.2	1.4	2	2	1
0	38	DPCD	6	0.2	3.1	2	2	1
0	39	DPCD	5	0.2	6.8	4	1	
0	39	control	5	0.3	6.1	4	2	1
0	39	heat	6	0.1	1.6	3	1	
0	40	DPCD	7	6.2	2.2	2	2	2
0	40	heat	7	3.4	4	1	1	
0	40	control	8	2.8	4.3	1	1	
0	41	heat	2	2.2	7	5	2	2
0	41	control	3	0.8	5.2	4	2	1
0	41	DPCD	3	0.9	4.8	4	2	1
0	42	heat	4	0.2	6.6	3	2	2
0	42	DPCD	6	0.2	5.4	1	2	1
0	42	control	7	0	0	1	2	1
0	43	control	3	0.9	0.1	3	2	2
0	43	DPCD	5	6.6	4.8	2	2	2
0	43	heat	5	5.4	5.7	2	2	2
0	44	control	5	0	0	1	2	2
0	44	heat	5	0.2	0.3	1	2	2
0	44	DPCD	5	0.1	0.4	1	2	1
0	45	DPCD	3	1	7.8	6	2	2
0	45	control	6	0.1	3.6	1	1	
0	45	heat	3	7	9.9	5	2	2
0	46	DPCD	6	7.1	1.6	3	2	1
0	46	heat	1	1.9	4.8	6	2	2
0	46	control	4	0	1	4	2	2
0	47	heat	5	0.8	3.3	2	2	1
0	47	control	7	1.5	2.7	1	1	
0	47	DPCD	6	3.5	2.6	1	1	
0	48	heat	6	7.6	3.8	5	2	2
0	48	DPCD	6	0.4	0.6	5	2	2
0	48	control	7	2.3	6.9	4	2	2
0	49	control	7	1	0	1	1	
0	49	DPCD	7	1	0	1	1	
0	49	heat	5	1	3	3	2	2
0	50	control	9	4	0	2	1	
0	50	heat	1	5.6	9.9	6	2	2
0	50	DPCD	7	5.8	5.6	2	1	
2	1	control	2	1	2	2	2	2
2	1	DPCD	6	0.5	1.5	1	1	

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
2	1	heat	4	0.5	3.5	2	2	2
2	2	control	1	2	4.4	4	2	2
2	2	heat	2	0.6	6.6	4	2	2
2	2	DPCD	1	7.9	8.4	3	2	2
2	3	DPCD	7	4.8	2.5	2	2	1
2	3	control	4	4	4.1	2	2	2
2	3	heat	3	3.3	1	2	2	2
2	4	DPCD	6	2.5	4.7	3	1	
2	4	heat	5	0.8	8.1	5	2	1
2	4	control	8	1.1	2	3	1	
2	5	heat	6	0.6	6.9	3	1	
2	5	control	7	0.6	0.7	3	1	
2	5	DPCD	5	0.6	4.9	4	2	1
2	6	heat	4	1.5	5.2	3	2	1
2	6	DPCD	3	2.5	6.4	3	2	2
2	6	control	2	1.1	6.6	5	2	2
2	7	control	5	0.2	1.5	2	1	
2	7	DPCD	4	0.1	3.8	3	2	1
2	7	heat	3	2.8	5.5	4	2	1
2	8	control	5	1.9	0.9	4	2	2
2	8	heat	7	0.8	4.3	2	1	
2	8	DPCD	5	0.5	0.9	3	2	2
2	9	DPCD	5	2.5	0.3	1	1	
2	9	control	5	2.2	4	2	1	
2	9	heat	2	4.4	7.8	4	2	1
2	10	DPCD	3	4.7	6.8	4	2	2
2	10	heat	4	0.1	8.3	4	2	2
2	10	control	3	0.1	2.8	3	2	2
2	11	heat	6	0.5	1	1	1	
2	11	control	7	8.4	0.2	1	1	
2	11	DPCD	8	0.4	0.7	1	1	
2	12	heat	6	0	2.3	3	2	2
2	12	DPCD	8	0.1	0.1	1	1	
2	12	control	7	0.1	2.6	3	1	
2	13	control	2	0.9	6.2	4	2	2
2	13	DPCD	5	0.8	6.3	3	2	1
2	13	heat	4	1.3	2	3	2	2
2	14	control	5	0.2	1.8	2	2	2
2	14	heat	5	0.2	0.1	1	2	2
2	14	DPCD	5	0.1	3	2	2	2
2	15	DPCD	5	0.2	6.1	4	1	

