Effects of high pressure processing on changes of bioactive compounds in honeydew melon juice and honeydew melon juice milk

by

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Abstract

Honeydew melon is a rich source of bioactive compounds, such as vitamin C and group B vitamins while milk is a rich source of vitamin E. The objective of this thesis is to investigate the changes of quality attributes and bioactive compounds in honeydew melon juice and melon milk using High Pressure Processing (HPP) treatments of 300-600 MPa/20 and 60 ºC/5 min. The total phenolics and total flavonoids content were analyzed by spectrometry. The retention of vitamin C, B3, folic acid and α-tocopherol were quantified using high performance liquid chromatography (HPLC). The total antioxidant capacity was determined by 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) inhibition assay and ferric reducing antioxidant power (FRAP) assay. Overall, HPP treatments had different effects on melon juice and melon milk matrices. In melon juice, significant increases of vitamin C content, and color parameters of greenness and lightness were observed, indicating an extraction effect of HPP. Folic acid was well retained by protection of vitamin C. Niacin showed variable results at different pressures. A decrease of total flavonoids content was found after HPP at 20 °C. In honeydew melon milk (semi-skim), HPP treatments decreased total phenolics content and total flavonoids content. And no significant effects were found in the retention of ascorbic acid, niacin, α-tocopherol and total antioxidant capacity. In honeydew melon milk (whole), significant increases were found in total phenolics content, ascorbic acid and α-tocopherol. Based on the positive effects of HPP on the quality attributes and most bioactive compounds contents of honeydew melon juice and melon milk (whole) and the minor effects of HPP on bioactive compounds contents of melon milk (semi-skim), HPP seems a promising technology for honeydew melon juice and melon milk (whole) beverages.

Acknowledgements

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

Ascorbic acid	Ascorbic acid
CFU	Colony forming unit
DPPH	2,2-Diphenyl-1-pic-ryl-hydrazyl
Folic acid	Folic acid
FRAP	Ferric reducing antioxidant Power
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
HPP	High pressure processing
M.F.	Milk fat
MMS	Melon milk (semi-skim)
MMW	Melon milk (whole)
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
ORAC	Oxygen radical absorbance capacity
PEF	Pulsed electric field
PME	Pectin methylesterase
PPO	Polyphenol oxidase
QE	Quercetin equivalent
TAC	Total antioxidant capacity
TEAC	Trolox equivalent antioxidant capacity

Chapter 1. Introduction

1.1 Rationale

Fruit juices and milk are the top popular drinks around the world. It was reported that adults in the USA consumed 0.36 servings (250 mL per serving) of fruits juices and 0.69 servings of milk per day (State News Service, 2015). An investigation showed that intake of fruit juice (apple, grape, blueberry and pomegranate) can prevent inflammation and oxidative stress caused by ingestion of high-energy or high-fat meal for overweight adults (Miglio et al., 2014). Other studies also showed -based products is beneficial for preventing lipid peroxidation (Prior, 2014), reducing plasma cholesterol and triglycerides (Peluso et al., 2014) and maintaining vascular health (Ellis et al., 2011). Traditional juice pasteurization uses a time-temperature scheme, usually of 85–92°C for 10–15 s (Ramaswamy et al., 1992). Pasteurization using heat is helpful for extending the shelf life, but detrimental for sensorial and nutritional properties and induces chemical reactions (Miller & Silva, 2012).

As a potential replacement of thermal pasteurization, High Pressure Processing (HPP) (400 to 600 MPa in commercial use) is able to inactivate same vegetative microorganisms and reduce the activity of enzymes (Gupta & Balasubramaniam, 2012). HPP-treated products available in the USA and Europe include fruit juices (orange, mango and banana), vegetable juices (pumpkin, cucumber and spinach), sliced ham, sauces, guacamole, oysters and onions (Patterson, 2005; Cano & de Ancos, 2004). HPP was shown to retain bioactive compounds (vitamin C, group B vitamins, carotenes and polyphenols) and preserve sensorial properties to a level comparable to

fresh products (Plaza et al., 2011; Carbonell-Capella et al., 2013).

Honeydew melon is one of the dominant fresh-cut fruits served all year round in North America (Saftner et al., 2006). The minimal processed honeydew melon is likely to lose firmness and flavor during storage (Supapvanich & Tucker, 2011). Chemical sanitation method using chlorine is able to extend the shelf life of honeydew melon pieces to 15 days (Ayhan & Chism, 1998). Due to the high sweetness (10% sugar content) and low acidity (pH=5.6), honeydew melon is highly perishable and hard to preserve. Currently, no honeydew melon juice is available in the beverage industry. Therefore, our study is the first to process honeydew melon into a juice product and treat it with HPP to investigate the retention of bioactive compounds, namely ascorbic acid, niacin, folic acid, α-tocopherol, total phenolics (total phenolics content), total flavonoids content and total antioxidant capacity (TAC).

1.2 Hypothesis

Main hypothesis are:

- HPP can preserve or improve the bioactive compounds contents in the honeydew melon juice and juice milk.
- The effects of HPP on each bioactive compound might be different among three samples depending on the food matrix

1.3 Objectives

The main objective of this thesis is to investigate the effects of HPP on the bioactive

compounds in honeydew melon juice and juice milk. The specific objectives are:

- To determine the retention of total phenolics, total flavonoids and other quality attributes change (pH and color parameters) in melon juice and melon juice milk after HPP treatments.
- To determine the retention of vitamins (vitamin C, vitamin B3, folic acid and vitamin E) in melon juice and melon milk after HPP treatments.
- To evaluate the total antioxidant capacity of melon juice and melon milk after HPP treatments.
- To compare the retention of bioactive compounds in HPP treated juice (semi-skim) milk and HPP treated juice (whole) milk.

Chapter 2. Literature review

2.1 Honeydew melon

Cucumis melo is a polymorphic species that has seven different cultivars, among which C. *melo* var. *reticulatus* Naud (cantaloupe) and C. *melo* var. *Inodorus* Naud (honeydew melon) are of commercial importance in North America.

Cantaloupe has a netted and warty surface, while honeydew melon has a smooth surface and does not form an abscission zone on the surface until it becomes overripe (McCreight, Nerson & Grumet, 1993). The flesh of cantaloupe is orange and it emits characteristic melon flavor or musty flavor. However, honeydew melon has white-greenish flesh, without a musky odor (McCreigh et al., 1993).

Honeydew melon is famous for its distinctive flavor and high content of bioactive compounds (Wyllie, 1995). The production season for honeydew melon in USA ranges from May to September. During off-season time, honeydew melons are available via importing from Mexico and Central America.

Honeydew melon is one of the widely consumed fruits in North America (Aguayo Escalona & Artés, 2004). According to the USDA Agricultural Statistics Board, the consumption of honeydew melon in the USA in 2011 was 0.68 kg per capita, which is comparable to the consumption of cauliflower and asparagus. Mostly in North America, honeydew melon is minimally processed which main products include cylinders (Portela & Cantwell, 1998), cubes

or slices (Lamikanra et al., 2000).

2.1.1 Bioactive compounds in honeydew melon

2.1.1.1 Vitamins

According to the USDA National Nutrient Database for Standard Reference (2015), honeydew melon contains water soluble vitamins as shown in Table 2-1, which are quantitively comparable to cantaloupe. However, honeydew melon has low concentrations of carotenoids compared with cantaloupe (Seymour & McGlasson, 1993). No vitamin B_{12} or vitamin D was found either in honeydew melon or cantaloupe.

Table 2-1 Composition of two melon cultivars.

Nutrient	Unit (per 100 g)	Honeydew melon	Cantaloupe
Water	g	89.82	90.15
Protein	g	0.54	0.84
Total lipid (fat)	g	0.14	0.19
Carbohydrate	g	9.09	8.16
Fiber, total dietary	g	0.8	0.9
Sugars, total	g	8.12	7.86
Water-soluble vitamins			
Vitamin C (ascorbic acid)	mg	18.0	36.7
Thiamin	mg	0.038	0.041
Riboflavin	mg	0.012	0.019
Niacin	mg	0.418	0.734
Vitamin B-6	mg	0.088	0.072
Folate, DFE	µg	19	21
Fat-soluble vitamins			
Vitamin A, RAE	µg	3	169
Vitamin A, IU	IU	50	3382
Vitamin E (alpha-tocopherol)	mg	0.02	0.05
Vitamin K (phylloquinone)	µg	2.9	2.5

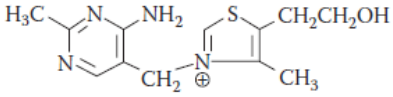
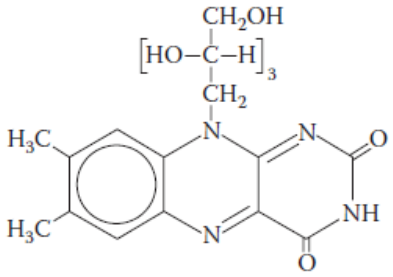
Adapted from the US Department of Agriculture (2015)

DFE: Dietary folate equivalent; RAE: Retinal activity equivalent; IU: International unit.

The diverse profile of bioactive compounds leads to a high antioxidant capacity in honeydew melon (Salandanan et al., 2009). It was reported that fruits that have high antioxidant capacity are efficient in scavenging causative agents of a great number of human diseases, like reactive oxygen species (Halliwell, 1996).

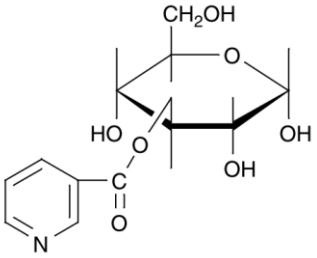
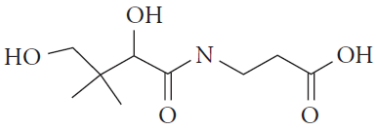
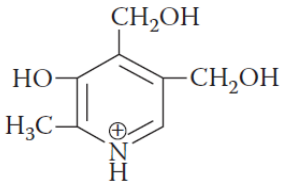
Vitamins are a critical group of bioactive compounds in fruits, which are generally divided into water-soluble and fat-soluble vitamins. Some vitamins functions in human metabolism are listed in Table 2-2.

Table 2-2 Molecular structures, properties and functions of vitamins (Adapted from Ötleş, 2005)

Vitamin	Structure and properties	Functions in metabolism
B_1 (thiamin)	 MW = 265.35 g/mol	Co-enzyme functions in energy metabolism (the conversion of protein, carbohydrates, and fat into energy). Facilitates the formation of acetylcholine for neural function. A role in detoxification and heart functions.
B_2 (riboflavin)	 MW=376.37 g/mol pKa = 9.888	Essentially functions in numerous oxidation and reduction reactions. Supports hormone production, energy production, neurotransmitter function and the production of red blood cells.

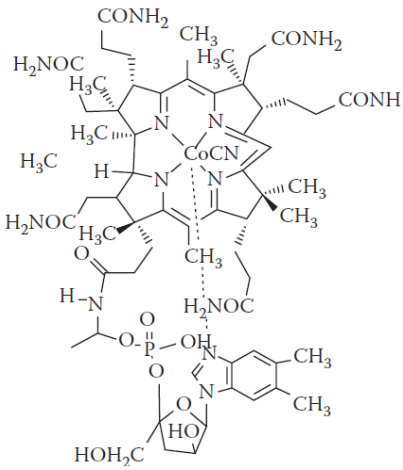
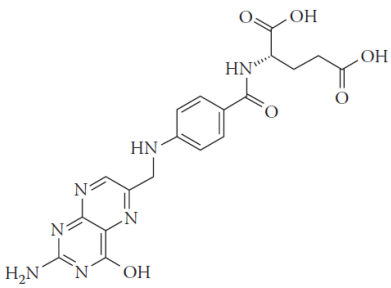
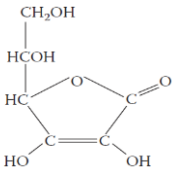
MW: molecular weight; MP: melting point; BP: boiling point.

Table 2-2 (Continued)

Vitamin	Structure and properties	Functions in metabolism
B_3 (nicotinic acid)	 MW = 123.11 g/mol $pKa_1 = 2.0$ $pKa_2 = 4.85$ MP = 237 °C	Exists within the redox-active co-enzymes, nicotinamide adenine dinucleotide (NAD), which function in dehydrogenase-reductase systems requiring transfer of a hydride ion Functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as lactate, alcohol.
B_5 (pantothenic acid)	 MW = 219.24 g/mol pKa = 4.41 MP = 183.8 °C	Co-substrate/co-enzyme for hydrogen transfer with numerous dehydrogenases. Funtions in intracellular respiration, which is vital for energy release.
B_6 (pyridoxine)	 MW = 169.18 g/mol MP = 159 - 162 °C	Important in protein synthesis and the manufacture of hormones, red blood cells, and enzymes. Serves as a carbonyl-reactive co-enzyme involved in the metabolism of amino acids. Involved with conversion of the omega-6 essential fatty acids and sphingoid base biosynthesis, which play a role in hormone health.

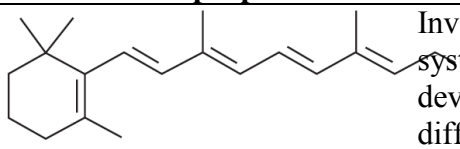
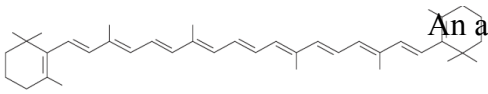
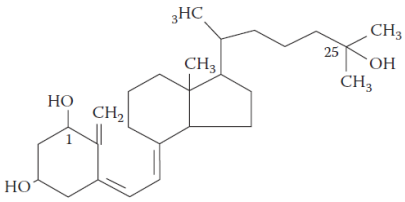
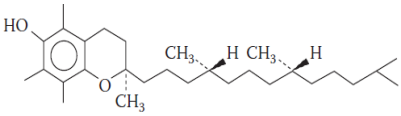
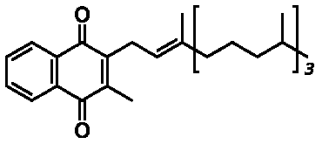
MW: molecular weight; MP: melting point; BP: boiling point.

Table 2-2 (Continued)

Vitamin	Structure and properties	Functions in metabolism
B_{12} (cyanocobalmin)	 MW = 1355.38 g/mol MP > 300 °C	Needed for normal red blood cell formation and neurological functioning. Aids in the replication of the genetic code within each cell. Involved in the processing of carbohydrates, protein, and fats.
Folic acid (or folate)	 MW = 441.40 g/mol pKa = 4.65(1^{st}), 6.75(2^{nd}), 9.00(3^{rd}) MP = 250 °C	Essential role in DNA synthesis. Regulates cell division and the transfer of inherited traits from one cell to another. Reduces the risk of neural tube birth defects. Heart health—helps regulate blood levels of homocysteine.
C (ascorbic acid)	 MW = 176.12 g/mol MP = 190 - 192 °C	An important antioxidant that facilitates hydroxylation reactions. Helps protect cells against damage caused by free radicals. Strengthens immune system (antibodies and white blood cells). Involved in collagen formation and helps iron absorption.

MW: molecular weight; MP: melting point; BP: boiling point.

Table 2-2 (Continued)

Vitamin	Structure and properties	Functions in metabolism
A (retinol)	 MW = 286.46 g/mol MP = 62 - 64 °C	Involved in normal eyesight, immune system response, embryonic development and cellular differentiation. Helps skin health and development of bones and teeth
β-carotene	 MW = 536.89 g/mol MP = 176 – 184 °C	An antioxidant. Precursor of vitamin A
D (calciferol)	 MW = 400.64 g/mol	Precursor of steroid hormones involved in regulating calcium and phosphate homeostasis, which is essential for bone and teeth strength.
E (α-tocopherol)	 MW = 430.71 g/mol MP = 2.5 – 3.5 °C BP = 200 – 220 °C	Fat-soluble antioxidant. Essential in membrane lipids to prevent oxidation of polyunsaturated fatty acids. Reduces oxygen requirement of muscles. Prevents degeneration of nerves and muscles.
K (phylloquinone)	 MW = 450.70 g/mol	Required for post-translational modification of glycoproteins, which is responsible for the synthesis of blood-clotting proteins. Involved in bone mineralization and the regulation of blood calcium levels.

MW: molecular weight; MP: melting point; BP: boiling point.

2.1.1.2 Phenolic compounds

Phenolic compounds are a complex group of secondary metabolites in fruits and chemically structured as a hydroxyl group bound to an aromatic ring (Bravo, 1998). The subclasses of phenolic compounds and their basic structures are shown in Table 2-3. A majority of phenolic compounds are biosynthesized from several components via either shikimate pathway or polyketide pathway and they naturally occur in the form of esters (Lee, 2004). Various phenolic compounds contribute to the taste, flavor and color of foods and exhibit antioxidant activity preventing lipid peroxidation (Lee & Widmer, 1996). Studies showed that dietary polyphenols contributed as one of the main sources of antioxidants for human health (Graf, Milbury & Blumberg, 2005) and related to intestinal health that prevented colon cancer in human being (Cardona et al., 2013). Guo, Xu, Wei, Yang & Wu (2008) found quercetin, kaempferol, apigenin, luteolin and myricetin in honeydew melon.

Flavonoids, structured as diphenylpropane derivatives, are the major polyphenol compounds in honeydew melon (Guo et al., 2008). As shown in Table 2-4, flavonoids mainly consist of five groups – flavanols, flavonols, flavones, flavanones and anthocyanins (Bravo, 1998). They are either sugar-free (aglycones) or glycosylate. Moieties including polysaccharides, amines and lipids have been found esterified to flavonoids (Bravo, 1998).

More than 50 different flavonoids were found in the species *Cucumis melo*, which is the family of honeydew melon (Rodríguez-Pérez, Quirantes-Piné, Fernández-Gutiérrez & Segura-Carretero, 2013).

Table 2-3 Basic skeletons of phenolic compounds (Adapted form Bravo, 1998)

Class	Basic skeleton	Class	Basic skeleton
Simple	C_6	Chromones	C_6-C_3
Benzoquinones	C_6	Naftoquinones	C_6-C_4
Phenolic acids	C_6-C_1	Xanthones	C_6-C_3
Acetophenones	C_6-C_2	Stilbenes	C_6-C_2-C_6
Phenylacetic acids	C_6-C_2	Anthraquinones	C_6-C_2-C_6
Hydroxycinnamic acids	C_6-C_3	Flavonoids	C_6-C_3-C_6
Phenylpropenes	C_6-C_3	Lignans	$(C_6$-$C_3)_2$
Coumarins, isocoumarins	C_6-C_3	Lignins	$(C_6$-$C_3)_n$

C_6 means aromatic ring

Quercetin was found to be the most abundant flavonoid (5.85-6.82 mg/100 g fresh weight) in honeydew emlon, which is comparable with that in apple and lemon (Guo et al., 2008). Other flavonoids distributed in honeydew melon include kaempferol, apigenin, luteolin and myricetin in low amounts (<0.40 mg/100g weight).