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
2	15	control	7	1.1	7.7	4	1	
2	15	heat	2	0.3	6.4	5	2	2
2	16	DPCD	5	1.8	0.9	3	1	
2	16	heat	4	0.9	5.8	3	2	1
2	16	control	6	1	1.5	2	1	
2	17	heat	3	1	4.3	5	2	2
2	17	control	2	0.5	0.2	5	2	2
2	17	DPCD	3	1.7	5.2	4	2	2
2	18	heat	3	6.6	1	4	2	2
2	18	DPCD	2	4.8	5.2	5	2	2
2	18	control	4	4.4	0.1	6	2	2
2	19	control	3	0.8	0.5	2	2	2
2	19	DPCD	3	0.8	3.2	3	2	2
2	19	heat	3	0.3	5.2	4	2	2
2	20	control	2	1.5	0.3	2	2	2
2	20	heat	9	6.1	9.4	5	1	
2	20	DPCD	3	4.7	1.5	3	2	2
2	21	DPCD	3	0.9	2.2	3	2	2
2	21	control	2	0.7	4.2	4	2	2
2	21	heat	6	1	5.6	4	1	
2	22	DPCD	7	0.1	3.6	2	1	
2	22	heat	5	1.5	1.1	3	2	2
2	22	control	6	0	0.1	4	1	
2	23	heat	3	5.7	7.4	5	2	2
2	23	control	5	0.4	4.1	4	2	2
2	23	DPCD	7	0.2	7.7	3	1	
2	24	heat	7	4.1	5.8	3	1	
2	24	DPCD	6	1.5	6	1	1	
2	24	control	7	5.6	4.9	2	1	
2	25	control	1	0.8	4.8	4	2	2
2	25	DPCD	1	0.4	9.1	5	2	2
2	25	heat	1	2.3	9.2	5	2	2
2	26	control	4	1.5	1.1	2	2	2
2	26	heat	5	0.1	1.9	3	2	2
2	26	DPCD	6	3.5	4.5	3	2	1
2	27	DPCD	6	0.6	8.8	4	2	2
2	27	control	3	6.8	1.9	5	2	2
2	27	heat	4	5.7	9.2	3	2	2
2	28	DPCD	5	0.7	0.9	1	2	2
2	28	heat	5	2.9	1.9	1	2	2
2	28	control	5	0.2	2	1	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
2	29	heat	4	0.6	2	3	2	2
2	29	control	4	0.5	1	2	2	2
2	29	DPCD	4	2.6	0.2	1	2	2
2	30	heat	4	1.5	6.7	3	2	2
2	30	DPCD	5	6.5	4.9	1	2	2
2	30	control	5	0.1	7.8	2	2	2
2	31	control	1	7.2	8.6	5	2	2
2	31	DPCD	3	0.2	8.1	4	2	2
2	31	heat	2	0.5	8.3	4	2	2
2	32	control	3	2	5.1	3	2	2
2	32	heat	5	0.2	4.2	3	2	2
2	32	DPCD	5	0.6	4.8	3	2	1
2	33	DPCD	6	6.1	5.9	3	1	
2	33	control	6	0.9	5.4	2	1	
2	33	heat	7	1.6	2.8	1	1	
2	34	DPCD	5	0.6	1.8	1	2	1
2	34	heat	3	1.5	9.2	4	2	1
2	34	control	1	1.1	8.3	6	2	1
2	35	heat	4	3.1	6.6	3	2	2
2	35	control	4	4	6.3	3	2	2
2	35	DPCD	4	0.2	0.1	2	2	2
2	36	heat	5	0.4	3.7	2	2	2
2	36	DPCD	5	2.2	4.8	1	2	2
2	36	control	5	4.1	3.8	1	2	2
2	37	control	6	4	1.9	1	1	
2	37	DPCD	5	3.5	4	2	1	
2	37	heat	6	0	0.9	1	1	
2	38	control	4	10	10	5	2	2
2	38	heat	4	10	10	4	2	2
2	38	DPCD	6	0.1	9.9	3	2	1
2	39	DPCD	5	1.2	1.7	2	2	2
2	39	control	6	0.6	3.4	1	2	2
2	39	heat	4	3.8	6.6	3	2	2
2	40	DPCD	6	6.5	6.2	2	2	1
2	40	heat	3	1.2	3.8	4	2	2
2	40	control	6	2.8	3.8	2	1	
2	41	heat	1	3.9	6.7	4	2	2
2	41	control	6	0.2	1.1	1	2	2
2	41	DPCD	6	2.7	2.7	1	2	2
2	42	heat	3	5.3	7.6	4	2	1
2	42	DPCD	3	0.1	8.2	4	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
2	42	control	3	2.2	1	3	2	1
2	43	control	5	0.1	0.2	3	2	2
2	43	DPCD	4	2.5	1.2	3	2	2
2	43	heat	2	0.3	7	4	2	2
2	44	control	4	3.6	4	4	2	2
2	44	heat	4	3.5	5.4	5	2	2
2	44	DPCD	4	3	5.3	5	2	2
2	45	DPCD	6	0.9	3.5	1	1	
2	45	control	7	0.3	0.9	1	1	
2	45	heat	5	0.8	5.4	4	2	1
2	46	DPCD	6	6.9	6.2	4	1	
2	46	heat	5	0.9	4.6	1	2	1
2	46	control	7	8.8	3.5	3	1	
2	47	heat	4	0.7	2.8	3	2	2
2	47	control	4	0	3.2	4	2	2
2	47	DPCD	6	0	6.1	1	2	2
2	48	heat	3	1.7	6.5	4	2	2
2	48	DPCD	6	6.6	4.1	4	1	
2	48	control	8	4	1.8	4	1	
2	49	control	1	8.1	9.9	6	2	2
2	49	DPCD	1	3.8	7.1	6	2	2
2	49	heat	1	0.1	0.2	2	2	2
2	50	control	3	0.1	2.2	4	2	1
2	50	heat	4	0.4	2	4	2	1
2	50	DPCD	4	0.5	2.1	4	2	1
3	1	control	6	5	8.7	4	1	
3	1	DPCD	3	0.1	1.6	3	2	2
3	1	heat	5	0.2	0.3	3	2	2
3	2	control	6	0.3	4.9	3	2	2
3	2	heat	7	0.1	8.9	3	2	2
3	2	DPCD	5	2.6	0	1	2	2
3	3	DPCD	5	1.5	1.4	4	2	2
3	3	control	6	4.1	2	4	2	2
3	3	heat	4	2	6.8	5	2	2
3	4	DPCD	7	10	0.1	2	1	
3	4	heat	5	9.9	9.8	4	2	1
3	4	control	4	10	10	5	2	2
3	5	heat	1	0.1	0.4	2	2	2
3	5	control	1	0.1	0.9	2	2	2
3	5	DPCD	1	0.1	0.3	2	2	2
3	6	heat	1	5.2	1.6	4	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
3	6	DPCD	5	1	5.5	4	2	2
3	6	control	3	1.9	4	2	2	2
3	7	control	2	6.1	2.6	2	2	2
3	7	DPCD	3	0.1	6.2	1	2	2
3	7	heat	3	0.9	3.7	1	2	2
3	8	control	1	0.3	9.6	6	2	2
3	8	heat	4	4.6	1.1	4	2	2
3	8	DPCD	6	1.2	7.3	3	1	
3	9	DPCD	1	5.3	0.6	1	2	2
3	9	control	6	0.3	8.4	1	2	2
3	9	heat	4	5.3	4.3	1	2	2
3	10	DPCD	7	3.5	4.3	3	1	
3	10	heat	3	0.1	5.9	4	2	2
3	10	control	6	0.1	6.3	3	1	
3	11	heat	4	3.8	1.7	4	2	2
3	11	control	6	0.8	0.2	1	2	1
3	11	DPCD	3	0.8	6.7	5	2	2
3	12	heat	2	0.7	0.9	4	2	2
3	12	DPCD	3	4.2	5.1	5	2	2
3	12	control	3	3.5	7.8	4	2	2
3	13	control	2	4	3	3	2	2
3	13	DPCD	5	0.4	5.5	4	1	
3	13	heat	5	2.3	4.9	4	1	
3	14	control	3	0.1	0.1	3	2	2
3	14	heat	3	0.6	2	4	2	2
3	14	DPCD	3	0.1	0.6	4	2	2
3	15	DPCD	5	5.3	4.1	4	2	2
3	15	control	5	4.2	7.3	3	2	2
3	15	heat	5	2.2	6.9	3	2	2
3	16	DPCD	2	7.6	7.2	4	2	1
3	16	heat	5	2.2	1.6	1	1	
3	16	control	2	2.5	1.7	4	2	1
3	17	heat	5	0.6	1.9	2	2	2
3	17	control	7	0	0	1	1	
3	17	DPCD	6	0.3	1	2	2	1
3	18	heat	4	1.1	1	1	2	1
3	18	DPCD	5	1	2	1	2	1
3	18	control	3	0.3	0.6	1	2	1
3	19	control	5	0.1	1	2	2	1
3	19	DPCD	4	0.2	2.7	3	2	2
3	19	heat	3	0.1	4.2	3	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
3	20	control	3	0.3	1.9	4	2	1
3	20	heat	5	1.7	4.5	4	2	1
3	20	DPCD	6	3.4	4.8	4	2	1
3	21	DPCD	6	1	3	3	1	
3	21	control	6	1	4	4	1	
3	21	heat	7	0	5	4	1	
3	22	DPCD	5	0.1	5.9	5	2	1
3	22	heat	3	1	6.6	4	2	2
3	22	control	8	0.5	0.2	2	1	
3	23	heat	5	2.6	0	4	2	1
3	23	control	5	1.7	0.9	4	2	1
3	23	DPCD	6	1.8	1	3	1	
3	24	heat	6	2.5	4.5	3	1	
3	24	DPCD	4	1.5	3.2	2	2	1
3	24	control	7	0.5	6	4	1	
3	25	control	4	0.4	0.2	3	2	2
3	25	DPCD	3	4.8	6.3	4	2	2
3	25	heat	2	5.2	7.8	5	2	2
3	26	control	6	0.3	0.3	1	2	2
3	26	heat	3	5.2	6.2	4	2	2
3	26	DPCD	4	3.4	5.8	2	2	2
3	27	DPCD	5	1.2	5.3	4	2	2
3	27	control	7	0.8	0.1	2	1	
3	27	heat	6	0.8	3	2	1	
3	28	DPCD	6	3	6.7	2	1	
3	28	heat	7	0.3	1.8	1	1	
3	28	control	6	0.8	3	2	1	
3	29	heat	7	0.5	5.5	2	1	
3	29	control	6	0.1	4.3	3	2	2
3	29	DPCD	8	0.2	0	1	1	
3	30	heat	2	9.9	8.3	3	2	1
3	30	DPCD	5	8.9	0.8	4	1	
3	30	control	6	8.6	2.2	3	1	
3	31	control	9	0	0	1	1	
3	31	DPCD	7	0	0.4	2	2	1
3	31	heat	7	0	2.2	3	2	1
3	32	control	1	0.3	5.6	2	2	2
3	32	heat	1	6.4	1.2	2	2	2
3	32	DPCD	1	7.2	8.1	2	2	2
3	33	DPCD	6	1.2	2	1	2	1
3	33	control	6	0.6	2.2	1	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
3	33	heat	6	0.6	3.2	1	2	1
3	34	DPCD	6	4.7	2.2	2	1	
3	34	heat	6	0.1	1.1	1	1	
3	34	control	7	0.1	1.9	1	1	
3	35	heat	6	0.3	4.7	3	1	
3	35	control	5	0.1	5.4	2	1	
3	35	DPCD	4	0.3	4.8	2	1	
3	36	heat	6	7.2	2.5	1	2	1
3	36	DPCD	5	1.4	4.1	1	1	
3	36	control	4	0.3	4.8	1	2	1
3	37	control	4	0	3.2	4	2	2
3	37	DPCD	6	4.9	1.9	1	2	2
3	37	heat	5	1.5	2.3	1	2	2
3	38	control	5	0.8	1.9	4	2	1
3	38	heat	4	4.9	6.9	3	2	2
3	38	DPCD	6	2.5	6.5	2	1	
3	39	DPCD	7	6.4	6.7	2	1	
3	39	control	8	1.1	4.9	1	1	
3	39	heat	6	0.4	3	2	1	
3	40	DPCD	5	0.8	4	2	2	2
3	40	heat	5	0	4.6	3	2	2
3	40	control	6	0	1.5	1	1	
3	41	heat	7	4.7	0.1	2	1	
3	41	control	6	0.1	1	2	1	
3	41	DPCD	7	0.3	3.2	3	1	
3	42	heat	7	0.8	5.2	2	1	
3	42	DPCD	7	1.9	6.2	3	1	
3	42	control	6	2.8	2.5	2	1	
3	43	control	6	0.9	0.3	3	1	
3	43	DPCD	6	0	0.2	4	1	
3	43	heat	6	0.1	0.2	4	1	
3	44	control	4	0	3.7	3	2	2
3	44	heat	3	0	6	4	2	2
3	44	DPCD	6	0	2	1	2	2
3	45	DPCD	5	0.2	4.7	2	2	2
3	45	control	6	0.2	8.1	3	2	2
3	45	heat	4	0.2	8.4	2	2	2
3	46	DPCD	7	0.6	5.9	1	1	
3	46	heat	7	0.8	3.5	1	1	
3	46	control	8	0.7	0.8	1	1	
3	47	heat	7	10	6.1	3	1	