Table 2-4 Chemical structures of flavonoids (Adapted from O'Connell & Fox, 2001)

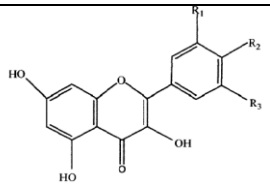
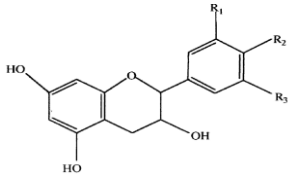
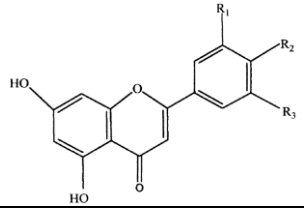
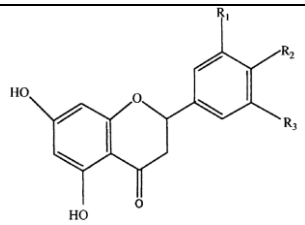
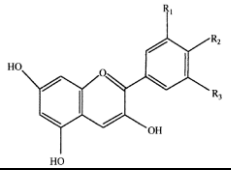
Flavonoids	Example	Structure
Flavonols	Quercetin, Kaempferol, Myricetin	
Flavanol	Catechin	
Flavones	Apigenin, Luteolin, Chrysoeriol	

Table 2-4 (Continued)

Flavonoids	Example	Structure
Flavanones	Hesperetin, Naringenin, Eriodictyol	
Anthocyanidins	Peonidin, Delphinidin, Cyanidin	

2.1.1.3 Antioxidant activity

Oxygen causes biomolecules oxidation in foods, and induces a series of oxidation reactions within the cells. Lipid constituents in foods, especially unsaturated fatty acids, have one or more allyl groups and are ready to oxidize into hydroperoxides through either autoxidation or lipid peroxidation catalyzed by lipoxygenase (Belitz, Gosch & Schrieberle, 2009). This reaction leads to off flavor, stale flavor, rancidity or other aroma changes.

In the human body, cellular redox process generates by-products, including reactive oxygen species and reactive nitrogen species, which have an unpaired electron. An imbalance between reactive species and antioxidant defense system produces oxidative stress (Singh, Kapoor & Bhatnagar, 2015) and causes oxidation of lipids, proteins and DNA with cell damage, which is related to the cardiovascular disease and cancer (Sen & Chakraborty, 2011).

The steps involved in autoxidation of lipids and the reaction with its inhibitors are shown in Figure 2-1, assuming one antioxidant scavenges two radicals and the oxygen is in large amount (R_2N_2: azo compounds; LH: substrate; AH: antioxidant)

Initiation

$$R_2N_2 \rightarrow 2R^{\cdot} + N_2 \quad (2.1)$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (2.2)$$

$$ROO^{\cdot} + LH \rightarrow ROOH + L^{\cdot} \quad (2.3)$$

Propagation

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad (2.4)$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad (2.5)$$

$$LO^{\cdot} + LH \rightarrow LOH + L^{\cdot} \quad (2.6)$$

Branching

$$LOOH \rightarrow LO^{\cdot} + {}^{\cdot}OH \quad (2.7)$$

$$2LOOH \rightarrow LOO^{\cdot} + LO^{\cdot} + H_2O \quad (2.8)$$

Termination

$$2L^{\cdot} \rightarrow \text{nonradical products} \quad (2.9)$$

$$LOO^{\cdot} + L^{\cdot} \rightarrow \text{nonradical products} \quad (2.10)$$

$$LOO^{\cdot} + LOO^{\cdot} \rightarrow \text{nonradical products} \quad (2.11)$$

Figure 2-1 Fundamental steps in the autoxidation reaction

The formation of peroxy radical ($LOO^{\cdot}$), alkoxy radical ($LO^{\cdot}$) and alkyl radical ($L^{\cdot}$) can be inhibited by intake of dietary antioxidants, which donate a hydrogen atom to the unpaired electron (Huang, Ou & Prior, 2005), as shown in Fig. 2-2.

Inhibition

$$LOO^{\cdot} + AH \rightarrow LOOH + A^{\cdot} \quad (2.12)$$

Figure 2-2 Inhibition of free radical by antioxidant

In fruit juices, the major antioxidants include phenolic compounds, ascorbic acid, tocopherols and carotenoids (Gardner, White, McPhail & Duthie, 2000). The rate constants of these antioxidants with the reactive species are presented in Table 2-5. However, studies showed that the presence of antioxidant compounds and their concentrations in different fruit juices have different contributions to the antioxidant potential (Gardner et al., 2000; Hertog et al., 1993; Pulido et al., 2000).

Based on the FRAP assay, Gardner et al. (2000) found that ascorbic acid accounted for more than 65-100% of TAC in citrus fruit juice, including orange and grapefruit juice while ascorbic acid contributed with less than 5% of TAC in apple and pineapple juice. For non-citrus fruit juice, it was shown that phenolic compounds had more contribution to the TAC. The major phenolic compounds in non-citrus fruit juices contain flavonoids, anthocyanins, chalones and hydroxycinnamic acids (Hertog et al., 1993). Among all polyphenols, quercetin showed the highest total antioxidant activity because of the presence of catechol-type structure (an o-dihydroxy substitution pattern on the aryl ring) and the presence of a 2,3-double bond in conjugation between two aryl rings (Pulido et al., 2000).

Table 2-5 Rate constants of reactions of reactive oxygen species with food constituents (Belitz et al., 2009)

Antioxidant	Free radical			
Vitamin	**$^1O_2$**	**$HO^{\cdot}$**	**O^-_2**	**$HOO^{\cdot}$**
β-Carotene	5.0×10^9			
Riboflavin	6.0×10^7	1.2×10^{10}		
Ascorbic acid	1.1×10^7	8.2×10^9		1.6×10^4
Vitamin D	2.3×10^7			
α-Tocopherol	13.2×10^7		No reaction	2.0×10^5

2.1.1.4 Phenolic-protein interactions

Phenolic compounds from different fruits or tea have interactions with proteins in milk, which may change the conformation of protein molecules (O'Connell & Fox, 2001). The phenolic group, as a source of hydrogen donor, helps form hydrogen bonds with the carboxyl group of the protein. Some phenolic compounds are small enough to penetrate inner protein structures, while large phenolic compounds can crosslink peptide chains at different points of the protein molecules (Mulaudzi, Ndhlala, Kulkarni & Staden, 2012). Besides the indigenous phenolic compounds in milk, milk can also be added with phenolic compounds, referred as exogenous phenolic compounds. Exogenous phenolic compounds were found to affect organoleptic properties and nutritious functions of milk or dairy products (O'Connell & Fox, 2001) due to the interactions between phenolic compounds and milk constituents.

The interaction between phenolic compounds and proteins include reversible and irreversible interactions (Ozdal et al., 2013). The reversible interactions mainly involve non-

covalent forces, whereas irreversible interactions are featured for the formation of covalent bonds. It was found that quercetin combined with soy protein by covalent bonds shifted the isoeletric point to a lower pH (from pH 5 in control to pH 3.5 in the derivatives) and affected the solubility of the soy protein (Rawel, Czajka, Rohn & Kroll, 2002). Another study found that cross-linking occurred between quercetin and gelatin, which improved the mechanical strength and reduced swelling of gels (Strauss & Gibson, 2004).

As a result of covalent and non-covalent interactions, flavonoids are able to bind with macronutrients (proteins, carbohydrates and lipids) (Bordenave et al., 2014). Riihimäki et al. (2011) reported that all flavonoids (hesperidin, luteolin, vitexin, kaempherol, myricetin, quercetin and genistein) slightly bound with β-lactoglobulin regardless of the pH used (pH 2-10).

Covalent binding of flavonoids and proteins resulted from the reaction between reactive amino acid residues and quinones formed by phenolic oxidation under specific conditions, like exposure to alkaline pH, high heat and reactive oxygen species. An *ortho*- or *para*-diphenol in the phenolics molecules is converted to corresponding *o*- or *p*-quinone. The extremely electrophilic nature of *o*-quinones can form crosslinks with N-terminal α-amino- and ε-lysine side chains that are nucleophilic in proteins (Matheis & Whitaker, 1984). Kaempferol, quercetin and myricetin were found to have higher reactivity compared with gallic acid and caffeic acid, while quercetin was the most reactive flavonoid (Rawel et al., 2002). The interaction between phenolic compounds-formed quinones and reactive amino acid residues were used to inhibit enzymes like rennin and prevent the dissociation of κ-casein from the casein micelles

(O'Connell & Fox, 2001). Therefore, it was recommended to add certain phenolic compounds into liquid milk to increase the heat stability of milk (Morgan et al., 1971).

Non-covalent binding, such as hydrogen bonding and hydrophobic force, is more dominantly between flavonoids and defined globular tertiary structure like milk immunoglobulins or non-globular/non-enzymatic proteins like milk β-casein (Bordenave et al., 2014). An increase of pH was found to improve the binding between quercetin and bovine serum albumin, because the low pH induced more negatively charged molecules, which had more affinity to quercetin (Rawel et al., 2005). High temperatures (60 and 90 °C) were found not favorable for the interaction between quercetin and proteins due to the denaturation of proteins.

Rawel et al. (2005) found that the covalent binding of phenolic compounds affected both the secondary and tertiary structures of proteins whereas the non-covalent binding had no influence on the secondary structure of proteins but affected the tertiary structure.

2.2 Beverages

2.2.1 Fruit juice

Fruit juice is becoming increasingly popular because of its health benefits, convenience and pleasant sensory properties (Zulueta, 2010). Various studies have demonstrated the prevention effects against diseases related to the consumption of fruit products that have vitamin C, carotenoids, polyphenols and minerals, such as K, Mg, Ca and P (Simon et al., 2001; Sánchez-Moreno et al., 2003).

2.2.2 Cow milk

Cow milk is highly consumed in North America and is a good source of proteins, vitamins and minerals. Milk contains diverse antioxidants, such as vitamin E, β-carotene, isoflavones, formononetin, selenium and peptides (Öste & Anderson, 1997). It was shown that α-tocopherol is the major form of vitamin E in cow milk (Pehrson & Hakkarainen, 1986). The selenoprotein glutathione peroxidase in cow milk has antioxidant capacity by degrading lipid peroxides while lactoferrin acts as an antioxidant by binding pro-oxidative iron ions (Lindmark-Mansson & Akesson, 2000). The low molecular-weight fractions like urate were also found to contribute to the ferric-reducing activity of whey in cow milk (Chen et al., 2003).

2.2.3 Mixed fruit juice milk beverages

Research and market trends show that milk-base fruit beverages are ideal products for delivering bioactive ingredients (Zulueta, 2010). Mixed juice milk beverages are becoming appealing as a new functional and ready-to-drink product (Zulueta et al., 2007). The study by Sharma (2005) shows that fruit juice-milk products are becoming common in the USA and Europe markets. It was proved that mixed juice milk beverages are an excellent source of bioactive compounds, such as vitamin C, vitamin B group and polyphenols with potential antioxidant capacity (Andrés, Villanueva, Mateos-Aparicio & Tenorio, 2014).

2.3 Processing technologies

2.3.1 Thermal treatment

Pasteurization is required for milk juice and dairy products for two reasons: one is to reduce viable bacteria that are harmful to human health in order to guarantee the safety of the products; the other is to extend the shelf life of the products. Thermal processing is the major pasteurization technology applied in fruit juice, dairy products and as well in mixed fruit juice milk beverages (Andrés et al., 2014).

It was reported that pasteurization caused up to 66% loss of vitamin A, D and E in milk (Miller, Jarvis & McBean, 2007). Studies showed that thermal treatment induced damage for vitamin C, phenolics (Klopotek, Otto & Bohm, 2005). Other studies also reported that thermal treatment (90 °C for 60 or 30 s) had detrimental effects on flavonols and anthocyanins (Dubrović, Herceg, Jambrak, Badanjak, & Dragović-Uzelac, 2011; Odriozola et al., 2008). Thus innovative technologies are needed to process fruit juice and mixed juice milk beverages to preserve bioactive compounds.

Currently, thermal treatment (85–92°C, 10–15 s) is commonly used in fruit juice processing to prevent enzymatic and microbial deterioration and produce shelf-stable products. However, thermal treatments compromise sensorial and nutritional attributes of juice products (Miller & Silva, 2012).

For example, according to Chaikham, Apichartsrangkoon, & Seesuriyachan (2014), HPP treatment of 500 MPa at 25 ^{o}C for 30 min induced a higher retention of bioactive compounds in longan juice than a thermal treatment at 90 ^{o}C/2 min. Ascorbic acid was retained at 93% after

HPP whereas the retention was only 44% after the thermal treatment.

Table 2-6 Effects of thermal treatment on vitamins in milk

Vitamin	Treatment	Mean difference	Reference
A	72°C, 15 s	-2.07×10-14 mg/L	Bector & Rani (1998)
	72°C, 15 s	+0.101 mg/L	Panfili et al. (1998)
	60°C, 30 min	No significant difference	
B_1	72°C, 15 s	-11.90%	Haddad & Lowenstein (1981)
	80°C, 16 s	-9.40%	
	110°C, 3.5 s	-7.90%	
	80°C, 15 s	-1%	Sierra & Vidal-Valverde (2000)
	90°C, 18 s	-1%	
B_2	60°C, 30 min	No significant difference	Bendicho et al. (2002)
	72°C, 15 s	-0.90%	Haddad & Lowenstein (1981)
	80°C, 16 s	-1.50%	
	110°C, 3.5 s	-2.10%	
C	72°C, 15 s	-4.2 mg/L	Haddad & Lowenstein (1981)
	80°C, 16 s	-5.32 mg/L	
	110°C, 3.5 s	-6.32 mg/L	
	72°C, 15 s	+3.82 mg/L	Pizzoferrato (1992)
	75°C, 15 s	+4.58 mg/L	
	80°C, 15 s	+6.55 mg/L	
	85°C, 15 s	+25.46 mg/L	
	90°C, 15 s	+5.86 mg/L	
E	63°C, 30 min	No significant difference	Bendicho et al. (2002)

+: increase; -: decrease

Similarly, the antioxidant capacity in the longan juice was higher (78.98%) after HPP treatment than that after the thermal treatment (66%). Other bioactive compound contents, such as gallic acid and total phenols were found to improve after the HPP treatment due to the extraction effects compared with the decrease after thermal treatment. Another example (Odriozola et al., 2008) reported that *p*-hydroxybenzoic content was enhanced slightly from 0.39 to 0.43 mg/100 mL while ellagic acid was substantially reduced from 4.5 to 4.1 mg/100 mL just after treatment of 90 °C for 60s. And the retention of phenolic acids was lower after thermal treatment at 90 °C/60s (43.6 mg/100 mL) than in the fresh sample (47.3 mg/100 mL). It was also

reported that 90 °C/60s treatment significantly decreased the content of anthocyanins (cyaniding-3-glucose and pelargonidin-3-glucoside), probably caused by hemiacetal base formed by flavylium cation. And the loss of anthocyanins was more than 50% after 56 days storage. It was found that the flavonol content (kaempferol, quercetin and myricetin) remained the same just after thermal treatment, whereas a decrease occurred during its storage. The vitamin C content was found to be 94-95% after thermal treatment at 90°C/60s.

Besides, previous studies found thermal treatments induced more decrease in the content of bioactive compounds of fruit juices compared with nonthermal technologies. Dubrović, Herceg, Jambrak, Badanjak, & Dragović-Uzelac (2011) found that the loss (5.3-5.8%) after thermal pasteurization (85°C/2 min) was higher than the loss (0.7-4.4%) after using different high intensity ultrasound conditions (temperatures of 25, 40 and 55 °C, amplitudes of 60, 90 and 120 μm and times of 3, 6, and 9 min) on the anthocyanins content in strawberry juice. Another study reported that the decrease of vitamin C content in thermal treated (90°C/60s) strawberry juice (below 24 mg/100 mL) was higher than the Pulsed Electric Field (PEF) treated sample (data not reported) (Odriozola et al., 2008).

2.3.2 High Pressure Processing (HPP)

High pressure processing (HPP) is one of the most innovative technologies that have been used instead of thermal processing in the food industry. It uses elevated pressures (100 to 900 MPa) with or without heat to inactive enzymes and certain types of food born microorganisms (Sukhmanov, Shatalov, Petrova, Birca & Gaceu, 2014). Since HPP technology uses high

hydrostatic force of a pressurized medium, it instantaneously affects the food matrix despite of the shape or size of the sample (Bridgman, 1958).

According to Martinez-Monteagudo & Saldaña (2014) (Table 2-7), HPP highly affects intermolecular forces including electrostatic bonds, van der Waals forces, hydrogen bonds, solvation and hydrophobic bonds, whereas it does not impact covalent bonds. Therefore, macromolecules like proteins that have hydrophobic bonds are more likely to be affected by HPP, whereas small mollecules like vitamins that only have covalent bonds are minimally affected by HPP (Balasubramaniam, Farkas & Turek, 2008).

HPP has the following advantages compared with traditional thermal processing:

(i) HPP implies all-direction effects and uniform transition through the product. Compared with thermal processing, HPP treatment is more uniform and does not cause the problem of gradient effects (Palou, Lopez-Malo, Barbosa-Canovas & Swanson, 2004).

(ii) As high pressure has no effects on covalent bonds (Plaza et al., 2011; Uckoo et al., 2013), HPP preserves bioactive compounds (vitamins and flavonoids) and maintains the natural sensory properties (flavor and color) of products (Polydera et al., 2005) whereas traditional thermal treatment at atmosphere pressure may destroy nutritional attributes and cause undesirable loss of flavor and color.

(iii) HPP is independent of the ratio of time/mass, so it acts instantaneously which reduces the processing time (Balasubramaniam, Ting, Stewart & Robbins, 2004).

(iv) HPP is environmently friendly because there is no waste generation (Zulueta, 2010).

Based on above, HPP is a promising technology to treat melon juice and melon milk. However, limitations of HPP were also pointed out as:

(i) Some enzymes (polyphenol oxidase and peroxidase) in foods are very resistant to pressure, so the HPP treatment has to be carried out at very high pressures or in combination with heat (Zulueta, 2010).

(ii) Products treated by HPP need to be in refrigeration storage and low temperature distribution to retain the sensory and nutritional attributes (Barba et al., 2013).