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
3	47	control	7	10	5	3	1	
3	47	DPCD	6	10	9.2	4	2	1
3	48	heat	5	2	6.1	3	1	
3	48	DPCD	4	2	0.5	3	2	2
3	48	control	5	2	3.5	3	2	2
3	49	control	5	8.1	8.6	4	2	2
3	49	DPCD	2	2	9.9	2	2	2
3	49	heat	5	9.6	7.9	3	2	2
3	50	control	4	7	2	2	2	2
3	50	heat	5	4.5	2.5	2	2	2
3	50	DPCD	4	5.1	2	2	2	2
5	1	control	2	7.1	7.7	2	2	2
5	1	DPCD	1	7.7	7.3	2	2	2
5	1	heat	1	7.4	0.2	1	2	2
5	2	control	6	0.5	3	4	1	
5	2	heat	8	4.2	3.8	3	1	
5	2	DPCD	7	6	6.5	3	1	
5	3	DPCD	4	2.5	7.9	5	2	1
5	3	control	5	0.8	4	4	2	1
5	3	heat	4	3	3.7	4	2	1
5	4	DPCD	6	0.1	0.3	1	1	
5	4	heat	6	0.1	0.6	2	1	
5	4	control	6	0.1	0.1	1	1	
5	5	heat	4	0.2	7.2	4	2	2
5	5	control	2	0.3	7	4	2	2
5	5	DPCD	4	3	6.3	3	2	2
5	6	heat	4	1	1	3	2	2
5	6	DPCD	3	2	2	3	2	2
5	6	control	4	1.6	5.8	4	2	2
5	7	control	3	5.2	4.8	4	2	2
5	7	DPCD	5	0.2	5.8	4	2	2
5	7	heat	4	0	3.2	4	2	2
5	8	control	4	1.1	1.2	1	2	1
5	8	heat	3	1.1	3.5	1	2	1
5	8	DPCD	3	0.8	9.1	1	2	1
5	9	DPCD	4	1	3	2	2	2
5	9	control	6	0	0	1	2	1
5	9	heat	7	0	0	1	1	
5	10	DPCD	1	0	3	5	2	2
5	10	heat	1	3.7	3.3	6	2	2
5	10	control	1	1.1	1.4	4	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
5	11	heat	5	2.2	3.2	2	2	2
5	11	control	5	3	1.5	1	2	2
5	11	DPCD	5	2.2	2.8	1	2	2
5	12	heat	7	0.9	1.5	2	1	
5	12	DPCD	5	0.2	4	1	2	1
5	12	control	5	0.9	1.9	2	2	1
5	13	control	7	1	0.2	2	1	
5	13	DPCD	6	0.6	3	2	1	
5	13	heat	7	1.6	2.7	2	1	
5	14	control	6	0.6	1	1	1	
5	14	heat	7	1	1	1	1	
5	14	DPCD	7	0.6	1.5	1	1	
5	15	DPCD	4	1	7	5	2	2
5	15	control	5	0.6	0.5	3	2	1
5	15	heat	4	0.1	4	4	2	2
5	16	DPCD	8	9.9	1.1	1	1	
5	16	heat	8	0.6	5.2	1	1	
5	16	control	8	1.4	1.5	1	1	
5	17	heat	6	0	0.9	2	2	1
5	17	control	7	0	0	1	2	1
5	17	DPCD	8	0	2.2	1	1	
5	18	heat	5	3.7	4.6	5	2	2
5	18	DPCD	5	4.3	4.3	5	2	2
5	18	control	6	1.5	6.1	4	2	2
5	19	control	4	6.1	3.5	3	2	1
5	19	DPCD	5	1	3.5	3	2	1
5	19	heat	5	1.5	3	2	2	1
5	20	control	2	2.3	0.1	1	2	1
5	20	heat	1	0.2	0.1	1	2	1
5	20	DPCD	2	0.2	2.4	2	2	2
5	21	DPCD	5	7.8	6.9	3	2	2
5	21	control	5	4.4	4.7	3	2	2
5	21	heat	5	4.5	5.4	3	2	2
5	22	DPCD	6	9.7	5.8	2	1	
5	22	heat	6	2.2	0.5	2	1	
5	22	control	6	4.2	8	4	2	1
5	23	heat	8	9.9	3.5	1	1	
5	23	control	8	0.5	0.8	1	1	
5	23	DPCD	6	0.8	5.9	2	2	2
5	24	heat	4	0.3	8.9	2	2	1
5	24	DPCD	3	0.7	9.9	2	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
5	24	control	6	0.9	0.7	1	2	1
5	25	control	5	6.6	6.9	3	1	
5	25	DPCD	7	0	3.5	3	1	
5	25	heat	7	3.1	3.5	1	1	
5	26	control	4	5	7.5	4	2	2
5	26	heat	6	5	6	4	2	2
5	26	DPCD	7	5	7	4	2	2
5	27	DPCD	2	0.1	5	6	2	2
5	27	control	1	0.2	5.4	6	2	2
5	27	heat	1	0.3	0.2	6	2	2
5	28	DPCD	1	0.6	0.9	1	2	2
5	28	heat	1	5.6	2.1	2	2	2
5	28	control	2	1.4	0.8	2	2	2
5	29	heat	3	2.9	6.6	3	2	2
5	29	control	6	4.6	1	1	2	2
5	29	DPCD	5	1.5	3.1	2	2	2
5	30	heat	1	0.9	5.8	2	2	2
5	30	DPCD	1	0.1	3.5	3	2	2
5	30	control	2	0.2	4.4	1	2	2
5	31	control	4	4.8	5.2	3	2	2
5	31	DPCD	5	1.5	3	2	2	2
5	31	heat	5	0.1	3.7	2	2	2
5	32	control	3	0.9	1.4	2	2	2
5	32	heat	3	1.5	4.6	3	2	2
5	32	DPCD	3	4.2	1.2	2	2	2
5	33	DPCD	6	1.5	1.2	1	1	
5	33	control	5	0.5	3.6	3	2	1
5	33	heat	5	1.1	1.9	2	2	2
5	34	DPCD	4	0	4.2	4	2	2
5	34	heat	6	3.7	5.8	3	1	
5	34	control	7	0	8.4	3	2	2
5	35	heat	9	1	6.5	1	1	
5	35	control	6	0.7	1.6	3	1	
5	35	DPCD	6	1.7	7.7	2	1	
5	36	heat	3	0.4	1.4	4	2	2
5	36	DPCD	2	0.8	0.8	4	2	2
5	36	control	4	1	3.5	3	2	2
5	37	control	6	2.8	1.3	1	2	1
5	37	DPCD	2	1.1	6.9	3	1	
5	37	heat	4	0.9	1.2	1	1	
5	38	control	3	2.5	7.8	4	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
5	38	heat	1	0.9	9.1	5	2	1
5	38	DPCD	7	0.2	10	4	1	
5	39	DPCD	6	0.1	5.8	2	1	
5	39	control	4	0.1	5.7	3	1	
5	39	heat	6	5.7	8.1	3	1	
5	40	DPCD	7	3.5	0.9	2	1	
5	40	heat	4	0.4	5.8	4	2	2
5	40	control	6	0.1	0.1	1	2	2
5	41	heat	7	0.6	0.2	1	2	1
5	41	control	7	0.3	2.5	2	2	1
5	41	DPCD	6	1	4	3	2	1
5	42	heat	4	1	4.6	4	2	1
5	42	DPCD	5	2.5	2.6	4	2	1
5	42	control	5	1	2.3	3	2	1
5	43	control	6	2.2	0.7	4	1	
5	43	DPCD	9	0.1	0.8	2	1	
5	43	heat	9	1.9	8.8	1	1	
5	44	control	7	0.9	1.1	2	1	
5	44	heat	6	2.5	4	3	2	1
5	44	DPCD	7	2.7	3.2	1	1	
5	45	DPCD	7	1.4	7.4	3	1	
5	45	control	2	6.1	2	4	2	2
5	45	heat	1	1.5	0.1	3	2	2
5	46	DPCD	3	8.4	8.6	4	2	2
5	46	heat	4	1.4	2.7	3	2	2
5	46	control	5	2.1	1.2	2	2	2
5	47	heat	5	6.1	7.6	2	1	
5	47	control	7	5.7	5.3	1	1	
5	47	DPCD	6	2.9	1.4	2	1	
5	48	heat	6	1.1	1.7	4	1	
5	48	DPCD	6	4.9	0.8	3	1	
5	48	control	6	3.5	6.6	5	1	
5	49	control	3	1	9	5	2	2
5	49	DPCD	8	9	2	1	1	
5	49	heat	6	2	2	2	2	2
5	50	control	5	0.3	6.5	4	2	1
5	50	heat	5	0.4	5.7	4	2	2
5	50	DPCD	4	0.5	6.1	4	2	2
9	1	control	6	0.9	2.8	4	2	2
9	1	DPCD	4	0.2	5	3	2	2
9	1	heat	6	0.8	0.8	3	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
9	2	control	4	8	7.6	3	2	2
9	2	heat	5	5.7	8.1	3	2	2
9	2	DPCD	5	8.9	3.5	3	2	2
9	3	DPCD	3	0.6	6.6	5	2	2
9	3	control	5	0.4	1.9	3	1	
9	3	heat	3	0.3	7.2	4	2	2
9	4	DPCD	2	1.1	1.9	4	2	2
9	4	heat	6	0.1	6.3	3	2	2
9	4	control	6	0.5	7.1	3	2	2
9	5	heat	5	0.5	4.9	3	2	2
9	5	control	4	0.2	0.6	2	2	2
9	5	DPCD	4	0.4	5	2	2	2
9	6	heat	3	0.4	7.5	4	2	2
9	6	DPCD	3	0.6	6.9	4	2	2
9	6	control	5	5.8	5.6	3	2	2
9	7	control	9	1	8	4	1	
9	7	DPCD	5	0.1	0.2	3	2	2
9	7	heat	7	0	8	5	1	
9	8	control	7	0.3	0.9	1	1	
9	8	heat	7	0.3	1.4	3	1	
9	8	DPCD	8	3.3	0.9	1	1	
9	9	DPCD	6	2.2	3.8	1	1	
9	9	control	7	1.8	0.3	1	1	
9	9	heat	6	1	4.8	1	1	
9	10	DPCD	7	0.4	2.2	1	2	2
9	10	heat	8	0.8	2.2	1	2	1
9	10	control	8	0	0	1	1	
9	11	heat	7	3.2	2.3	1	1	
9	11	control	7	0.1	0.1	1	1	
9	11	DPCD	5	0.1	8.4	2	2	1
9	12	heat	7	5.7	4.4	3	1	
9	12	DPCD	6	2.2	5.9	2	2	1
9	12	control	5	1	3.5	4	2	2
9	13	control	5	0.3	0.5	2	2	2
9	13	DPCD	5	0.2	0.5	1	2	2
9	13	heat	5	0.3	4.7	1	2	2
9	14	control	3	1.5	0	4	2	2
9	14	heat	2	2.5	2.5	5	2	2
9	14	DPCD	3	0.1	0.5	5	2	2
9	15	DPCD	5	0.1	0.8	2	2	1
9	15	control	5	0.1	0.1	2	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
9	15	heat	5	0.3	0.6	2	2	1
9	16	DPCD	7	4.5	1.9	3	1	
9	16	heat	6	1.3	5.3	2	1	
9	16	control	7	2.3	3.8	2	1	
9	17	heat	8	0	0	2	1	
9	17	control	8	0	0	2	1	
9	17	DPCD	6	1.4	6.7	2	1	
9	18	heat	7	0.6	0.3	1	1	
9	18	DPCD	8	0.5	0.3	1	1	
9	18	control	7	0.4	0.8	1	1	
9	19	control	7	0.1	0	2	2	2
9	19	DPCD	7	2.5	2.8	1	1	
9	19	heat	8	1.2	3	4	1	
9	20	control	6	4.1	4.8	4	2	1
9	20	heat	5	5.8	3.8	3	2	2
9	20	DPCD	6	4.3	1.1	1	1	
9	21	DPCD	3	7.6	0.6	5	2	2
9	21	control	7	9.6	8.8	3	1	
9	21	heat	1	4.1	0.1	6	2	2
9	22	DPCD	2	6.4	7.1	5	2	2
9	22	heat	4	1.1	3.7	3	2	2
9	22	control	5	0.6	7	5	2	2
9	23	heat	3	1.3	4	4	2	2
9	23	control	5	0.3	3.5	2	2	2
9	23	DPCD	5	1.1	2.5	2	2	2
9	24	heat	1	9	10	6	2	2
9	24	DPCD	5	8	2	2	2	2
9	24	control	6	2	3.5	2	2	1
9	25	control	6	2.8	3.5	3	1	
9	25	DPCD	2	0.1	9.2	5	2	2
9	25	heat	7	6.2	2.2	1	1	
9	26	control	5	0.9	2.2	2	2	2
9	26	heat	4	3.8	3.2	3	2	2
9	26	DPCD	3	2	4.3	3	2	2
9	27	DPCD	3	3.5	2.7	5	2	1
9	27	control	4	3.9	6.1	4	2	1
9	27	heat	6	6.3	8.1	3	2	1
9	28	DPCD	6	9.1	4.9	2	2	1
9	28	heat	6	2	7.6	2	2	1
9	28	control	6	2	3	2	2	1
9	29	heat	4	4.8	2.2	4	2	1

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
9	29	control	2	0	8.7	5	2	2
9	29	DPCD	3	1.4	6.7	4	2	2
9	30	heat	8	0.2	1.4	1	1	
9	30	DPCD	5	8.7	6.4	3	2	1
9	30	control	6	4	0.9	2	1	
9	31	control	2	6.2	5.8	4	2	1
9	31	DPCD	6	5	7.8	3	1	
9	31	heat	6	0	7.9	3	2	2
9	32	control	6	7.9	2.2	3	2	1
9	32	heat	6	0.6	7.6	3	2	1
9	32	DPCD	7	0.6	3.5	4	1	
9	33	DPCD	6	1	3	3	1	
9	33	control	4	0.5	6	4	2	2
9	33	heat	6	0.2	5	3	1	
9	34	DPCD	5	0	0.3	1	2	2
9	34	heat	5	0.2	0.6	1	2	2
9	34	control	5	0	0.2	1	2	2
9	35	heat	8	8.4	5.3	4	1	
9	35	control	6	4.9	6.3	6	2	1
9	35	DPCD	7	5	4.8	3	1	
9	36	heat	3	4.8	5.9	4	2	2
9	36	DPCD	4	4.2	4.4	3	2	2
9	36	control	4	1.5	2.5	3	2	2
9	37	control	3	0.2	0.6	4	2	2
9	37	DPCD	2	2.3	3.8	5	2	2
9	37	heat	2	0.7	4.7	5	2	2
9	38	control	7	0.4	0.6	1	1	
9	38	heat	4	0	5.3	4	1	
9	38	DPCD	2	0	10	5	2	2
9	39	DPCD	7	3	2.8	2	2	1
9	39	control	6	7.9	7	4	2	1
9	39	heat	3	2	8	5	2	1
9	40	DPCD	4	0.3	3.3	3	2	1
9	40	heat	6	1.5	3.3	2	1	
9	40	control	7	0.1	0.4	1	1	
9	41	heat	8	1.5	4.5	1	1	
9	41	control	8	2.4	4.2	1	1	
9	41	DPCD	6	0.2	1.9	1	1	
9	42	heat	3	0.2	9.6	5	2	2
9	42	DPCD	5	5.7	0.3	1	2	2
9	42	control	5	6.3	0.3	1	2	2