The investigation of HPP on food products firstly started in milk (Hite, 1899). Japan became the first to use HPP technology in the food industry supported by the Ministry of Agriculture in 1980's and then HPP treated food products began to be commercialized during 1990's (Zulueta, 2010). HPP has been used by the juice industry since 1990. Currently, there are various food industrial plants operating with HPP, available HPP juice products in the market include orange, apple, cantaloupe and watermelon juices. However, no HPP research was undertaken for honeydew melon yet. Therefore this thesis will be the first to investigate HPP effects on honeydew melon.

Table 2-7 Pressure effects on intermolecular forces based on distances (Adapted from Martinez-Monteagudo & Saldaña, 2014)

Type of interaction	Working distance (nm)	Effects by pressure
Electrostatic bond	20	Highly affected
van der Waals	1-20	Highly affected
Hydrogen bond	0.2	Affected
Solvation	>2	Affected
Hydrophobic bond	>2	Affected
Covalent bond	0.2	Not affected

2.4 Effects of HPP on microbial safety in juices

HPP treatments of 300-600 MPa at ambient temperature are able to inactivate vegetative cells, such as yeast and moulds, whereas the inactivation of pathogens and spoilage microorganisms requires temperatures of 45-50 °C (Knorr, 1995). Depending on pH, compositions, water activity and microorganism types in foods, HPP treatments may require higher pressure, higher temperature and longer holding times.

For example, Health Canada (2014) announced a new HPP fruit smoothie (pH=3.8-4.2), which can reach microbiological specifications of coliform count less than 10 CFU/mL, *Esherichia coli* count lower than 10 CFU/mL, yeast count less than 104 CFU/mL and mold count less than 104 CFU/mL after an HPP treatment at 593 MPa/ambient temperature/2 min. However, for raw bovine milk which has a more complex nutrients profile and more pathogen types, including *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and other species (Hahn, 1996), HPP treatments are required to be 200 MPa/20 °C/4 h to significantly reduce coliforms by 10^6 CFU/mL and HPP treatment at 600 MPa/55 °C/5 min to reduce aerobes mesophiles by 4.5 log CFU/mL (Huppertz et al., 2006).

Therefore, HPP treatments at ambient temperature is enough to pasteurize fruit juice products, whereas HPP combined with temperature is better to pasteurize low acid (pH>4.6) foods, like milk (Rastogi et al., 2007).

2.5 Effects of HPP on plant cells

2.5.1 Plant cell structure

The structure of a plant cell is mainly composed of a cell wall, a plasma membrane, a nucleus, a central vacuole, cytoplasm and specific plastid types (Figure 2-3). The characteristic that differentiate fruit cells from other plant cells is that intracellular space in fruit cells has a large vacuole (Figure 2-3), a watery compartment that contains bioactive compounds such as organic acids and polyphenols, which provide flavor, color and other features to fruits (Shiratake & Martinola, 2007). The vacuole is surrounded with a membrane called tonoplast. Because of the presence of the various solutes in the big vacuole, the consequent turgidity leads to the turgor pressure of cells. Cell walls, as the most durable organic material, counteract the turgor pressure (Carpita & McCann, 2000).

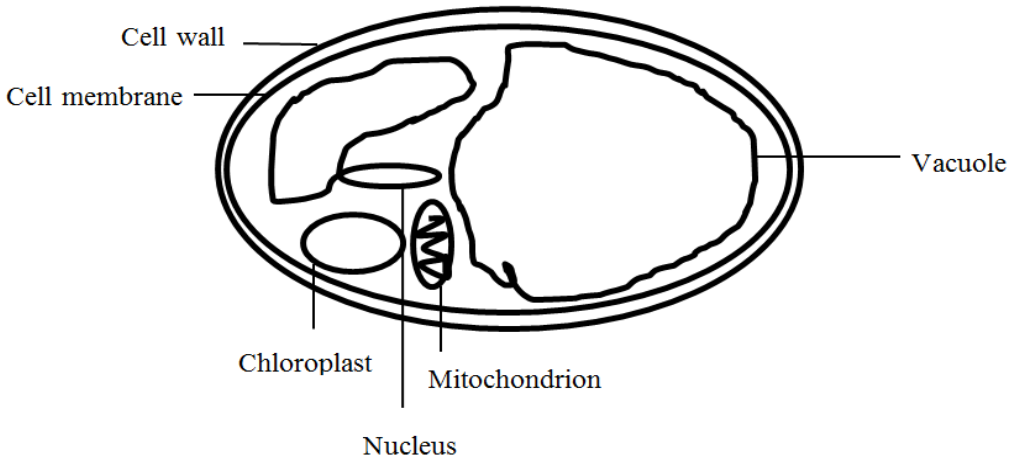


Figure 2-3 A schematic representation of a single plant cell

Cell walls are generally structures with various polysaccharide components. The stress-bearing fibrils – cellulose are paralleled and linearly embedded in a pressure-bearing consisting of hemicelluloses and pectins. Pectin mollecules that contain galacturonic acid are cross-linked by Ca^{2+} bridges, forming a second net. The third net structure in primary cell walls consists of cross-linked glycoproteins (Moerschbacher, 2002). This inner surface of cell wal is lined with cell membrane, which forms the boundary outside the plasma and is attached or embedded with proteins (Coyle, 2005).

The structure of cell walls and cell membranes provides the support and texture for fruits and protects intracellular compounds from leaking (Carpita & McCann, 2000). In addition, recent research results show that phenolics are associated with cell wall polysaccharides (Saura-Calixto, 2012), which might be bioaccessible (release from the food matrix) and bioavailable (absorbed in the small intestine) after digestion (Saura-Calixto et al., 2007).

2.5.2 Effects of HPP on plant cell walls

During food processing, various technologies might affect cell structure and modify cell permeability (Moerschbacher, 2002). It is reported that optical microscopy results showed that HPP treatments are able to cause loss of turgor pressure and loss of cell wall integrity in carrots (Basak & Ramaswamy, 1998) and cauliflower and spinach (Prestamo & Arroyo, 1998). Since cellular structures of fruits are much fragile and soft, HPP are likely to increase cell wall

permeability (Hills et al., 2005).

The effects of HPP on cell structures depend on the types of compartments in plant cells, the treatment time and processing temperature (Furfaro et al., 2009; Dörnenburg & Knorr, 1998; Sila et al., 2008). Studies showed that cell walls are the most resistant in plant cells: it is found that plasmalemma membranes in potato tissues were ruptured by HPP treatments at 100-500 MPa/room temperature/15 min, whereas cell walls were intact after these treatments (Hills et al., 2005). Tonoplasts in onion cells were ruptured by HPP treatment of 100 MPa/room temperature/30 min, at which cell walls were not affected (Butz et al., 2004). The destruction of cell membranes was because proteins embedded in the cell membranes are affected by pressure treatments (Dörnenburg & Knorr, 1998) and as well HPP resulted in crystallization of phospholipids in the cell membranes (Rastogi, 2009). The rupture of plasma membranes and tonoplasts then led to the destruction of cell walls and the release of bioactive compounds from vacuoles (Dörnenburg & Knorr, 1998). As well, the increased cell walls permeability caused texture degradation and certain biochemical reactions (Rastogi, 2009). It was reported that pulse HPP treatments of 100-300 MPa at 35 °C induced cell wall damage in pineapple and mass transfer was enhanced due to the increased cell permeability (Rastogi & Niranjan, 1998). The study by Tangwongchai et al. (2000) showed that HPP treatments at 200-400 MPa/25 °C/20 min damaged tomato tissues by observing with scanning electron microscope.

By detecting the changes of compositions (cellulose, hemicellulose, vacuolar water, pectins, proteins and starch) in cell membranes using NMR cross-correlation relaxometry with optical

microscopy, it was shown that HPP had an impact on plant cell permeability caused by both the breakdown of cell wall compartments and the denaturation of proteins on cell membranes, which is attached to cell walls (Dörnenburg & Knorr, 1998; Butz et al., 2004; Hills et al., 2005; Sila et al., 2008). This pressure induced rupture of cells, which affected both the texture of products and the biosynthesis and release of metabolites during processing (Dörnenburg & Knorr, 1998). Krebber et al. (2002) reported that the green color in HPP treated green beans became more intense due to the leakage of intracellular chlorophyll compounds.

Marigheto, Vial, Wright & Hills (2004) observed cell rupture and loss of natural angular morphology on cell walls in strawberry juice after HPP treatment of 100 MPa/5 min at room temperature. And, major tissue was observed after the HPP treatment of 300 MPa/5 min. Kurenda et al. (2014) reported that HPP treatments of 100-200 MPa/10-20 °C/10 min induced cell disintegration, loss of turgor and transformation of cell walls of apple fruit using methods of chlorophyll fluorescence, VIS/NIR spectroscopy as well as biospecle and backscattering. Angersbach, Heinz & Knorr (1997) reported lower cell viability in grape (*V. vinifera*) cell cultures after HPP treatment at 100 MPa/5 min/25 °C by observing a reductase activity of 56.4% and a cell permeability of 5.4% compared with untreated sample.

2.6 Effects of HPP on vitamins, phenolics and total antioxidant capacity in juices

Since the HPP effects on nutritional properties are greatly dependent on food matrix, pH and water activity (Balasubramaniam, Farkas & Turek, 2008), studies are investigating the

influence of HPP on bioactive compounds in various fruit juices (De Ancos, Sgroppo, Plaza & Cano, 2002; Sánchez-Moreno et al., 2005). Popular fruit juices that have been researched using HPP include fruits that have soft structure (strawberry juice), fruits that are intense in color (carrot juice, tomato juice and berry juice) and fruits that have high in vitamin content (orange juice).

2.6.1 Strawberry juice

Strawberry, as a rich source of anthocyanins, has been under HPP research for a long time. Sancho et al. (1999) reported that 200/400/600 MPa/ 25 °C/30 min had no significant effect on the pyridoxal and thiamin content either in the strawberry coulis or the multivitamin system (ascorbate, thiamin and pyridoxal). Vitamin C level in the multivitamin system had a significant ($p<0.05$) decrease of 11.42 -12.17%. Ascorbic acid level in the strawberry coulis had no significant change at 200 and 600 MPa while 400 MPa caused 11.32% reduction on the ascorbic acid content. Comparatively, pasteurization at 0.1 MPa, 72 °C for 20 s caused 8.75% decrease on the ascorbate content in strawberry coulis while about 32.9% reduction was reported for the sterilized sample (0.1 MPa, 120 °C for 20 min).

Using HPP treatments of 700 MPa at 60, 90 and 110 °C for 0 - 250 min, Verbeyst, Bogaerts, Plancken, Hendrickx & Loey (2013) found at 700 MPa, anaerobic degradation in HPP treated sample above 90 °C while anaerobic degradation in 0.1 MPa treated samples only occurred above 120 °C. Therefore, the use of a temperature below 90 °C would be helpful to avoid vitamin C degradation.

Patras, Brunton, Da Pieve & Butler (2009a) studied the change of ascorbic acid content and TAC of strawberry and blackberry purées subjected to HPP treatment of compared with the thermal treated sample. HPP treatments at 600 MPa resulted in the highest retention of ascorbic acid content (94%) among all treatments (400-600 MPa/10-30 °C/15 min), while thermal (70 °C/2 min) treated strawberry purée had a loss of 22.6% of ascorbic acid content. All HPP treatment had higher retention of TAC in strawberry purée than the thermal treated sample, whereas there was no significant difference in the retention of TAC in blackberry purée after different treatments. HPP treatment at 500 and 600 MPa significantly increased the total phenolics content in strawberry purée compared with the untreated one while 400 MPa treated sample had no significant change. The same result was reported for blackberry purée.

Terefe et al. (2013) found that the content of bioactive compounds strongly depend on the cultivars cultivars (Camarosa, Festival, and Rubygem) in HPP treated strawberry purée. The total phenolics content in all cultivars treated by HPP (600 MPa/20 °C/5 min) decreased between 9-24% whereas thermal (88 °C/2 min) treated sample had a total phenolics content loss of 14-24%.

2.6.2 Apple juice

Baron, Dénes & Durier (2006) found that the hydroxycinnamic acid in apple juice (106 mg/L in untreated sample) became significantly higher after HPP treatments of 200-300MPa/20 °C/5-10 min (115-123 mg/L) and the highest was found after treatment of 400 MPa/20 °C/10 min (139 mg/L). The procyanidins content (69 mg/L in untreated sample) increased after all HPP treatments (75-117 mg/L). The dihydrochalcones (21.3 mg/L in untreated

sample) were also stable after all HPP treatments (20.7-23.5 mg/L) except increased after the treatment of 400 MPa/20 °C/10 min (26.2 mg/L). However, catechin content (9.1 mg/L in untreated sample) changes after HPP treatments (2.7 to 26.6 mg/L) probably due to oxidation where the oxidative products inhibited the PME activity.

Valdramidis, Graham, Beattie, Linton, McKay, Fearon & Patterson (2009) reported a vitamin C reduction of 4.8-8.5 mg/100 g in apple juice after HPP treatment of 350-550 MPa/20 °C/1-25 min.

2.6.3 Orange juice

Using HPP treatments of 50-350MPa/30 and 60 °C/ 2.5-15 min, De Ancos, Sgroppo, Plaza & Cano (2002) found that HPP caused significant improvement of carotenoids content in orange juice at 100 and 350 MPa and no significant change occurred at 50 and 250 MPa. A corresponding increase of carotenoids occurred as the holding time increased from 2.5 to 15 min. 50-350 MPa/30 °C/5 min treated orange juice presented lower free radical-scavenging (DPPH) capacity than that of untreated sample. The lowest DPPH inhibitation occurred at 350 MPa/30 °C/5min.

Bull et al. (2004) reported that the initial amounts of ascorbic acid in orange juices (Valencia and Navel) were 406 mg/L and 498 mg/L, respectively, which were not significantly affected by HPP treatments (600 MPa/ 18-20 °C/1min).

Sánchez-Moreno et al. (2005) found no significant effect on the ascorbic acid content in

orange juice by HPP treatment of 400MPa/40 °C /1 min, while a significant increase occurred in the content of carotenoids (α-carotene increased by 33.76%, β-carotene increased by 30.24%, α-cryptoxanthin increased by 45.87%, β-cryptoxanthin increased by 43.21%, zeaxanthin increased by 44.52%, and lutein increased by 75.43%) and flavanones (hesperetin increased by 20.16% and naringenin increased by 39.88%). In contrast, thermal pasteurization (70 °C/30s) exerted no change on all types of carotenoids evaluated in the orange juice sample and pasteurization at 90 °C/1 min only increased some types of carotenoids (R-cryptoxanthin increased by19.19%, zeaxanthin increased by 37.49% and lutein decreased by 23.10%). In terms of flavanone, an increase of 28.12% of narirutin was observed with no change of hesperetin.

Fernández García, Butz, Bognar & Tauscher (2001) reported that vitamin C contents in both orange juice and orange-lemon-carrot juices were not affected by 500 and 800 MPa treatments for 5 min (temperature not reported). The authors reported that the retention of L-ascorbic acid in orange juice was higher than that in orange-lemon-carrot juice.

2.6.4 Tomato juice

Comparing HPP treatments of 100-500 MPa and 4/20/50 °C for 10 min and thermal treatment (60 °C/2 min and 92 °C/2 min), Hsu (2008) found that pressure of 300-500 MPa induced substantial increase in the total carotenoids up to 62% and increased in lycopene content up to 60%.

Patras, Brunton, Da Pieve, Butler & Downey (2009b) found thatHPP treated (400- 600

MPa/ambient temperature/15 min) tomato purée had higher values for total phenols (337.36 ±15.31 ~ 371.73±15.15 mg GAE/100g) compared with thermal treated sample (341.13 ±4.83 mg GAE/100g). Thermal treatment (70 °C for 2 min) and HPP at 400 and 500 MPa were found to significantly reduce the content of total carotenoids and ascorbic acid, whereas HPP at 600 MPa increased the content of total carotenoids by 172% and had a relatively high retention of ascorbic acid (93.7%) compared with the untreated sample. The TAC measured by the DPPH assay after HPP of 500-600 MPa/ambient temperature/15 min was higher (TEAC of 33-37 mg/L) than those of a thermal treated sample (TEAC of 32 mg/L) and untreated sample (TEAC of 27 mg/L).

2.6.5 Melon and watermelon juice

Wolbang, Fitos & Treeby (2008) reported a loss of vitamin C in three melon (*Cucumis melo* L.) cultivars (Northern Sky, Southern Cross and Chantele) occurred during cutting and packing before HPP treatment of 600 MPa/ambient temperature/10 min. Both vitamin C content and ferric ion reducing capacity (FIRC) decreased during HPP treatments and the reduction of vitamin C greatly depended on the cultivar, whereas FIRC was not affected by cultivar. In contrast, the β-carotene content increased after HPP treatment compared with untreated sample (data not reported).

Zhang, C., Trierweiler, B., Li, W., Butz, P., Xu, Y., Rufer, C., Ma, Y. & Zhao, X. (2011) found that HPP treatments of 600 and 900 MPa for 5-60 min at 60 °C were effective to reduce the browning in treated watermelon juice, whereas the thermal treatment (60 °C/5-60 min) significantly increased the browning degree. The concentration of all-trans-lycopene (17.0-25.1

nmol/L) was found to be lower after thermal and all HPP (300-900 MPa/60 °C/5-60 min) treatments compared with untreated sample (27.8 nmol/L). At the same, the content of all-trans-lycopene was higher after HPP treatment than thermal treatment, which was explained by the extraction effects of high pressure.

2.6.6 Grapefruit juice

Uckoo et al. (2013) reported that HPP treated (400 MPa/31.8 °C/3 min) sample had significantly higher content of ascorbic acid than thermal treated (85 °C for 45 s) sample and were similar to the degree of ascorbic acid in fresh samples (data not reported). Thermal treated sample had more reduction of ascorbic acid compared with HPP treated sample (data not reported). No significant variation was observed for the contents of β-carotene and all-trans-lycopene in the HPP treated samples. With respect to flavanones (narirutin, naringin, neohesperidin, didymin and poncirin), there was no significant change found after HPP treatments. However thermal treated samples had a significant increase in the contents of flavanones compared with untreated sample (narirutin increased from 7.01 to 8.01 mg/100 mL; naringin increased from 21.26 to 23.24 mg/100 mL; neohesperidin increased from 0.79 to 0.96 mg/100 mL).

2.6.7 Blueberry juice

In a study of blueberry juice treated by HPP at 200/400/600 MPa and 42 °C for 5/9/15 min, Barba, Esteve & Frigola (2012) observed a retention of over 92 % of ascorbic acid after HPP treatment at 400 and 600 MPa while HPP treatment at 200 MPa preserved all the ascorbic acid

content. Phenols in blueberry juice after treatment had an increment of 13-27% after 200 MPa for 5/9/15 min and an increment of 24% after 400MPa/15min compared with the fresh juice. However, the antioxidant capacity measured by TEAC dramatically decreased from 32.2 μmol/g to 26.3 and 26.9 μmol/g after 600MPa/5 and15 min, respectively.