Table E-1 Continued

Storage time (Weeks)	Judges	Treatment	Overall likeability	Aroma difference from control	Taste Difference from control	Off-flavor	Purchase Intent	Ask again
9	43	control	4	0.1	1.7	5	2	2
9	43	DPCD	3	2.2	9.2	6	2	2
9	43	heat	2	0.1	9.9	6	2	2
9	44	control	4	0.6	2	2	2	2
9	44	heat	5	0.6	2.8	1	2	2
9	44	DPCD	4	0.6	1	2	2	2
9	45	DPCD	5	0	2.6	4	2	2
9	45	control	7	0	7.8	4	2	2
9	45	heat	4	0	0	2	2	2
9	46	DPCD	7	1.1	0.7	4	1	
9	46	heat	2	8.2	8.6	5	2	2
9	46	control	6	0.9	2.3	2	2	1
9	47	heat	5	6.5	4.2	3	2	1
9	47	control	6	2.8	1.1	3	1	
9	47	DPCD	5	1.2	1.4	3	2	1
9	48	heat	6	8.2	3.8	2	1	
9	48	DPCD	7	0.8	5	2	1	
9	48	control	4	3.2	7.5	3	2	1
9	49	control	8	0.1	5	3	1	
9	49	DPCD	3	0.1	9	4	2	1
9	49	heat	5	0.1	4.5	4	1	
9	50	control	3	0.2	5.2	2	2	2
9	50	heat	1	0.2	9.8	5	2	2
9	50	DPCD	7	0.2	3.7	4	1	

[a] Purchase intent: Panelists answering “Yes” to the “Would you buy this product?” question chose score “1” and those answering “No” to the same question chose score “2”.
[b] Ask Again: Panelists choosing score “2” were asked a second question; ”Would you buy this product if you knew its rehydrating properties”. If their answer was “Yes”, they chose score “1” and if “No”, they chose the score “2”. This column is empty if the panelist was not asked the second question.
*Control (untreated); Heat (heat treated at 74^{o}C, 15 s); DPCD (DPCD treated at 34.5 MPa, 25^{o}C, 13%CO_2, 6 min); Storage at 4^{o}C

Table E-2. SAS software output of analysis of variance (ANOVA) for "overall likeability" data for untreated, DPCD and heat treated coconut water by panelists

```
                              The ANOVA Procedure
                        Dependent Variable: likeability
                                          Sum of
Source                      DF         Squares     Mean Square    F Value    Pr > F
Model                      259     1906.118667        7.359532       4.35    <.0001
Error                      490      829.040000        1.691918
Corrected Total            749     2735.158667

                R-Square     Coeff Var      Root MSE    likeability Mean
                0.696895      26.79355      1.300738            4.854667

Source                      DF        Anova SS     Mean Square    F Value    Pr > F
week                         4       47.565333      11.891333       7.03    <.0001
panelist(week)             245     1798.260000       7.339837       4.34    <.0001
treat                        2       29.090667      14.545333       8.60    0.0002
week*treat                   8       31.202667       3.900333       2.31    0.0197

                              The ANOVA Procedure
                    Duncan's Multiple Range Test for likeability
  NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise
error rate.

                            Alpha                          0.05
                            Error Degrees of Freedom        490
                            Error Mean Square          1.691918
                            Number of Means                2          3
                            Critical Range            .2286      .2407
                Means with the same letter are not significantly different.
                     Duncan Grouping         Mean      N     treat
                             A              5.0320    250    control
                             A              4.9520    250    DPCD
                             B              4.5800    250    heat
```

Table E-3. The weekly mean "overall likeability" scores for untreated, DPCD and heat pasteurized samples during storage

Storage time (Week)	Control (Untreated)*	DPCD*	Heat*
0	5.36±0.29	5.34±0.25	4.38±0.31
2	4.38±0.29	4.76±0.24	4.08±0.24
3	5.06±0.27	4.88±0.25	4.68±0.25
5	4.80±0.26	4.90±0.30	4.76±0.32
9	5.56±0.23	4.88±0.25	5.00±0.29

*Mean weekly score ± Std error

Table E-4. SAS software output of analysis of variance (ANOVA) for "aroma difference from control scores" (corrected data) of different treatments during storage study

The ANOVA Procedure
Dependent Variable: aroma

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	224	1449.077612	6.469096	2.08	<.0001
Error	420	1303.573488	3.103746		
Corrected Total	644	2752.651101			

R-Square	Coeff Var	Root MSE	aroma Mean
0.526430	101.7484	1.761745	1.731473

Source	DF	Anova SS	Mean Square	F Value	Pr > F
week	4	4.652651	1.163163	0.37	0.8267
panelist(week)	210	1315.925116	6.266310	2.02	<.0001
treat	2	112.821054	56.410527	18.17	<.0001
week*treat	8	15.678791	1.959849	0.63	0.7514

Duncan's Multiple Range Test for aroma

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05	
Error Degrees of Freedom	420	
Error Mean Square	3.10374	
Number of Means	2	3
Critical Range	.3340	.3516

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	2.1242	215	DPCD
A	1.9181	215	heat
B	1.1521	215	control

Table E-5. The weekly mean "aroma difference from control" scores for untreated, DPCD treated (34.5 MPa, 25^{o}C, 13% CO_2, 6 min) and heat treated (74^{o}C, 15 s) coconut water during storage (4^{o}C)

Week	Control*	DPCD*	Heat*
0	0.99±0.16	2.09±0.40	1.82±0.33
2	1.37±0.21	2.18±0.34	1.77±0.27
3	0.91±0.18	2.09±0.32	1.88±0.32
5	1.35±0.20	2.31±0.43	1.79±0.30
9	1.15±0.20	1.95±0.37	2.34±0.43

*Mean weekly score ± Std error

Table E-6. SAS software output for analysis of variance (ANOVA) for "taste difference from control scores" (corrected data) of different treatments during the storage study

```
                                The ANOVA Procedure Class Level Information
Class    Levels  Values
week        5    0 2 3 5 9
panelist   50    1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
                 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
treat       3    DPCD control heat
                           Number of Observations Read         597
                           Number of Observations Used         597
                                  The ANOVA Procedure
                             Dependent Variable: tastediff
                                      Sum of
Source                      DF       Squares        Mean Square    F Value    Pr > F
Model                      208     2171.954631       10.442090       2.39     <.0001
Error                      388     1698.488317        4.377547
Corrected Total            596     3870.442948
                  R-Square     Coeff Var      Root MSE    tastediff Mean
                  0.561164      63.23168      2.092259          3.308878
   Source                      DF        Anova SS     Mean Square    F Value    Pr > F
   week                         4       40.635925       10.158981       2.32    0.0564
   panelist(week)             194     1591.380356        8.202992       1.87    <.0001
   treat                        2      477.201642      238.600821      54.51    <.0001
   week*treat                   8       62.736708        7.842089       1.79    0.0772
                       Duncan's Multiple Range Test for tastediff
  NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise
                                        error
                           Alpha                        0.05
                           Error Degrees of Freedom      388
                           Error Mean Square        4.377547
                           Number of Means          2          3
                           Critical Range         .4124      .4341

               Means with the same letter are not significantly different.
               Duncan Grouping          Mean     N     treat
                        A             4.1744    199    heat
                        B             3.6744    199    DPCD
                        C             2.0779    199    control
```