2.6.8 Bayberry juice

In a study of Chinese bayberry juice treated by HPP at 400/500/600 MPa for 10 min, Yu et al., (2013) found that the pH for HPP treated samples (pH ~ 2.84) was more stable than untreated sample (2.74-2.82) during storage of 8 days at 4 °C. The content of ascorbic acid after treatments at 500 and 600 MPa/10 min at room temperature had retention above 96% of the initial content (21.21 mg/100 mL) while HPP treatment at 400 MPa resulted in a significant decrease in the ascorbic acid content (17.26 mg/100 mL).

2.6.9 Fruit smoothie

Keenan, Roble, Gormley, Butler & Brunton (2012) studied HPP (450MPa/20 °C/5min and 600 MPa/20 °C/10 min) treated juice smoothie (strawberry, apple, banana and orange) and found that TAC in samples treated at 400MPa/20 °C/5min was similar to fresh sample, which was higher than 600MPa/20 °C/10min treated sample. And, the 400 MPa treated sample had a 15% higher level of total phenolic contents than the 600 MPa treated sample.

2.6.10 Effects of HPP on vitamins and total antioxidant capacity in milk

To date, HPP has not been applied in the dairy industry, with the exception of some

cheese-based spreads in Australia (Datta & Deeth, 2011). Some studies have been conducted in HPP or Pressure assisted thermal pasteurization (PATP) treated dairy products about microbial and enzymes inactivation (Udabage et al., 2010), protein (Gaucheron et al., 1997), fatty acids (Martinez-Monteagudo, 2014; Kim et al., 2007), lactose (Martinez-Monteagudo & Saldaña, 2015), volatile compounds (Sampedro et al., 2009; Valdez-Fragoso et al., 2011) or chemical reactions like acidification (Famelart et al., 1997). However, few studies are available on the effects of HPP or PATP on the vitamins and TAC.

Mainville, Montpetit, Durand & Farnworth (2001) studied the effects of HPP (200 MPa for 1.5 s, 300 MPa/1.5 s and 400 MPa/ 1.5 s/5 min/30 min at 5 °C) on the inactivation of microflora and microstructural changes in kefir, and compared with kefir autoclaved (110 °C/ 3 min), irradiation (1, 2, 3, and 5 kGy) and ohmic heating (50/60/70 °C for 10s) treatment. It was found that HPP treated kefir had no physical change, while autoclave caused a clumped mass after treatment. Autoclave, irradiation at 5kGy, ohmic heating at 72 °C and HPP at 400 MPa for 5 or 30 min were all able to inactivate bacteria and yeasts completely in kefir. It was revealed by scanning electron microscopy that kefir had become visibly thicker with small molecules intact after HPP treatment.

Chen et al. (2003) showed that TAC measured by ABTS (reduction of the cation radical of 2,20-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid)) did not change significantly during heating at 63 °C for 0-60 min, while TAC (ABTS) in the whey samples increased after the same heating treatment and became 20-50% higher at 60 min than the initial value. In the study by

Cilla et al. (2011), TAC in fruit juice (grape, orange, apricot and peach) (9299 μM/L Trolox Equivalent) mixed with milk (11%, V_{milk}/V_{juice}) became lower (6617 μM/L Trolox Equivalent), but TAC value increased significantly in fruit juice mixed with milk after 45 days of storage at 2-4 °C (7285 μM/L Trolox Equivalent) whereas TAC in single fruit juice decreased after the same storage period (7254 μM/L Trolox Equivalent).

Table 2-8 indicates that HPP in previous research of fruit juice were mainly using pressures of 0-800 MPa, temperatures of 0-40 ºC and holding time of 0-60 min. The results of HPP treated juice showed that vitamin C content was not affected or reduced by no more than 12% (strawberry juice); carotenoids contents were significantly increased; vitamin B group (pyridoxal and thiamin) contents were not significantly affected; phenolic compounds contents were improved by 3-30% and TAC had different results in different research.

Table 2-8 HPP effects on vitamins in fruit juice beverages

Treatment	Food matrix	Effects on vitamins	Reference
200/400/ 600 MPa ambient T 30 min pH=3.64	Strawberry coulis	No significant effect on pyridoxal and thiamin contents. Ascorbic acid had no obvious change at 200/600 MPa while at 400 MPa had 11.32% reduction.	Sancho et al. (1999)
400/500/600 MPa 10, 30 °C 15 min	Strawberry and blackberry purée	No significant effects on ascorbic acid. HPP had higher retention of TAC in strawberry purée than thermal treatment, while no such difference occurred in blackberry purée. Blackberry purée had a higher TAC value than that in strawberry purée. 500 MPa and 600 MPa significantly accelerated total phenolics content in strawberry and blackberry purée. 400 MPa treated sample had no significant change in phenolics	Patras et al. (2009a)
350-550 MPa 20 °C 1-25 min pH=3.3	Cloudy apple juice	Vitamin C decreased after HPP and degraded during storage at 4, 8 and 12 °C for 36 days.	Valdramidis et al. (2009)
200–600 MPa 20 °C 5-10 min pH=3.5	Cloudy apple juice	Hydroxycinnamic acid increased from 106 to 115-139 mg/L after all treatments. Procyanidins increased from 69 to 75-117 mg/L after all treatments. Dihydrochalcones were stable after all HPP treatments and catechins had various changes after different HPP treatments.	Baron et al. (2006)
400 MPa 40 °C 1 min	Orange juice	45.19% increase of carotenoid content	Plaza et al. (2011)

Table 2-8 (Continued)

Treatments	Food matrix	Effects on vitamins	Reference
350 MPa 30 °C 5min	Orange juice	Significant increase in the amounts of carotenoids (α-carotene increased by 60%, β-carotene increased by 50%, α-cryptoxanthin increased by 63%, β-cryptoxanthin increased by 42%). No further increase in carotenoids was observed when the holding time increased up to 15 min.	de Ancos et al. (2002)
400MPa 40 °C 1min, pH=3.59	Orange juice	Increased the content of carotenoids (α-carotene increased by 33.76%, β-carotene increased by 30.24%, α-cryptoxanthin increased by 45.87%, β-cryptoxanthin increased by 43.21%, zeaxanthin increased by 44.52%, and lutein increased by 75.43%).	Sánchez-Moreno et al. (2005)
600 MPa 1 min Room T pH=4.3 (Valencia) pH=3.7 (Navel)	Valencia and Navel orange juice	Ascorbic acid, cryptoxanthin and β-carotene contents in both samples were not significantly affected by HPP.	Bull et al. (2004)
500/800 MPa 5 min pH=3.42	Orange juice and orange-lemon-carrot (OLC) juice	Vitamin C was not affected after HPP treatment for both samples but had a reduction of 5-23% during storage at 4 °C for 21 days. TAC of orange juice had 84% retention after 21 days of storage at 4 °C. TAC of OLC juice was higher than that in orange juice after 21 storage days of storage at 4 °C.	Fernández García et al. (2001)
300-500MPa 4 and 25 °C 10 min, pH=3.8-4.2	Tomato juice	Total carotenoids increased by 62% and lycopene contents increased by 60%.	Hsu (2008)

Table 2-8 (Continued)

Treatments	Food matrix	Effects on vitamins	Reference
400-600 MPa Ambient T 15 min	Tomato purée	Total phenolics increased from 341. To up to 371.73 mg GAE/100 g. Total carotenoids were reduced after HPP at 400-500 MPa but increased by 172% after HPP at 600 MPa. Ascorbic acid was reduced by HPP at 400-500 MPa but had retention of 93.7% after HPP at 600 MPa.	Patras et al. (2009b)
600 MPa 10 min	Melon (*Cucumis melo* L.) juice	Vitamin C and FIRC were reduced after HPP and β-carotene content increased after HPP (data not reported).	Wolbang et al. (2008)
400 MPa 31.8 °C 3 min, pH=3.58	Grapefruit juice	β-carotene and all-trans-lycopene had no significant change.	Uckoo et al. (2013)
200/400/600 MPa 42 °C 5-15 min, pH=2.95	Blueberry juice	Vitamin C retention was more than 92 % after HPP, 13-27% higher of phenols. TEAC decreased from 32.2 μmol/g to 26.3 and 26.9 μmol/g.	Barba et al. (2012)
300/600/900 MPa 5/20/40/60 min 30 °C	Watermelon juice	All-trans-lycopene and total lycopene were reduced with increasing pressure and holding time.	Zhang et al. (2011)
300-500 MPa 20 ºC 0.5-2.5 min pH=2.98-3.02	Pomegranate Juice	Total phenolics content increased by 3.38-22.99%.	Varela-Santos et al. (2012)
400-600 MPa 20 °C 5min	Smoothie (strawberry, apple, banana and orange)	450 and 600 MPa have similar retention of ascorbic acid (98.7 and 90.5 mg/100mL) as in fresh one (81.13 mg/100mL).	Keenan et al. (2011)

Table 2-8 (Continued)

Treatments	Food matrix	Effects on vitamins	Reference
63 °C 0-60 min pH=5-6	Milk	TAC (ABTS) of milk had no significant change, while TAC (ABTS) in the whey increased after the same treatment (20-50% higher at 60 min than the initial value).	Chen et al. (2003)

Previous studies were commonly conducted on fruit juices and milk separately and not many studies used the mixture of juice and milk, which led to the objective of combining honeydew melon juice and milk of different fat contents in this thesis. HPP treatments of fruit juices in previous research mainly used mild temperatures. This thesis aimed to investigate the effects of both room temperature (20 ºC) and a temperature of 60 ºC during HPP treatments. According to Balasubramaniam et al. (2004), the temperature of sample increased by 3-8 ºC/100 MPa because of adiabatic heating during HPP treatment.

Chapter 3. Materials and Methods

3.1 Chemicals

Acetonitrile (HPLC grade), and methanol (HPLC grade) were purchased from Fisher Scientific (Ottawa, ON, Canada). Isopropanol (purity>99.7%), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), dichloromethane (HPLC grade), hexane (HPLC grade), Folin-Ciocalteu reagent, sodium carbonate ($NaCO_3$), gallic acid (GA), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$), acetic acid ($C_2H_4O_2$), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 2,2-Diphenyl-1-pic-ryl-hydrazyl (DPPH), quercetin, ascorbic acid, nicotinic acid (niacin), riboflavin, folic acid, thiamine, β-carotene, α-tocopherol, potassium hydroxide (KOH) and sodium chloride (NaCl) were purchased from Sigma Aldrich (St. Louis, MO, USA)

3.2 Sample preparation

Honeydew melon fruit, *Cucumis melon var.inodorous* from Del Monte Company in Guatemala was purchased from a local market (Safeway, Edmonton, AB, Canada). Among, one melon was selected based on its uniformity (no mechanical damage and no disease) and external color, which is a greenish-white and medium matured. The honeydew melon was washed in sanitizing solution using chlorine bleach (200 ppm, v/v) for one minute according to the procedure reported by Troller (1983). Then, the fruit was peeled, seed removed and cut into

cubes. Melon juice was obtained by extracting the juice from melon cubes using a domestic juice extractor (67900, Hamilton Beach, ON, Canada). The extractor was cleaned using ethanol before its use. The extracted juice was then transferred into a blender and mixed uniformly. The prepared sample was immediately chilled at 4 °C until treatment.

Raw (whole) milk and semi-skim (2% M.F.) milk were provided by Lucerne Milk Plant (Edmonton, AB, Canada). Melon milk, whole (MMW) was obtained by mixing melon juice with raw milk (1:1, v:v) and then homogenized in a blender (Hamilton Beach, Picton, ON, Canada) for 1 min. The same procedure was used for melon milk, semi-skim (MMS). The nutritional composition of milk used in the melon milk was provided by Lucerne Company (Table 3-1). For the HPP treatments, three samples (melon juice, MMW and MMS) were used. All prepared samples were immediately chilled at 4 °C until treatment.

Table 3-1 Nutritional composition of whole and semi-skim milk

Composition	Whole milk (%)	Semi-skim (2% M.F.) milk (%)
Fat	3.87	2.00
Protein	3.30	3.49
Lactose	4.64	4.27
Solid non-fat (SNF)	8.99	8.79
Total solid	12.83	10.79

3.3 High pressure processing treatment

HPP treatment procedure was similar to a previous study (Martínez-Monteagudo et al., 2012). HPP samples were prepared by filling untreated samples into 3-mL polypropylene tubes

(Cryogenic vial, Fisher Scientific, St. Louis, MO, USA) and stored in the refrigerator at 4 °C before treatment. Samples that would be treated at 60 °C, were preheated in an oil bath (80 °C) for 1.5 min to the targeted temperature of 60 °C (initial temperature was 4 °C). Then, the tubes were transferred to the HPP vessels that were preset at the desired processing temperature. The closed vessels were pressured at a rate of 10 MPa/s and holding time was recorded once the pressure reached the targeted value. HPP experiments were performed at 300, 400, 500 and 600 MPa, 20 and 60 °C for 5 min. The holding time was recorded using a separate timer. Three different samples were placed into three vessels at a time. Each treatment condition was replicated by running twice at the same conditions. After the set holding time, samples were decompressed, removed immediately from the HPP vessels and cooled down in an ice-water bath. Treated samples were stored at -18 °C for further analysis.

The HPP system (Apparatus U111 Unipress, Warszawa, Poland) consisted of four parallel high pressure vessels. The HPP unit is coupled with a thermostat (Lauda Proline RP 855 Low Temperature). Pressure was built up at a high rate (set pressure at 10 MPa/s). Each vessel has an internal volume of 8 mL and the transmission fluid was propylene glycol. During heating and compression, a K type thermocouple equipped in each vessel kept record of the temperature and pressure profiles of the transmission fluid. It was assumed that the sample temperature is similar to the transmission fluid temperature since the sample volume in the tube is small (3 mL) compared to the transmission fluid volume (5 mL).

3.4 Measurement of two quality parameters

3.4.1 pH measurement

Untreated and treated samples pH was measured using a traceable pH meter (Control Company, Friendswood, TX, USA). Calibrations were made at pH of 4.0 and 7.0. Duplicate measurements were done for each sample.

3.4.2 Color assessment

Color of samples was measured using a Hunter lab Colorimeter (CR-400/CR-410, Konica Monolta, Ramsey, NJ, USA) at room temperature. The results were presented as L* (lightness), a* (+:red; -:green) and b* (+:yellow; -:blue) values, which are three dimensions of CIE (International Commission on Illumination) Lab color space. The tests were conducted following ASTM D2244 method (American Society for Testing and Materials, 2011) using a D65 illuminant with a 14 mm opening and a 10° standard observer. Before testing, the colorimeter was calibrated on a standard white plate (L^* = 93.49, a^* =-0.25 and b^* =-0.09). Total color difference ΔE^* and hue angle (h°) were calculated by the equations 3.1 to 3.2, in which L^*_0, a^*_0 and b^*_0 are the control values from untreated samples and $\Delta L^* = (L^* - L^*_0)$; $\Delta a^* = (a^* - a^*_0)$ and $\Delta b = (b^* - b^*_0)$. Duplicate measurements were performed and results were averaged.

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3.1)$$

$$h° = \text{arctangent}\ [(b^*)\ (a^*)^{-1}] \quad (3.2)$$

Table 3-2 Color difference classification of ∆E* values (Salva & Salva, 1999)

∆E*	Color difference classification
0.0-0.2	Not perceptible
0.2-0.5	Very small
0.5-1.5	Small
1.5-3.0	Distinct
3.0-6.0	Very distinct
6.0-12.0	Great
>12.0	Very great

Salva & Salva (1999) classified the color difference according to different ∆E* values as shown in Table 3-2.

3.5 Measurement of bioactive compounds

3.5.1 Extraction of bioactive compounds of samples

Before analysis, a step to remove clouds, proteins and fats was conducted according to Aqel et al. (2014) & Vázquez et al., (2015) with slight modifications. Briefly, 0.8 mL sample was mixed with 0.8 mL extraction solvent of isopropanol-acetonitrile (1:1, v:v) in a 2 mL centrifuge tube. After vortexed for 20 s, the mixture was centrifuged using a counter centrifuge (5415R, Eppendorf, Germany) at 10000*g*, 4 °C for 10 min. By this process, extracellular bioactive compounds from the juice and juice milk were drawn to the organic layer (supernatant) and the supernatant was used for analysis of vitamin C, vitamin B, total phenolics content, total flavonoids and total antioxidant capacity. Therefore, the results represent the bioactive compounds content from extracellular space in HPP treated melon juice or melon milk.

3.5.2 Total phenolics content

Total phenolics content was measured by mixing 0.04 mL sample extract with a reaction solution composed of 3.16 mL water and 0.2 mL Folin-Ciocalteau's phenol reagent (Singleton & Rossi, 1965). The mixture was vortexed for 10 seconds and incubated for 6 min. Then, 0.6 mL sodium carbonate solution was added and vortexed for 10 seconds. After incubation in dark for 2 hours, the absorbance was read at 765 nm in a plastic cuvette. The standard curve was generated by using gallic acid at different concentrations following the same procedures just described for samples. Experiments were conducted in triplicates. Results were expressed as milligrams of gallic acid equivalents per gram of fresh weight.

3.5.3 Total flavonoids content

Total flavonoids content was analyzed according to Cabrera et al. (2009) with some modifications. Sample extract was diluted to 5 times by adding 150 μL of extract in 600 μL water. A 200 μL of this diluted sample was mixed with 60 μL of $NaNO_2$ solution (5g/100 mL in Milli-Q water). The mixture was mixed well and incubated at room temperature for 5 min. Then, 60 μL of $AlCl_3$ solution (10g/100 mL in Milli-Q water) was added to the mixture to react within 1 min. After that, the mixture was added with 400 μL 1 N NaOH and 480 μL Milli-Q water. The absorbance of the mixture was measured at 510 nm in a spectrometer (Genova, Barioworld Scientific, Essex, UK) and the results were presented as mg quercetin equivalent (QE) per mL sample. The standard curve was generated by using quercetin at different concentrations.

Measurements were conducted in triplicates.

3.5.4 Water-soluble vitamins

Water-soluble vitamins were quantified by gradient reversed-phase HPLC (RP-HPLC) with UV/VIS detection according to Zafra-Gómez, Garballo, Morales & García-Ayuso (2006) with some modifications. The RP C18 column, endcapped (4.6 x 150 mm, 5 μm particle size; Supelco, Sigma Aldrich, St. Louis, MO, USA) was used for all analyses. The mobile phase was composed of a gradient of water (HPLC grade, solution A) and methanol (HPLC grade, solution B). Stock solutions and working solutions of standards water-soluble vitamin were prepared as reported in Table 3-3.

Table 3-3 Concentration of stock and working solutions of water-soluble vitamin standards

	Stock solution		**Working solution (mg/mL)**					
Vitamin	**m (g)**	**V_{H2O} (mL)**	**S10**	**S20**	**S40**	**S80**	**S100**	**S200**
Amount of each stock solution in water to fill up to 15 mL(mL)			10	20	40	80	100	200
Ascorbic acid	0.1133	25	0.0030	0.0060	0.0121	0.0242	0.0302	0.0604
Niacin	0.1014	25	0.0027	0.0054	0.0108	0.0216	0.0270	0.0541
Folic Acid	0.1039	25	0.0027	0.0054	0.0109	0.0217	0.0272	0.0543

Each working solution is a multi-vitamin (ascorbic acid, niacin, folic acid) aqueous system composed of every vitamin stock solution of the same amount into water to fill up to15 mL. After preparation of standard solutions, the extraction was performed following the protocol

described in Section 3.5.1. Briefly, 1 mL of working solution was mixed with 1 mL of extraction solvent of isopropanol-acetonitrile (1:1, v: v) in a 2 mL centrifuge tube. The mixture was then centrifuged in a centrifuge at 10000*g*, 4°C for 10 min.The HPLC samples were prepared by inserting 300 μL of sample extract or standard solution extract into a 300 μL polyspring insert (Thermo Scientific, Waltham, MA, USA) and then the polyspring insert was placed inside a 2 mL glass HPLC tubes (Fisher Scientific, Ottawa, ON, Canada).

HPLC samples were all prepared in duplicates. After vials were loaded in the autosampler, the mobile phase was programed. The starting eluent was 100% A for 5 min; then B was raised to 13% (v/v) for 20 min, 35% for 15 min and eventually 100% for 2 min. The mobile phase was then adjusted to its initial composition, and the elution was continued for 4 min. The flow rate was 1 mL/min, and the injection volume was 20 μL. The wavelength for detecting ascorbic acid was 260 nm and the wavelength for detecting niacin and folic acid was 290 nm. Data were quantified using the Galaxy data acquisition system.

3.5.5 Fat-soluble vitamins

HPLC was used to quantify α-tocopherol in the samples and standards according to the methodology by Eitenmiller, Ye & Landen (2008). Before injection in the HPLC, pretreatment was conducted for all samples and standard solutions. In a 2 mL microcentrifuge tube (Fisher Scientific, Ottawa, ON, Canada), 1 mL of sample (melon juice, MMS, MMW and standard solution) was mixed with 150 μL isopropanol (HPLC grade) to denature the proteins. Then, 100

μL ascorbic acid solution (0.0013 mol/ml) was added right after to prevent oxidation of α-tocopherol. Later 100 μL potassium hydroxide solution (1.5 g/mL) was added for saponification. After vortexing for 2 min, the mixture was incubated in a 50 °C water bath for 50 min and then the mixture was cooled in ice water bath for 2 min. Then the mixture wasadded with 700 μL hexane for extraction of α-tocopherol and finally 50 μL saturated sodium chloride solution (6.02 M) was added to remove the emulsion. After being mixed well on a vortex, the tubes were centrifuged in an accuSpin 400 centrifuge (Fisher Scientific, Ottawa, ON, Canada) at 7000 g for 20 min. A 150μL supernatant from the microcentrifuge tubes was transferred into a 300 mL polyspring insert (Thermo Scientific, Waltham, MA, USA) and then inserted in a 2 mL brown glass HPLC vial (Thermo Scientific, Waltham, MA, USA). The vials were then loaded in the autosampler.

The HPLC system used a Supelcosil 5 micron LC-18 reverse phase column (4.6 x 250 mm; Supelco, Sigma Aldrich, St. Louis, MO, USA). The mobile phase was composed of 0.6% isopropanol (100% purity) and 99.6% hexane (100% purity). The flow rate of the mobile phase was 1 mL/min.

Standard solution was made by adding 0.0692 g α-tocopherol into 6.4792 g hexane, which had a final concentration of 0.0067 g/mL. Six different concentrations of working solutions by adding 5 μL, 10 μL, 20 μL, 40 μL 100 μL and 150 μL, respectively, into 50 mL hexane were prepared. The concentrations for each working solution are shown in Table 3-3.

Table 3-4 Concentration of stock and working solutions of α-tocopherol vitamin standards

Working solution code	S5	S10	S20	S40	S100	S150
Amount of stock solution in 50 mL hexane (mL)	5	10	20	40	100	150
α-tocopherol (mg/mL)	0. 000673	0. 00135	0.00269	0. 00538	0. 0135	0. 0202

3.6 Determination of total antioxidant capacity

The total antioxidant capacity (TAC) was evaluated through the determination of ferric reducing antioxidant power (FRAP) according to the method reported by Alvarez, Cahyadi, Xu & Saldaña (2014) and measurement of the inhibition rate of DPPH radical as described by Eklund et al. (2005) with slight modifications. Both methods are described below.

3.6.1 DPPH method

DPPH solution (105 μM/L) was prepared by dissolving 20.702 mg DPPH crystals in 500 mL ethanol (>95% purity). Extracted sample (0.1 mL) was mixed with 0.9 mL acetic acid buffer (pH=5.5) and 2 mL DPPH solution in a test tube (13 x 100 mm, Fisher Scientific, Ottawa, ON, Canada). The mixture was vortexed well and incubated at room temperature in dark for 1h. Then the absorbance of the mixture was measured at 517 nm using a spectrometer (Genova, Barioworld Scientific, Essex, UK) and the reading was recorded as Absorbance 1 (A1). DPPH solution (2 mL) was used as a control to procode the reading Absorbance 0 (A0). The inhibition rate, which represented TAC in the sample, was calculated usingt he following equation:

$$\text{Inhibition of DPPH (\%)} = 100 \times (A0-A1)/A0 \qquad (3.4)$$

3.6.2 FRAP method

Ferric Reducing Antioxidant Power (FRAP) method used a reagent solution which consisted of acetate buffer (pH=3.6), acidic TPTZ solution (10 mmol/L in HCl) and ferric chloride solution (20 mmol/L water) at a ratio of 10:1:1 (v:v:v). A 0.1 mL of sample (extract from section 3.5.1) or standard solution was mixed with 3 mL of reagent solution and 0.3 mL Milli-Q water. The mixture was incubated in a 37 °C water bath in dark for 30 min. Then, the absorbance was measured at 593 nm in a spectrometer (Genova, Barioworld Scientific, Essex, UK). The reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ, which represented TAC in sample, was monitored by the absorbance at 590 nm. Gallic acid was used as a standard and each sample was analyzed in triplicates.

3.7 Statistical analysis

For each chemical analysis, duplicate results of each treated sample were averaged and then data were recorded. Data of duplicate treatments were processed by using both Microsoft Excel 2010 and Minitab 17 Statistical Software for windows (Minitab Inc., State College, PA, USA). Averages and standard deviations were generated by Excel. Significant differences between pressure levels at the same temperature were evaluated by Tukey test (ANOVA, one-way) at 0.05. The significant differences at 20 °C were recorded using capital letters. The significant differences at 60 °C were recorded as lower case letters. The confidence level was 95% with two-sided confidence intervals. Figures in the next chapter and tables in the appendix were generated using Excel. Superscripts were noted using group letters in the tables and figures in

next chapter. Data that share same group letter means no significant difference ($p<0.05$).

Chapter 4. Results and Discussions

4.1 Two quality parameters in melon juice and melon milk

The pH changes of HPP treated melon juice are reported in Table 4-1. Melon juice was treatd by HPP of 300-600 MPa/20 and 60 ºC. 5 min. There was a significant increase in the pH of melon juice at all pressures and temperatures treatments compared with the untreated sample, which could indicate that more anions are generated at the pressures used. According to Martinez-Monteagudo & Saldaña (2014), HPP favors electrostriction, which caused ionization. These ionization reactions induce pH changes in food systems during pressurization. For example, it was reported that citric acid become ionized under pressure (Kitamura & Itoh, 1987). Another study (Hamann & Linton, 1974) found that phenols were susceptible to ionization by pressure due to the charge delocalization between the oxygen and the aryl ring, producing phenoxide anions. As well, according to previous studies (Datta & Deeth, 2011; Samaranayake & Sastry, 2010; Khoshtariya et al., 2004), pressure treatment weakened hydrogen-bonds between water molecules, causing rearrangements of water molecules into denser clusters (Khoshtariya et al., 2004), which influenced pH change in water. Since fruit juice is composed of approximately 90% water, it is believed that the structure change of water molecules is part of the reason that induced pH changes in HPP treated juice. However, Martinez-Monteagudo & Saldaña (2014) stated that the pH changes in buffer solution or model systems caused by HPP cannot be applied to food matrices as they are more complex systems and also, pH was reversible upon pressure release. Therefore, pH values measured after HPP treatment cannot represent the pH changes

during pressurisation. Since the equipment used in our study was unable to measure the pH during HPP treatment, the results cannot reflect real pH changes affected by HPP.

MMS had no significant change in pH after all HPP treatments, whereas MMW had significant increase after HPP treatments compared with the untreated sample. The difference in pH values of HPP treated milk samples with different fat contents were earlier reported by Altuner et al. (2006), who observed significant increases of pH in milk samples (skim (0.1% M.F.), semi-skim (1.5% M.F.) and raw whole (3.2% M.F.)) milk after HPP treatments of 110 – 440 MPa/25 ºC/10 and 20 min. Schrader, Bucheheim & Morr (1997) reported that HPP of 400 MPa/20 ºC for 5 min induced a pH increase of 0.09 in skim milk. One reason for the pH increase of milk was explained by the effect of pressure to disintegrate colloidal calcium phosphate and increase the content of soluble calcium (Femalart et al., 1997 & Schrader et al., 1997).

Table 4-1 pH of melon juice and melon milk after HPP treatments.

Treatments (5 min)	Melon juice	MMS	MMW
Untreated	5.72±0.01c/C	6.55±0.07a	6.40±0.01a
300MPa/20ºC	5.98±0.02B		
400MPa/20ºC	6.01±0.02B		
500MPa/20ºC	6.03±0.02AB		
600MPa/20ºC	6.08±0.01A		
300MPa/60ºC	5.87±0.01b	6.60±0.01a	6.62±0.01b
400MPa/60ºC	5.89±0.01ab	6.61±0.03a	6.56±0.01c
500MPa/60ºC	5.89±0.01ab	6.58±0.02a	6.57±0.00c
600MPa/60ºC	5.93±0.01a	6.59±0.03a	6.44±0.01a

Mean ± standard deviation of duplicates treated samples;
Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C;
MMS: melon milk, semi-skim) and MMW: melon milk, whole.

Color parameters of HPP treated melon juice are shown in Table 4-2. Values a* and b* for untreated melon juice were -6.92±1.00 and 17.41±0.69, respectively, which represented a greenness and a yellowness, respectively. The decreased a* and increased b* values compared with untreated sample after HPP treatments indicated that the color of melon juice shifted toward more green-yellow. This can be related to the chlorophyll compounds released from melon flesh cells due to the pressurization effects. The highest yellowness (b*=24.61±0.29) was induced by the HPP treatment of 300 MPa/20 ºC/5min and the lowest yellowness (b*=22.31±0.47) was caused by HPP treatment of 600 MPa/20 ºC/5min. The lightness or luminosity (L*) of the initial melon juice was 84.21±0.26 and the L* values were all enhanced after all HPP treatments. The highest lightness (L*=89.35±0.06) occurred after 600 MPa/20 ºC/5min treatment and the lowest lightness (L*=85.59±0.29) occurred after 300 MPa/60 ºC/5min treatment. An increase in L* values after HPP treatments were also reported by previous studies in tomato purée (Sanchez-Moreno et al., 2006), Granny Smith apple purée (Landl et al., 2010), guava purée (Yen & Lin, 1996) and mango pulp (Kaushik et al., 2014). According to Coyle (2005), intercellular fluid and space give the brightness and lightness for fruits. The increase of lightness can be ascribed to the release of cytosol into intercellular space by pressurization which improved lightness and opaqueness (Kaushik et al., 2014).

The ΔE* values of HPP treated melon juice ranged from 7.30±0.32 to 8.76±0.25. According to Table 3-2, ΔE* values ranging from 6 to 12 indicate great color difference. Therefore, all HPP treatments in our study led to very significant color differences in melon juice

compared with the untreated sample. It was found that the biggest color difference (ΔE*=8.76±0.25) occurred at 300 MPa/20 ºC, whereas 300 MPa/60 ºC caused the lowest color difference compared with untreated sample. Hue angle (h°) represents the true color of samples. The initial h° value of untreated melon juice was 68.30±3.63, which is a color closer to yellow. No significant change was found in $h°^{*}$ after all HPP treatments, which suggested that the color remained as yellow-green.

In general, HPP increased the greenness and yellowness and lightness in melon juice. HPP treatments of 300-600 MPa at both 20 and 60 °C for 5 min induced significant differences in the color (ΔE*) of melon juice compared with untreated samples. However, the color range (h°) of all treated is within yellow-green.

Table 4-2 Color parameters of melon juice after HPP treatments

Treatment (5 min)	a*	b*	L*	h°	ΔE*
Untreated	-6.92±1.00A/a	17.41±0.69C/c	84.21±0.26C/c	68.30±3.63A/a	0.00
300MPa/20ºC	-10.44±0.15B	24.61±0.29A	87.66±0.04A	67.02±0.05A	8.76±0.25A
400MPa/20ºC	-10.14±0.06b	23.79±0.16AB	88.85±0.01B	66.92±0.01A	8.56±0.14AB
500MPa/20ºC	-10.30±0.12B	23.96±0.29AB	87.09±0.18B	66.74±0.01A	7.95±0.17AB
600MPa/20ºC	-9.34±0.21B	22.31±0.47B	89.35±0.06A	67.28±0.04A	7.55±0.38B
300MPa/60ºC	-8.6±0.04ab	23.11±0.17a	85.59±0.29b	69.59±0.05a	6.15±0.21b
400MPa/60ºC	-8.70±0.04ab	23.40±0.08ab	87.90±0.11a	69.61±0.01a	7.30±0.32a
500MPa/60ºC	-9.48±0.00b	24.03±0.01ab	87.31±0.07a	68.47±0.01a	7.79±0.17a
600MPa/60ºC	-9.51±0.34b	24.43±0.01b	87.25±0.59a	68.73±0.07a	8.12±0.06a

Mean±standard deviation of duplicates treated samples;. Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C;L*: lightness; a*: red-green; b*: yellow-blue; △E*: total color difference; h° : hue angle (0° = red purple, 60° =yellow, 120° =green, 180° = bluish-green, 240° = blue).

Color parameters of HPP treated MMS and MMW are shown in Table 4-3. HPP treated MMS had no significant difference regarding all color parameters: a* (-9.09±0.13 to -9.38±0.01), b* (16.87±0.27 to 17.39±0.01), L* (83.43±0.13 to 84.12±0.16) and h° (60.87±0.14 to 61.68±0.05) compared with untreated MMS (a*=-9.03±0.24, b*=16.57±0.49, L*=83.32±0.04 and h°=61.40±0.07). Consistently, ΔE* of HPP treated MMS ranged between 1.06±0.27 and 1.26±0.04, meaning a small difference in total color compared with untreated sample. This indicated that the pigments distribution in extracellular space of MMS was maintained after HPP treatments. However, significant changes were found in all color parameters of MMW. The a* values of MMW significantly increased (-4.61±0.03 to -5.33±0.01) after HPP treatments compared with the untreated sample (-7.32±0.09), indicating a lessned greenness in MMW. The change of a* might be due to the effects of pressure that induced release of β-carotene from milk fat globules, which gives more redness in the sample. The b* value in untreated MMW was 14.64±0.12 which decreased after HPP treatments: the lowest value (8.63±0.06) was obtained at 300 MPa/60 ºC/5min while the highest value (10.11±0.78) occurred at 400 MPa/60 ºC/5min. The L* values increased from 84.44±0.08 (untreated sample) up to 86.18±0.11 to 86.83±0.03 after HPP treatments. The highest L* value (86.83±0.03) was induced by 300 MPa/60 ºC/5min. The change of L* indicates that cell ruptures occurred andcytosol was released into extracellular space, which improved the lightness and opaqueness (Kaushik et al., 2014). The initial h° value of untreated MMW was 63.44±0.10 which is closer to yellowness and lower than the h° in melon juice (68.30±3.63). This could be attributed to the fat which contains β-carotene.

According to Table 3-2 and Table 4-4, the "greatest" total color difference in HPP treated MMW is induced at 300 MPa/60 ºC/5min (∆E* =7.03±0.07), whereas other HPP treatments (400-600 MPa/60 ºC/5min) caused "very distinct" total color differences (∆E*=5.31±0.03 - 5.89±0.30). In conclusion, HPP treatments of 300 - 600 MPa/60 ºC/5 min had no significant effects on color parameters of MMS, except that HPP improved the lightness. However, HPP treatments had significant differences in the color parameters of MMW and the biggest color difference occurred after HPP treatment of 300 MPa/60 °C/5 min. It is believed that the different results between MMS and MMW are related to the fat content in melon milk. But, further studies are required to elucidate this conclusion.

Table 4-3 Color parameters of melon milk (semi-skim) and melon milk (whole) after HPP treatments

Treatment (5 min)	a*	b*	L*	h°	∆E*
			MMS		
Untreated	-9.03±0.24a	16.57±0.49a	83.32±0.04a	61.40±0.07a	
300MPa/60ºC	-9.09±0.13a	16.87±0.27a	84.12±0.16a	61.68±0.05a	1.06±0.27a
400MPa/60ºC	-9.28±0.00a	17.16±0.04a	83.94±0.01a	61.59±0.06a	1.17±0.06a
500MPa/60ºC	-9.38±0.01a	17.39±0.01a	83.58±0.03a	61.66±0.02a	1.26±0.04a
600MPa/60ºC	-9.31±0.13a	17.35±0.09a	83.43±0.13a	60.87±0.14a	1.17±0.05a
			MMW		
Untreated	-7.32±0.09c	14.64±0.12a	84.44±0.08c	63.44±0.10a	
300MPa/60ºC	-4.61±0.03a	8.63±0.06d	86.83±0.03a	61.89±0.01b	7.03±0.07a
400MPa/60ºC	-5.33±0.01b	10.11±0.78b	86.18±0.11b	62.07±0.12b	5.31±0.03b
500MPa/60ºC	-5.13±0.17b	9.60±0.19c	86.49±0.18ab	61.87±0.31b	5.89±0.30b
600MPa/60ºC	-5.33±0.05b	9.56±0.04c	86.59±0.11ab	60.87±0.14c	5.89±0.05b

Mean±standard deviation of duplicates of each sample; Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C;L*: lightness; a*: red-green; b*: yellow-blue; ∆E*: total color difference; h°: hue angle (0°= red purple, 60°=yellow, 120°=green, 180° = bluish-green, 240°= blue).