Table E-7. The weekly mean "taste difference from control" scores for untreated, DPCD treated (34.5 MPa, 25°C, 13% CO_2, 6 min) and heat treated (74°C, 15 s) coconut water during storage (4°C)

Week	Control*	DPCD*	Heat*
0	1.66±0.26	2.79±0.33	4.37±0.46
2	2.32±0.25	3.99±0.40	4.76±0.41
3	2.21±0.27	3.72±0.42	3.72±0.38
5	2.20±0.29	3.99±0.43	3.45±0.40
9	2.01±0.29	3.92±0.48	4.56±0.47

*Mean weekly score ± Std error

Table E-8. SAS software output for analysis of variance (ANOVA) of "off flavor" scores of different treatments during storage study

```
                              The ANOVA Procedure
                          Dependent Variable: offflavor
                                       Sum of
Source                      DF         Squares     Mean Square    F Value    Pr > F
Model                      259      953.312000        3.680741       4.23    <.0001
Error                      490      425.946667        0.869279
Corrected Total            749     1379.258667

                  R-Square     Coeff Var      Root MSE    offflavor Mean
                  0.691177      33.55391      0.932351          2.778667

   Source                     DF        Anova SS     Mean Square    F Value    Pr > F
   week                        4      18.6186667       4.6546667       5.35    0.0003
   panelist(week)            245     903.9733333       3.6896871       4.24    <.0001
   treat                       2      16.4826667       8.2413333       9.48    <.0001
   week*treat                  8      14.2373333       1.7796667       2.05    0.0395
                      Duncan's Multiple Range Test for offflavor
  NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise
                                       error
                           Alpha                        0.05
                           Error Degrees of Freedom      490
                           Error Mean Square        0.869279
                           Number of Means        2          3
                           Critical Range       .1639      .1725
               Means with the same letter are not significantly different.

                  Duncan Grouping          Mean       N     treat
                        A                2.98800    250    heat
                        B                2.68400    250    control
                        B                2.66400    250    DPCD
```

Table E-9. The weekly mean "off flavor" scores for untreated, DPCD treated (34.5 MPa, 25^{o}C, 13% CO_2, 6 min) and heat treated (74^{o}C, 15 s) coconut water during storage (4^{o}C)

Storage time (Week)	Control (Untreated)*	DPCD*	Heat*
0	2.40±0.18	2.40±0.20	3.14±0.22
2	3.06±0.20	2.72±0.19	3.28±0.17
3	2.60±0.18	2.64±0.17	2.78±0.17
5	2.64±0.19	2.64±0.19	2.64±0.19
9	2.72±0.18	2.92±0.20	3.10±0.21

*Mean weekly score ± Std error

Table E-10. Sample ballots that were used in sensory panels throughout the storage study (Output obtained by Compusense software).

Carbonated Coconut Water

***NOTE: Today's samples contain an artificial sweetener (Sucralose, Brand Name: Splenda). If you are, or suspect you are, allergic, sensitive, or otherwise not able to consume artificial sweeteners, please DO NOT taste today. Thank you.**

Panelist Code: _____

Panelist Name: _____

Question # 1.

Please indicate your gender.

- ❍ Male
- ❍ Female

Question # 2.

Male:
Please indicate your age range.

- ❍ Under 18
- ❍ 18-29
- ❍ 30-44
- ❍ 45-65
- ❍ Over 65

Question # 3.

Female:
Please indicate your age range.

- ❍ Under 18
- ❍ 18-29
- ❍ 30-44
- ❍ 45-65
- ❍ Over 65

Table E-10 Continued

Question # 4 - Sample _____

Review Instructions

Do not taste any of the samples at this time. The first test will be smelling the samples. Please read the directions on the next screen.

You are being presented with a reference sample marked 000. Please SMELL the reference sample. Then SMELL sample %01 and compare it to the reference sample. Please mark how different the sample SMELLS from the reference sample on the line scale.

Sample Aroma

Not Different At All — Very Different

Question # 5 - Sample _____

Review Instructions

Take a bite of cracker and a sip of water to rinse your mouth.

The next 4 questions are related to your tasting experince. Please make the sample last, we have limited source. Click on the 'Continue' button below.

You are being presented with a reference sample marked 000. Please TASTE this sample. Then TASTE sample %01 and compare it to the reference sample. Then mark how different the sample TASTES from the reference sample on the line scale.

Taste Difference

Not Different At All — Very Different

Question # 6 - Sample _____

How much do you like the sample %01 OVERALL?

Sample %01

dislike extremely			neither like nor dislike			like extremely		
1	2	3	4	5	6	7	8	9

Question # 7 - Sample _____

Table E-10 Continued

Please rate the intensity of the OFF FLAVOR, if any.

Off-flavor

None	Just Detectable	Slightly Detectable	Moderately Intense	Very Intense	Extremely Intense
1	2	3	4	5	6

Question # 8 - Sample _____

Would you buy this product?

❍ Yes
❍ No

Question # 9 - Sample _____

Would you buy this product if you knew coconut water had rehydrating properties?

❍ Yes
❍ No

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BIOGRAPHICAL SKETCH

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