4.2 Total phenolics and total flavonoids content in melon juice and melon milk

4.2.1 Total phenolics content in melon juice and melon milk

Total phenolics content results of untreated and HPP treated melon juices at 20 and 60 ºC are shown in Figure 4-1 (I). The initial total phenolics content in the untreated melon juice was 333.72±1.10 μg GAE/mL. At 20 ºC, pressurizations of 300-500 MPa had no significant effects on the total phenolics content of melon juice, whereas pressurization of 600 MPa resulted in a significant increase by 6.28% compared with untreated sample. At 60 ºC, all HPP treatments of 500 and 600 MPa significantly increased total phenolics content by 7.79-11.41% in honeydew melon juice compared with untreated sample (See Table B.1). The higher total phenolics content in 500 MPa treated melon juice at 60 ºC than that at 20 ºC indicates a synergistic extraction effect of temperature on the extraction of phenolic compounds from intracellular space during HPP treatments. The increase of total phenolics content in melon juice was within the range of the increase reportedby previous studies in HPP treated strawberry purée (Patras et al., 2009a), tomato purée (Patras et al., 2009b), pomegranate juice (Varela-Santos et al., 2012) and pineapple purée (Chakraborty, Rao & Mishra, 2015).

The study of Patras et al. (2009a) showed that HPP at 400 and 500 MPa and 20 ºC for 15 min had no effects on total phenolics content in tomato puree, whereas the treatment of 600MPa/20 ºC/15 min significantly increased the total phenolics content by 9.8%; and total phenolics content in carrot puree significantly increased after all HPP treatments (400-600

MPa/20 ºC/15 min). Another study (Patras et al., 2009b) reported that total phenolics content in strawberry purée had no significant change after HPP treatments of 400-500 MPa/20 ºC/15 min, whereas it increased by 9.8% after 600 MPa/20 ºC/15 min.compared with untreated sample. Therefore, food matrix is an important factor when studying the effects of HPP on total phenolics content of fruit purées. Varela-Santos et al. (2012) reported a 3-12% increase in total phenolics content of pomegranate juice after HPP treatments at 350-550 MPa/20 ºC/0.5-2.5 min. Kaushik et al. (2014) reported an increase of 9-19% in total phenolics content of mango pulp after HPP treatments of 100-600 MPa at room temperature for 1s-20 min. The increase in total phenolics content was attributed to the use of high pressure that improved mass transfer rates, enhancing the membrane permeability and resulting in higher extraction of intracellular bioactive compounds into the juice (Gamlath & Wakeling, 2011).

There is no clear conclusion about the relationship between the increase of total phenolics content and HPP treatments. Ferrari, Maresca & Ciccarone (2010) found that total phenolics content in pomegranate juice increased from 1.36 mg/mL to 1.48 mg/mL after HPP treatment at 50 ºC and 400 MP for 5 min and this value increased to 1.92 mg/mL after HPP treatment at 50 ºC and 400 MP for 10 min. However, other pressure levels and temperature combinations (400 MPa/25 ºC/5-10 min and 500-600 MPa/25-50 ºC/5-10 min) had no significant effects on total phenolics content in pomegranate juice. At room temperature, 400 MPa is a benchmark where total phenolics content in pomegranate juice began to drop after HPP treatments if the pressure increased. At 45 ºC, total phenolics content changes were independent of pressure levels; at 50 ºC, increasing pressure induced increasing total phenolics content in pomegranate juice.

Therefore, the effect of HPP on total phenolics content has no linear relationship wtih pressure, but also related to the treatment temperature. Chakraborty et al. (2015) found that treatments at 200-600 MPa and 50 and 60 ºC for 10-20 min significantly improved the total phenolics content in pineapple purée by 11%, where the increase of pressure level led to increasing total phenolics content values. However, the same pressure levels (200-600 MPa) at 70 ºC and time for 10-20 min led to significant low total phenolics content with a maximum loss of 10%. Those results indicated that mild temperatures 50 and 60 ºC had synergistic effects on the total phenolics content in pineapple purée, whereas a temperature of 70 ºC caused detrimental effects on total phenolics content in pineapple purée. It was stated that the different effects of temperature during HPP treatments on total phenolics content results might be attributed to both the solubility and degradation effects by temperatures above 60 ºC. Phenolic compounds are extracted from tissues under high pressure, which are readily subject to degradation at high temperatures. Reversely, a decrease in total phenolics content occurred when the degradation effect is faster than the extraction effect at high pressures.

The total phenolics content of both MMS and MMW after HPP treatments are shown in Figure 4-1 (II) and (III). The total phenolics contents of untreated MMS and MMW were 690.26±7.91 and 773.44±18.26 μg GAE/mL, respectively, which were almost twice the value of untreated melon juice. Using the same Folin-Ciocalteu method, Vázquez et al. (2015) reported that total phenolics content in raw cow milk was as low as 38.24±0.18 to 64.00±0.00 μg GAE/mL depending on the species. Therefore, milk is not a contributor of phenolic compounds

in our sample. The dramatically high total phenolics content in melon milk might come from the blending step during sample preparation. It was reported blending induced higher phytochemical contents and phenolics content compared with other juicing techniques (Rajasekar, Akoh, Martino & MacLean, 2012; Uckoo, Jayaprakasha, Balasubramaniam & Patil, 2012; Pyo, Jin & Hwang, 2014). Rajasekar et al (2012) found that pomegranate juices of 15 different cultivars made by blending (15.59% total solids) had significantly higher content of total phenolics, measured by Folin-Ciocalteau method, compared with juices made by mechanical press using a juice extractor (14.94% total solids). Uckoo et al. (2012) compared phytochemical compounds in grapefruit juice made by three juicing methods: a house citrus juicer, hand squeezing and a blender. It was reported that except ascorbic acid content, all other phytochemicals were higher in blended juice (12.73% total solids) than juices made by juicer (11.48 total solids) or squeezing (11.86% total solids). Citric acid in blended grapefruit juice was 809.70±90.56 mg/100 mL, whereas the value in squeezed juice was 606.08±73.91 mg/100 mL. Other phytochemicals (flavonoids, limonoids and furocoumarins) in blended pomegranate juice were found about 2-6 times as high as the value in juices made by juicer or squeezing, because of the higher pulp content and the mechanical stress of the blender. Similar results were reported by Pyo et al. (2014), who found that the contents of citric acid and malic acid were significantly higher in four types of blended juice (apple, persimmon, pear and mandarin) than the juices made by the juicer. The study also reported that the total phenolics content in blended persimmon juice (610±2.6 GAE/250 mL) was much higher than that in juiced one (217.5±2.2 GAC/250 mL). Since melon milk in our study was prepared by 1 min of blendeing milk and melon juice, which is one more

step compared with the melon juice sample, the higher total phenolics content in the result can be attributed to the mechanical effects of the blender which induced the cell walls damage and released intracellular phenolic compounds.

For MMS, HPP treatments caused significant ($p<0.05$) reductions in total phenolics content of MMS. The lowest total phenolics content (585.86±7.81 μg GAE/mL) was found after an HPP treatment of 300 MPa/60 ºC/5min and the highest total phenolics content (733.45±55.77 μg GAE/mL) was detected after 600 MPa/60 ºC/5min. It is believed that high pressure at 60 ºC for 5min had negative effect on total phenolics content of MMS as total phenolics content decreased after 300-400 MPa/60 ºC/5min compared with untreated. For MMW, HPP treatments were found to improve the total phenolics content (968.18 ±45.54 to 1062.07±24.09 μg GAE/mL) compared with untreated sample. The highest total phenolics content was detected after HPP treatment of 400 MPa/60 ºC/5 min. Due to interactions between PCs and milk proteins may occur in our sample (discussed in Chapter 2), it can be speculated that the addition of melon juice into milk lead to the non-covalent binding between PCs and the tertiary structures of proteins molecules. Therefore the improvement of total phenolics content in HPP treated MMW can be explained by the dissociation of non-covalent bonds between PCs and protein during pressurization. The different effects of HPP on total phenolics content in MMS and MMW may suggest that the presence of fat content had a protective effect against the degradation of phenolic compounds after HPP treatments (300-600 MPa/60 °C/5 min).

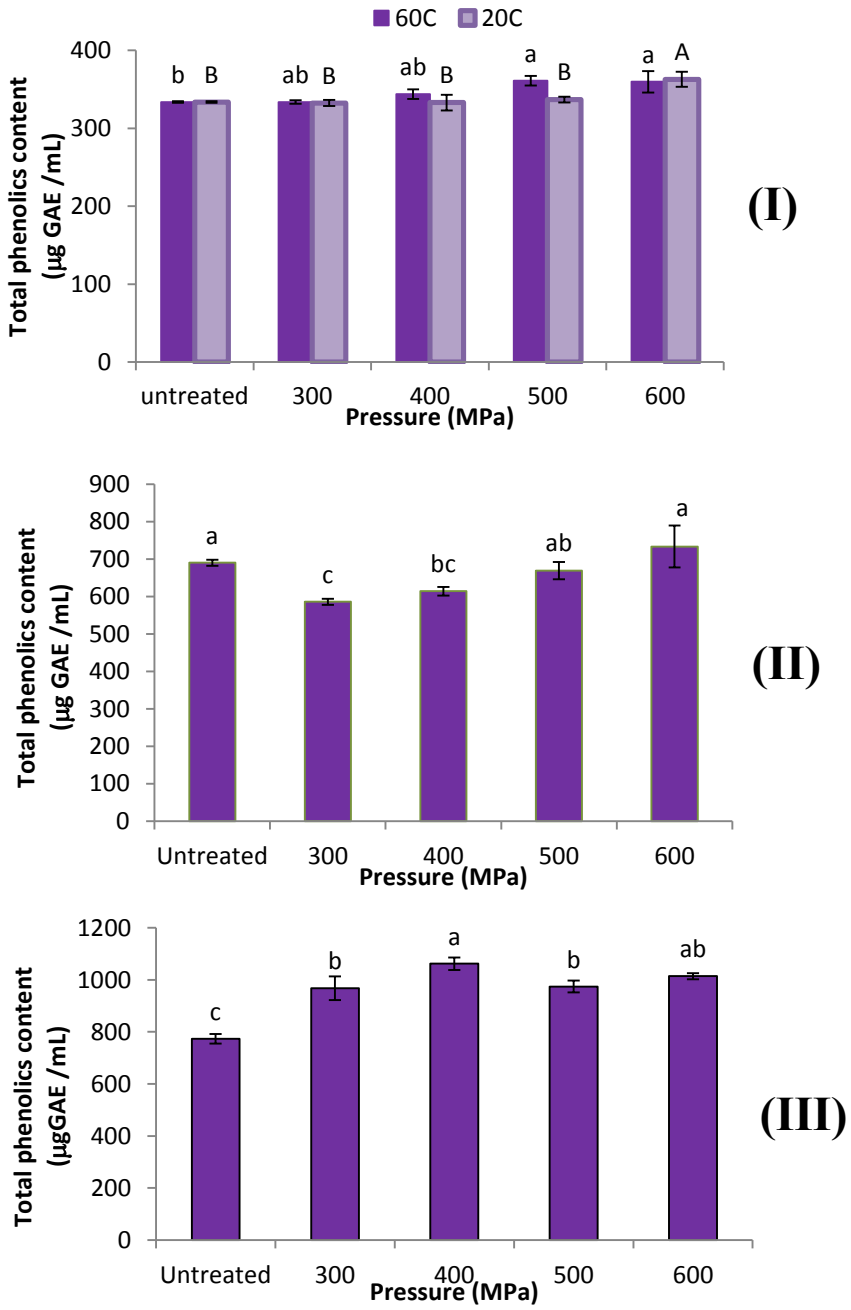


Figure 4-1 Total phenolics content after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). GAE: gallic acid equivalent. Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

The results from our study indicate that HPP treatments preserved total phenolics content in melon juice and MMS, with the exception of 300-400 MPa/60 ºC/5 min. But, there was an increase on the total phenolics content of HPP treated MMW. For melon juice, 60 ºC resulted in a higher total phenolics content compared with the untreated sample. Therefore, only 60 ºC was evaluated for MMS and MMW.

4.2.2 Total flavonoids content in melon juice and melon milk

Figure 4-2 (I) shows the effects of HPP treatments on total flavonoids content in honeydew melon (See Table B.1). The initial content in fresh honeydew melon juice was 0.193±0.005 mg QE/mL. Our study shows that HPP treatments at 20 ºC caused significant decrease (6.51-12.55%) on the total flavonoids content in honeydew melon juice, which might be attributed to the degradation by quercetinase, whose activity was activated by high pressure. However, HPP treatments at 60 ºC had no significant change on the total flavonoids content, indicating that the extraction effect of high pressure at this temperature, which offsets the degradation of total flavonoids content.

Chauhan, Raju, Ravi, Roopa & Bawa (2011) reported significant decreases in total flavonoids content in grape juice after HPP treatments of 400-600 MPa/40-60 ºC for 2-4 min. The authors revealed a second-order polynomial regression relationship between pressure (400-600 MPa) and temperature (40- 60 ºC) on total flavonoids content in grape juice when treated at a fixed holding time. Therefore temperature had to be reduced and pressure had to be increased

to reach a certain total flavonoids content. Plaza et al. (2011) revealed that the total flavanone content in orange juice was increased by 15.5% after HPP treatment of 400 MPa/40 ºC/1 min, which was attributed to the release of flavanone compounds from cells by extractability of HPP. Therefore, the effect of pressure on total flavonoids content is not necessarily linear and it has to consider the temperature during treatment.

Figure 4-2 (II) and (III) show the contents of total flavonoids in HPP treated MMS and MMW. The initial total flavonoids content were 0.356±0.015 mg/mL and 0.295±0.001 mg/mL for MMS and MMW, respectively, which is much higher than the flavonoids content in untreated melon juice. This phenomenon can be due to the mechanical stress caused by blending of milk with melon juice. The step of using a blender might increase cell walls permeability, which induced higher leaking of intracellular flavonoids into extracellular space. The study by Uckoo et al. (2012) found that blended grapefruit juice (12.73% total solids) had significantly higher content of flavonoids content than juices made by a juicer (11.48% total solids) or hand squeezing (11.86% total solids). For example, naringin content in blended juice was 160.80±18.68 mg/100 mL whereas naringin content in grapefruit juice that was made by a juicer was 26.25±5.44 mg/100 mL and 22.51±7.91 mg/100 mL in the juice made by squeezing. The study by Pyo et al. (2012) found that mandarin orange juice made by a blender had higher total flavonoids content (160.1±1.2 QE/250 mL) than the content in juice made by a juicer (72.5±0.6 QE/250 mL) using the same flavonoids assay method with our study.

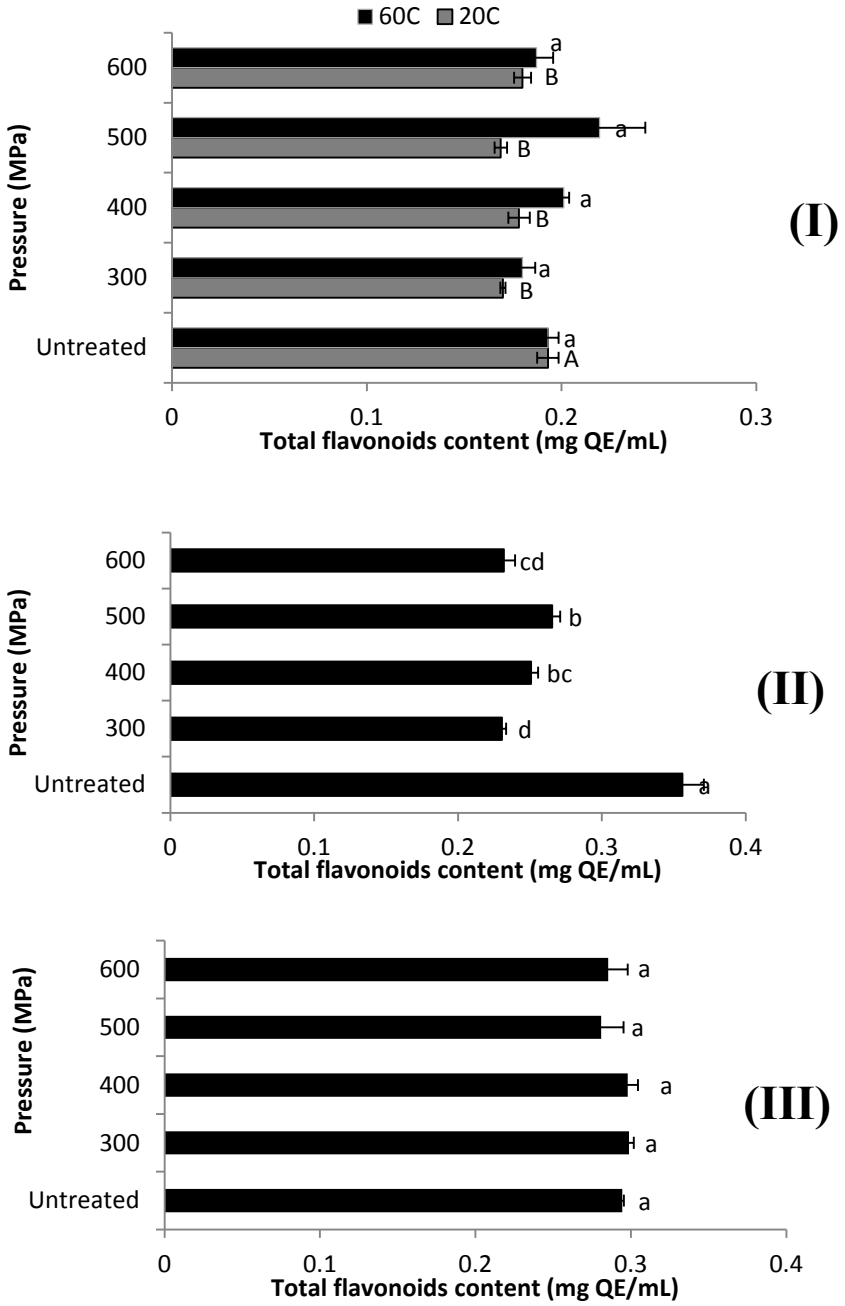


Figure 4-2 Total flavonoids content after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

HPP treatment of 300-600 MPa/60 ºC/5 min significantly reduced total flavonoids content in MMS (0.231±0.003 to 0.265±0.006 mg/mL). But there was no significant change ($P<0.05$) detected in the total flavonoids content in MMW after all HPP treatments (0.281±0.014 to 0.298±0.003 mg/mL). The different results of total flavonoids content between MMS and MMW suggest that the presence of fat globules protect flavonoids against degradation. Similiarly, Tadapaneni, Edirisinghe & Burton-Freeman (2015) recently reported that HPP treatment had a detrimental effect on flavonoids in juice milk product. It was found that flavonoids including quercetin-3-*O*-glucoside, kaempferol-3-coumaroylglucoside, quercetin-3-*O*-rutinoside, kaempferol-3-malonylglucoside and quercetin-3-*O*-malonylglucoside, in a strawberry juice milk (skim) beverage underwent significant reductions after HPP treatments of 600-800 MPa/18-22 ºC/1 min and 200-600 MPa/18-22 ºC/15 min.

4.3 Vitamin C in melon juice and melon milk

Vitamin C (ascorbic acid) content in melon juice was measured as an indicator of oxidative stress during the processing (Barba, Esteve & Frigola, 2012). The results of ascorbic acid content in melon juice affected by HPP treatments are shown in Figure 4-4 (I) (See Table B.2). The ascorbic acid content determined in the cloudy melon juice was 109.2±34.1 μg/mL, which is much higher than the value reported (39±4 μg/g fresh weight) by Leong & Shui (2002). Compared with other fruits, the ascorbic acid content in honeydew melon is higher than the vitamin C content in banana (87 μg/g fresh weight) and grape (American type) (4 μg/g fresh weight), which indicated that honeydew melon is a good source of vitamin C (USDA, 2015).

HPP treatments at both 20 ºC and 60 ºC significantly improved the ascorbic acid content in melon juice, where 300 MPa/60 ºC/5 min resulted in the lowest ascorbic acid value (167.32±26.99 μg/mL) and 600 MPa/60 ºC/5 min induced the highest value (353.39±2.68 μg/mL). The increment in ascorbic acid content can mainly be attributed to the rupture of melon pulp cells caused by compression effects of high pressure, leading to the release of intracellular compounds into the juice. The results also show that high pressure induces high cell permeability.

This trend of the results is consistent with the results reported by previous studies using fruit pulps (Briones-Labarca et al., 2013; Uribe et al., 2015; Torres-Ossandón et al., 2015; Kaushik, Kaur, Rao & Mishra, 2014). Kaushik et al. (2014) found that the content of ascorbic acid in mango pulp (1.234 mg/g untreated sample) improved with increased pressure (200 - 600 MPa/30 ºC/5 min) one with the maximum value sample after treatment of 600 MPa/30 ºC/5 min (1.587 mg/g). Briones-Labarca et al. (2013) showed that HPP treatments of 500 MPa/room temperature/0.5-1.5 min induced 9-53% increase in ascorbic acid content in Cape gooseberry purée compared with untreated sample.

Ascorbic acid content may also decrease due to oxidation caused by HPP. Yen & Lin (1996) reported a loss of 11% in the ascorbic acid content of guava purée after HPP treatment at 400MPa/20 ºC for 30min. Barba, Esteve & Frigola (2010) reported that HPP treatments of 100-400 MPa and 30 ºC resulted in no significant changes in the ascorbic acid of a vegetable beverage (pH=4.2), composed of tomato (*Lycopersicon esculentum* Mill., 33%), green pepper

(*Capsicum annuum* L., Italian pepper, 17%), green celery (*Apium graveolens* L., 8.5%), cucumber (*Cucumis sativus* L., 4%), onion (*Allium cepa* L., 4%), carrot (*Daucus carota* L., 4%), lemon (*Citrus limon* L, 1.7%), salt (1.7%), virgin olive oil (SOS Cuétara, SA, Madrid, Spain, 0.8%), and water when the treatment time was below 7 min but a reduction of less than 9% occurred when the treatment time was above 7 min. The study by Chakraborty et al. (2015) showed that HPP treatments at 200-600 MPa/20 to 40 ºC for 10- 20 min caused a maximum reduction of 4% in the ascorbic acid content in pineapple purée. However the same treatments at higher temperatures 50- 70 ºC caused higher loss of ascorbic acid by 7.3-23.5%. Wolbang, Fitos & Treeby (2008) reported that HPP treatment at 600 MPa/ambient temperature (data not reported) /10 min significantly reduced the content of vitamin C in three different cultivars of melon (*Cucumis melo L.*) juice (data not reported) where the effect of HPP on melon juice is related to the cultivar. Sancho et al. (1999) reported 87.8-88.6% retention of ascorbic acid in strawberry coulis after HPP treatments at 200-600 MPa/room temperature (data not reported)/30 min. Therefore, a long holding time and higher temperature may reduce ascorbic acid content by oxidation. The increment of ascorbic acid content in our study indicated that ascorbic acid did not have oxidation reaction during HPP treatments or the oxidation rate is much lower than the extraction rate.

According to Nagy (1980), ascorbic acid is susceptible to oxidative reactions that are facilitated by enzymes such as cytochrome oxidase and peroxidase in fruits, or anaerobic oxidation. Figure 4-3 shows the aerobic oxidation process in the presence of oxygen. Ball (2006)

reported that chemical oxidation of ascorbic acid can be minimized during processing by using a vacuum deaertion on inert gas. In our study, oxygen was removed by pumping nitrogen in the melon juice, which helped to prevent chemical oxidation.

The concentrations of ascorbic acid in treated and untreated MMS and MMW are shown in Figure 4-4 (II) and (III). The ascorbic acid contents of untreated MMS and MMW were 139.63±22.73 μg/mL and 268.5±1.81 μg/mL, respectively. The higher content of ascorbic acid in melon milk than melon juice can be explained by extracting effects in the blending step. Our results indicated that melon juice mixed with whole milk had a higher content of ascorbic acid compared with that mixed with skim milk, which was also reported in other studies (Cilla et al., 2012; Salvia-Trujillo et al., 2011).

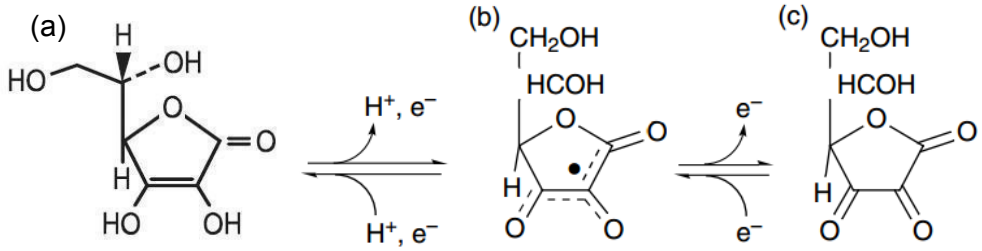


Figure 4-3 Oxidation of ascorbic acid (a) L-ascorbic acid, (b) ascorbyl radical anion, and (c) dehydroascorbic acid (adapted from Ball, 2006).

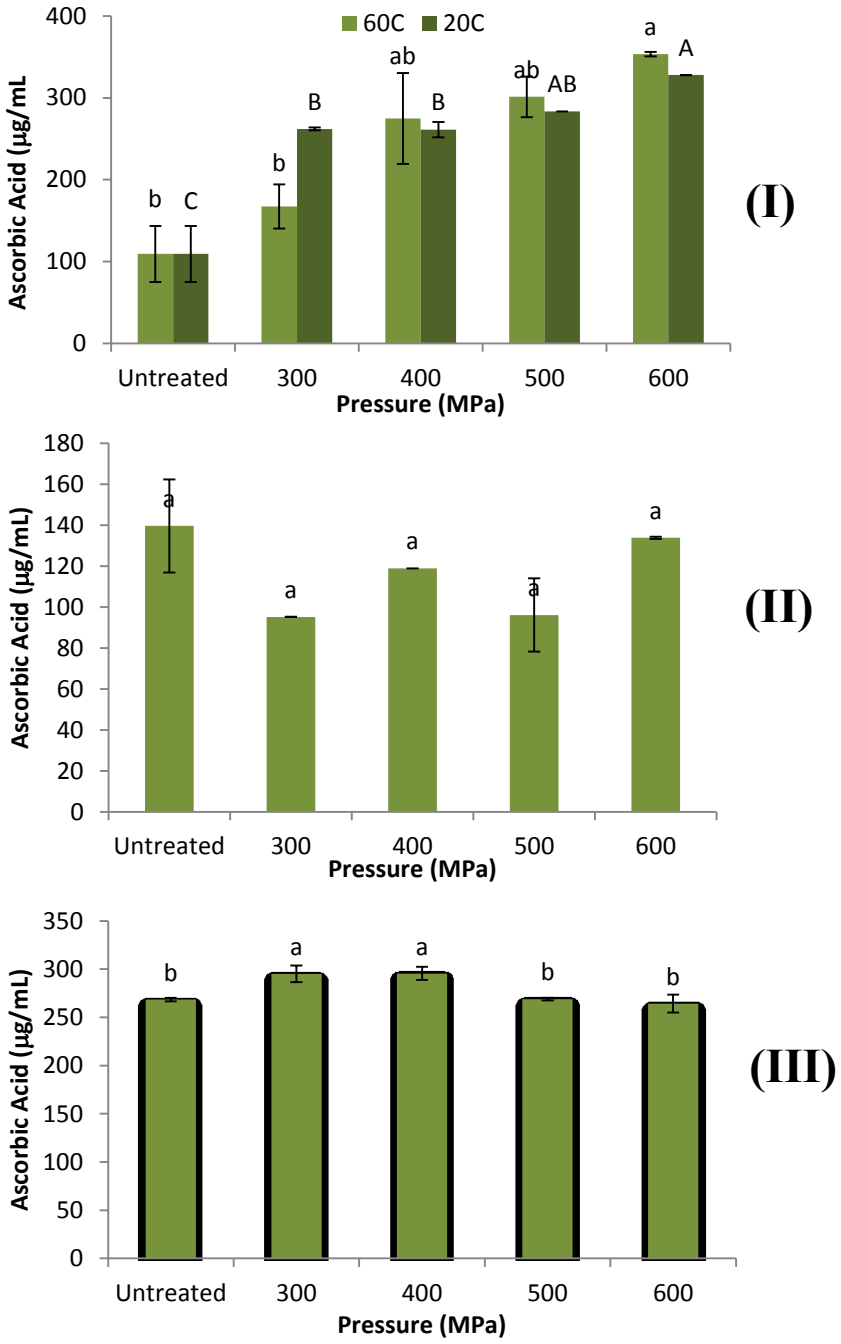


Figure 4-4 Vitamin C content after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

Our study showed that all HPP treatments had no significant effect on ascorbic acid content in MMS samples compared with untreated sample. But, HPP treatments of 300 and 400 MPa/60 ºC/5min induced significant increase of ascorbic acid content up to 295.16±8.57 and 295.52±6.82 μg/mL in MMW, respectively. However, HPP treatments of 500 and 600 MPa/60 ºC/5min caused no significant change on ascorbic acid in MMW. Cilla et al. (2012) found that HPP had differenct effects on ascorbic acid content in whole milk juice compared with that in skim milk juice affected by HPP. They reported that HPP treatment of 400 MPa/40 ºC/5min significantly decreased the content of ascorbic acid in orange juice milk (skim, 0.3% M.F.) but retained the initial content of ascorbic acid in orange juice milk (whole, 3% M.F.). Therefore, HPP treatments of 300-400 MPa/60 ºC/5min had a positive effect on juice milk with a higher fat content (MMW) compared with the juice milk with a lower fat content (MMS).

4.4 Folic acid in melon juice and melon milk

Effects of HPP on folic acid in melon juice are shown in Figure 4-5 (I). The initial content of folic acid in melon juice was 1.037±0.041 μg/mL. There was no significant change either in folic acid content in melon juice after all HPP treatments used (1.016±0.223 μg/mL to 1.266 ±0.021 μg/mL), probably due to the high ascorbic acid content in HPP treated melon juice which maintained the folic acid content . Similiarly, Oey et al. (2006) reported that folate was protected by ascorbic acid against oxidation during HPP treatments.

Different effects of HPP between MMS and MMW were found in folic acid content

(Figure 4-5 (II) and (III)). Our study showed that in MMS, folic acid content significantly increased from 0.820±0.149 μg/mL (untreated MMS) to 1.003±0.047-1.149±0.047 μg/mL after HPP treatments at 400-600 MPa/60 ºC/5 min, whereas HPP treatment of 300 MPa/60 ºC/5 min had no impact on the folic acid content in MMS. However, reductions were found in the folic acid content in MMW after HPP treatments of 300-500 MPa/60 ºC/5 min (0.407±0.000 to 0.468±0.039 μg/mL) compared with the folic acid content in the untreated MMW (0.721±0.021 μg/mL), whereas HPP treatment of 600 MPa/60 ºC/5 min significantly increased the folic acid content up to 0.975±0.000 μg/mL. According to Belitz et al. (2009), folic acid in foods exists as a conjugated form, bound to oligo-γ-L-glutamates. And folic acid has many different tetrahydrofolate derivatives. Therefore, HPP of 300-500 MPa/60 ºC/5 min might facilitate the isomerization of folic acid into derivatives.

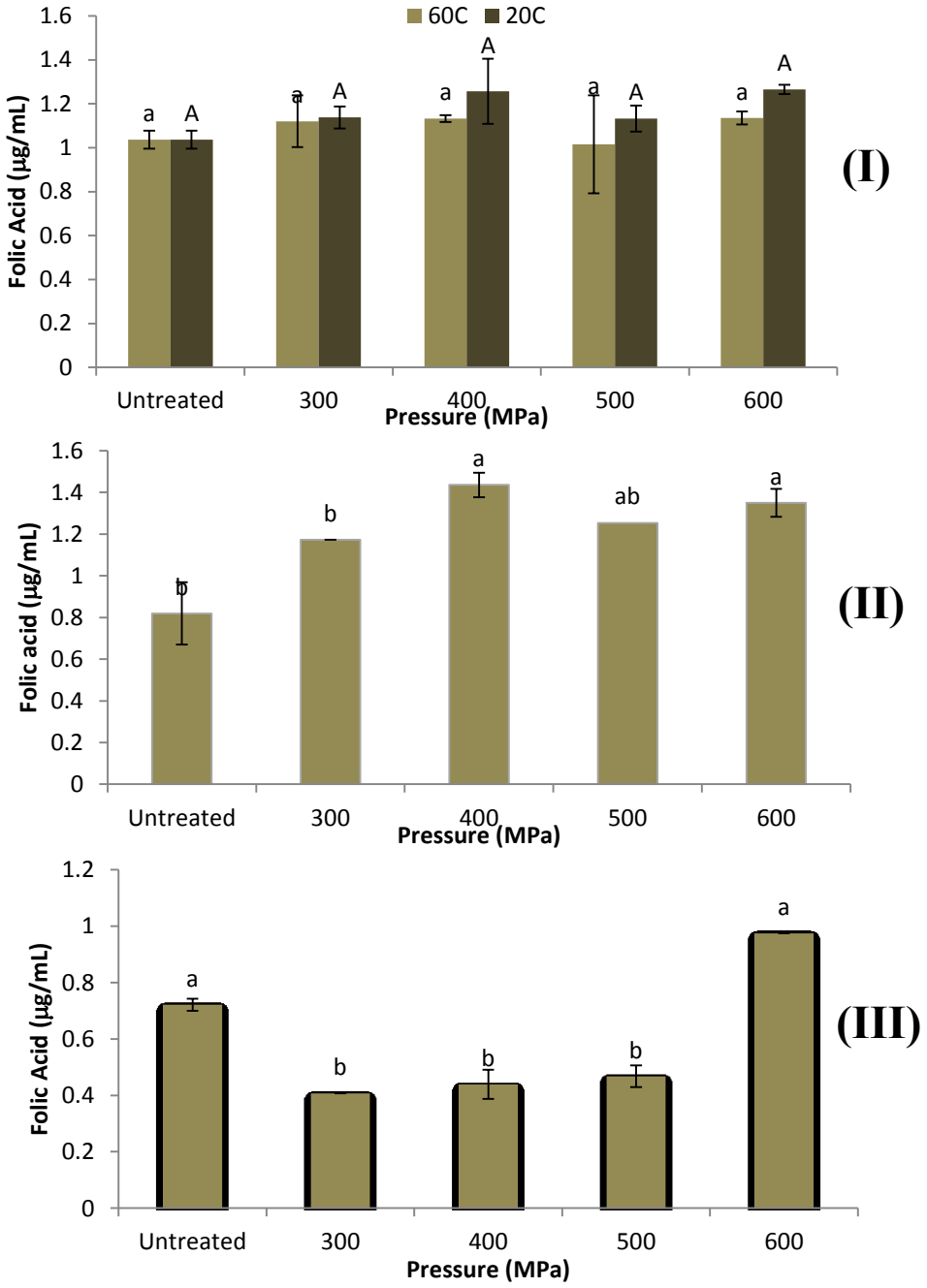


Figure 4-5 Folic acid content after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

4.5 Niacin in melon juice and melon milk

Results of vitamin B3 (niacin) are shown in Figure 4-7 (I). Niacin in untreated melon juice was 5.736±0.055 μg/mL, which is a little higher than the value (4.18 μg/mL) reported by the USDA National Nutrient Database for Standard Reference (2015). All HPP treatments at 60 ºC had no significant effect on niacin content in melon juice, whereas HPP treatments at 20 ºC induced significant changes in niacin content in melon juice, which might be attributed to enzyme activation. HPP treatment at 300 MPa/20 ºC/5 min significantly increased the niacin content up to 7.259±0.897 μg/mL while other treatments had no significant effect in the niacin content (5.050±0.127 – 6.980±0.432 μg/mL). Available data reported that niacin was stable in fruit products during most processing treatments, either in sterilization (data not reported) (Belitz et al., 2009) or high-intensity PEF treatment at 35 kV/cm for 1800 μs (Salvia-Trujillo et al., 2011). But, a loss of 31-65% was found in niacin content in vegetable products (asparagus, lima beans, green beans, spinach and baby corn-cobs) during canning and cooking (Belitz et al., 2009).

Based on limited studies available (Vidal-Valverde, Prodanov & Sierra, 1997; Nisha, Singhal & Pandit, 2009), niacin is the most stable water-soluble vitamin during processing (Eitenmiller et al., 2008), and unaffected by atmospheric oxygen, light and heat (Ball, 2006). Vidal-Valverde et al. (1997) reported that niacin in lentils significantly increased after fermentation at 28- 42 ºC (lentil flour with water) and 24-96 hours, which indicated that thermal conditions of 28-42 ºC/24-96 h did not affect niacin content. Nisha et al. (2009) investigated the

isothermal kinectics of niacin in potato plus water solution, reporting slow niacin degradation occurred where niacin has a half life of 693 min at 50 ºC and a half life of 224 min at 120 ºC. And the degradation rate of niacin is slower in potato than that in pure water solution (data not reported), probably due to the protection effects of phytochemicals present in potatoes. They also stated that the degradation of niacin in potato by pressure-cooking followed the similar trend as the isothermal degradation kinetics of potato in water. However, Prodanov, Sierra & Vidal-Valverde (2004) showed that alkali treatment with or without cooking (soaking in boiled water for 35 min) significantly decreased the niacin content by 32% in faba beans soaked in water, which indicated that niacin is alkali-labile and leaching was the route of niacin loss during food processing.

It is known that niacin moiety is contained in a pseudonucleotide, which is composed by the coenzyme - nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) (Figure 4-6). Most cellular NADP was found to be stored in the cytoplasm as bound either within a polysaccharide named niacytin (Kodicek & Wilson, 1960) or within a polypeptide named niacinogen (Das & Guha, 1960). Most of the bound niacin was found to be resistant to heating (Wall & Carpenter, 1988). Therefore it can be concluded that niacin content retention in HPP treated samples was ascribed to the stable structure of intracellular NADP.

Other group B vitamins like thiamin and riboflavin, were detected as traces in our study (data not shown) due to their thermolability. Besides, honeydew melon is not good sources of thiamin (Rickman, Barrett & Bruhn, 2007).

Niacin

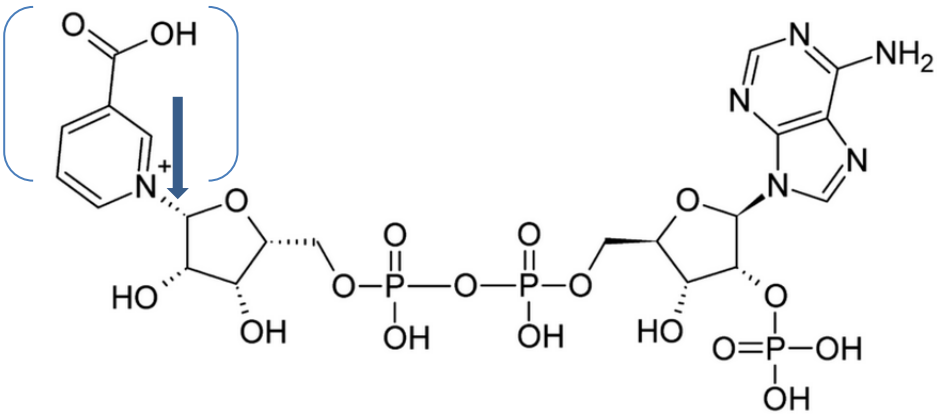


Figure 4-6 Structure of nicotinamide adenine dinucleotide phosphate (NADP) (adapted from Ball, 2006).

The niacin content in HPP treated MMS and MMW are shown in Figure 4-7 (II) and (III), respectively. The initial niacin contents were 5.436±0.862 and 1.195±0.091 μg/mL in MMS and MMW, respectively. The niacin content in MMS after all HPP treatments was between 5.632±1.713 and 7.372±0.880 μg/mL, which had no significant difference compared with the untreated sample. As discussed above, niacin in melon juice was stable after HPP treatments of 300-600 MPa/20-60 ºC/5 min. However, significant difference was found in MMW after HPP treatments. HPP treatment of 300 MPa/60 ºC/5 min significantly decreased niacin in MMW by 31.6% (0.817±0.014 μg/mL) compared with the untreated sample (1.195±0.091 μg/mL), whereas HPP treatment of 500 MPa/60 ºC/5 min significantly increased the niacin content to 1.624±0.067 μg/mL. It was reported that vitamin B3 in meat and milk are mainly in form of free

niacin or nicotinamide (Ball, 2004). Therefore, the loss of niacin in MMW after HPP treatments at 300 MPa/60 ºC/5 min might be attributed to the action of HPP on the free niacin of milk.

Group B vitamins are found in honeydew melon such as 4.18 μg/g of niacin, 0.88 μg/g of vitamin B-6, 19 μg/100 g of folate (USDA, 2015). Cow milk has also group B vitamins such as 0.89 μg/g of niacin, 36 μg/100 g of vitamin B-6, 5 μg/100 g of folate (USDA, 2015).

Data on group B vitamin content in fruit juice milk affected by HPP treatments is limited to one study. Salvia-Trujillo et al. (2011) reported that niacin content in a fruit juice milk (skim and whole) which was composed of orange (30%), kiwi (25%), mango (10%), pineapple (10%), milk (17.5%), and sugar (7.5%), was not significantly affected by the thermal treatment (90 ºC/1 min) or HIPEF treatment, which suggested that niacin in juice milk matrix may not be affected by HPP processing. However, group B vitamin including biotin, folic acid, and riboflavin in an orange-juice milk beverage were reported to have a noticeable loss of 18-23% after thermal treatment at 95 ºC/45 s (Rivas et al., 2007).

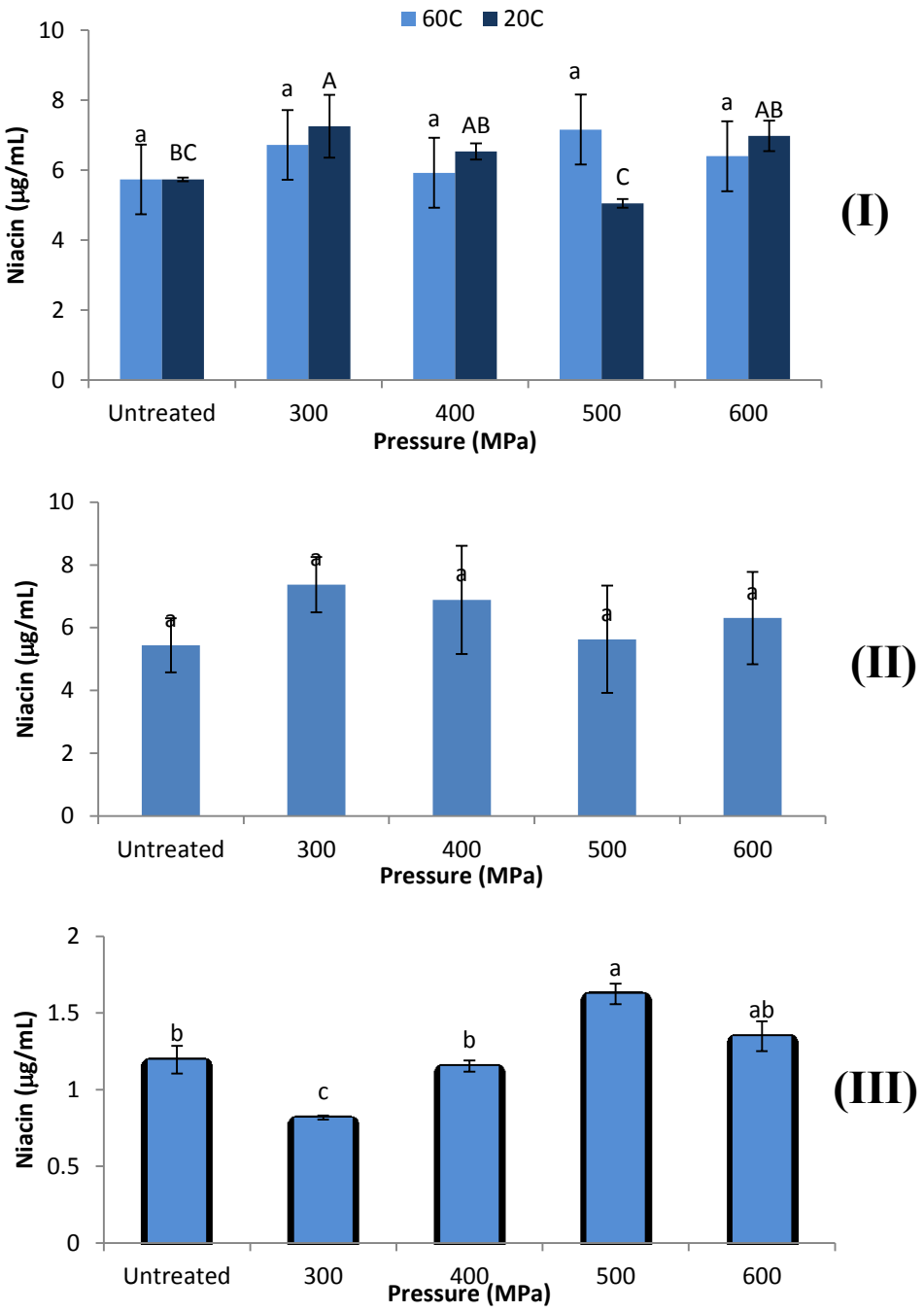


Figure 4-7 Vitamin B3 content after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

4.6 α-Tocopherol in melon milk

α-Tocopherol of untreated and HPP treated MMS and MMW are shown in Figure 4-8. The initial contents of α-tocopherol in untreated MMS and MMW were 0.177±0.034 μg/mL and 0.445±0.014 μg/mL, respectively. These values are consistent with the value (0.634±0.003 μg/mL) reported by Barba, Esteve & Frigola (2012) for a homemade orange juice milk. The content of α-tocopherol in MMW was higher than that in MMS, because of the higher fat content in the whole milk (3.87%) than the fat content in the milk with reduced fat (2%)It was found that commercial juice milk products with 3.6% M.F. had twice α-tocopherol as products that had only 1.5% M.F. (Herrero-Barbudo, Granado-Lorencio, Blanco-Navarro & Olmedilla-Alonso, 2005). Our study showed that HPP treatments of 300-600 MPa/60 ºC/5 min in MMS had no significant effects on α-tocopherol content (0.201±0.004 – 0.230±0.023 μg/mL). However, the content of α-tocopherol in MMW significantly improved up to 0.597±0.007 - 0.686±0.000 μg/mL after HPP treatments of 300-600 MPa/60 ºC/5 min. Among all HPP treated MMW samples, the lowest α-tocopherol content was found in MMW after treatment of 500 MPa/60 ºC/5 min (0.597±0.007 μg/mL) and all other treated samples had similar values in the α-tocopherol content. The obtained values can be ascribed to the tocopherols extraction effects of HPP on in milk fat.

α-Tocopherol is a lipophilic vitamin found as the dominant form of vitamin E in dairy products (Belitz et al., 2009). α-Tocopherol is stable to heat when oxygen is removed (Bramley

et al., 2000), but when exposed to free O_2, the compound was likely to be degraded by heat, trace metal and light (Ball, 2006). Barba et al. (2012) reported that α-tocopherol in an orange-juice milk (0.1% M.F.) was stable during thermal treatment at 90 ºC/15 s in the absence of oxygen.

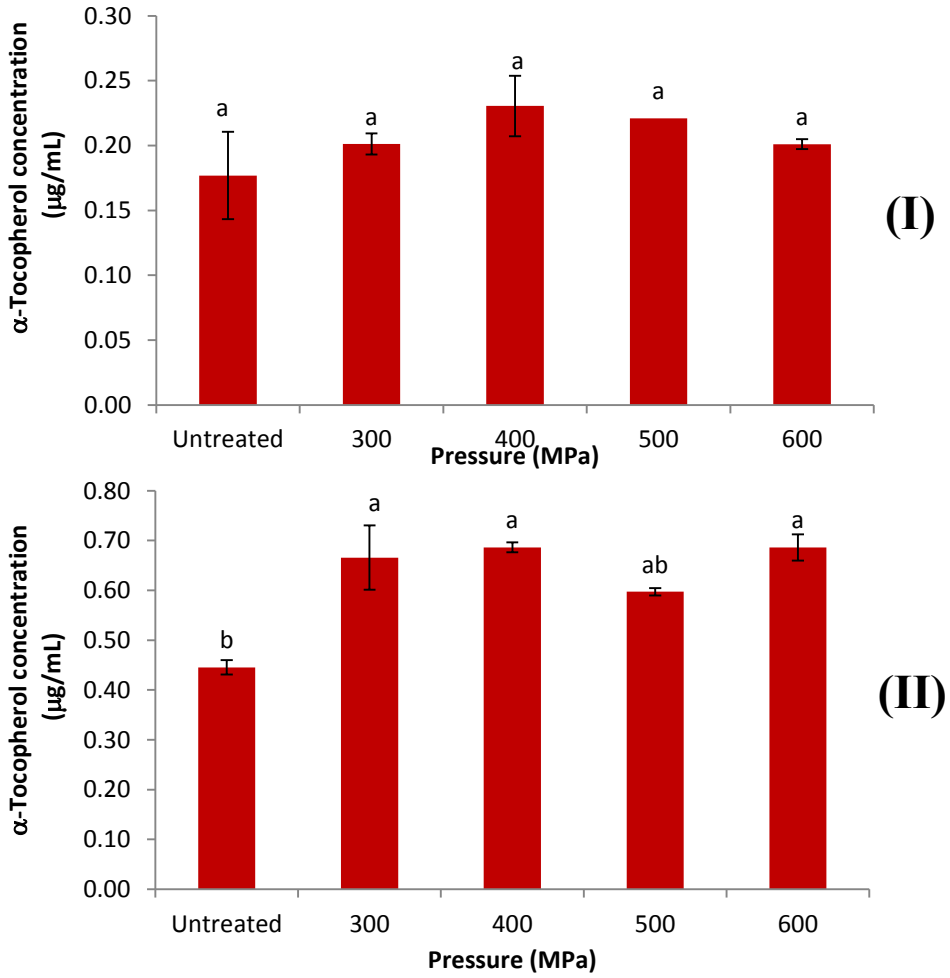


Figure 4-8 Vitamin E content after 5 min HPP treatments of: (I) melon milk (semi-skim) and (II) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C.

The MMW results of α-tocopherol after HPP treatments in our study were in consistent

with previous studies. A significant increase (7-28%) of α-tocopherol was induced after HPP treatment of 200-400 MPa at room temperature (data not reported) for 9 min (Barba et al., 2012). The study inferred that this increment of α-tocopherol could be due to the disruption of intracellular chloroplasts, which liberated α-tocopherol. Another study reported that HPP (588-784 MPa/25 ºC/30 min) treated egg white and yolk retained the total tocopherol and folate contents (Hayashi et al., 1989). Cilla et al. (2012) found that the α-tocopherol content was significantly increased by 22% in juice (orange, kiwi, pineapple and mango)-soymilk beverage.

4.7 TAC in melon juice and melon milk

4.7.1 Inhibition rate of DPPH in melon juice and melon milk

Results of TAC measured by DPPH are shown in Figure 4-9 (I). The DPPH value for the untreated sample was 21.51±1.94%. After all HPP treatments, DPPH values significantly decrease by 29.31 - 49.31%. No difference of DPPH inhibition rates was found between different pressure levels except that HPP treatment of 500 MPa/60 ºC/5min resulted in the highest DPPH inhibition rate in melon juice (15.20±0.48%) among all HPP treated samples. DPPH results cannot be related to the bioactive compounds contents studied in melon juice. Similar phenomena was observed by de Ancos et al. (2002), who found that HPP caused cell rupture and release of bioactive compounds (β-carotene, α-carotene, zeaxanthin and lutein) in orange juice after HPP treatments at 50-350 MPa/30 and 60 ºC/2.5-15 min, whereas significant decrease (from 37.5% to 22.5%) occurred in DPPH inhibition rate of the sample.

Reduction of free radical scavenging capacity was reported by previous HPP studies in fruit juices. Barba, Esteve & Frigola (2010) observed significant reduction of the TAC (from 1.61±0.10 to 1.10±0.15 - 1.58±0.03 mM Trolox) in a vegetable juice measured by ABTS radical scavenging ability after HPP treatments of 100-400 MPa/30 ºC/2-9 min. Chakraborty, Rao & Mishra (2015) also observed DPPH scavenging capacity in pineapple purée decreased by less than 5% when treated at 200-600 MPa and a temperature lower than 50 ºC for 10-20 min, whereas HPP at 60 ºC led to 6-9% loss of TAC and HPP at 70 ºC led ot 17-30% loss of TAC. Kaushik et al. (2014) reported that a long holding time (from single pulse up to 20 min) of HPP treatment (100-600 MPa/30 ºC induced a loss (maximum 9.5%) of DPPH inhibition rate in mango pulp. Varela-Santos et al. (2012) proved that HPP treatments of 450 MPa at ambient temperature (data not reported) for 0.5-2.5 min led to significant decreases in DPPH scavenging activities by 19.2-25.6% in pomegranate juice. However, other studies observed no significant change or increases in the radical scavenging ability in fruit juices after HPP treatments. For example, Sanchez-Moreno et al. (2004) reported that the DPPH scavenging activity was unaffected in tomato purée after an HPP treatment of 400 MPa/25 ºC/15min. Garcia et al. (2001) also found that treatments of 500 and 800 MPa at room temperature for 5 min caused no significant changes on the ABTS free radical scavenging capacity in orange juice. Patras et al. (2009a) demonstrated that 400-600 MPa/10-30 ºC/15 min caused no evident changes in TAC of strawberry and blackberry purées. But, DPPH radical scavenging capacity increased from TEAC of 2.86±0.23 g /L (untreated sample) to TEAC of 3.87±1.11 - 4.80±1.79 g/L in blackberry purée

after treatments of 400-600 MPa/20 °C/15min (Patras et al., 2009a), from TEAC of 0.37±0.04 g/L (untreated sample) to TEAC of 0.43±0.01 - 0.47±0.03 g/L in tomato purée after treatments of 400-600 MPa/20 °C/15min (Patras et al., 2009b) and from $FeSO_4$/7H2O Equivalent of 2070.73 μmol/mL (untreated sample) to 2546.34 μmol/mL in grape juice after HPP treatments of 400–600 MPa/40–60 °C/2–4 min (Chauhan et al., 2011).

TAC of HPP treated MMS and MMW measured by DPPH assay are shown in Figure 4-9 (II) and (III). DPPH inhibition rate in untreated MMS was 12.14±1.26%. All HPP treatments had no significant influence on the DPPH inhibition rate in MMS samples (14.64±0.51% to 16.49±0.68%). The untreated MMW had a DPPH inhibition rate of 13.53±0.55%, which is similar to the value in MMS (12.14±1.26%). After HPP treatments of 300-600 MPa/ 60 ºC for 5 min, the DPPH inhibition rate in MMW significantly improved up to 24.38±0.57%.

Barba et al. (2012) found different results for TAC measured by ABTS radical inhibition capacity and ORAC in orange juice milk after HPP treatments of 100-400 MPa/26.6 ºC/2-9 min. Treatments of 100 and 300 MPa/26.6 ºC/2-9 min significantly decreased the ABTS radical inhibition capacity with the exceptions of 200 and 400 MPa/26.6 ºC/2-9 min. However, ORAC results showed no significant change after all HPP treatments. They found no clear correlation between the content of antioxidant compounds, such as vitamin C or carotenoids, and TAC, but a negative relationship between TAC and total phenolics was observed.

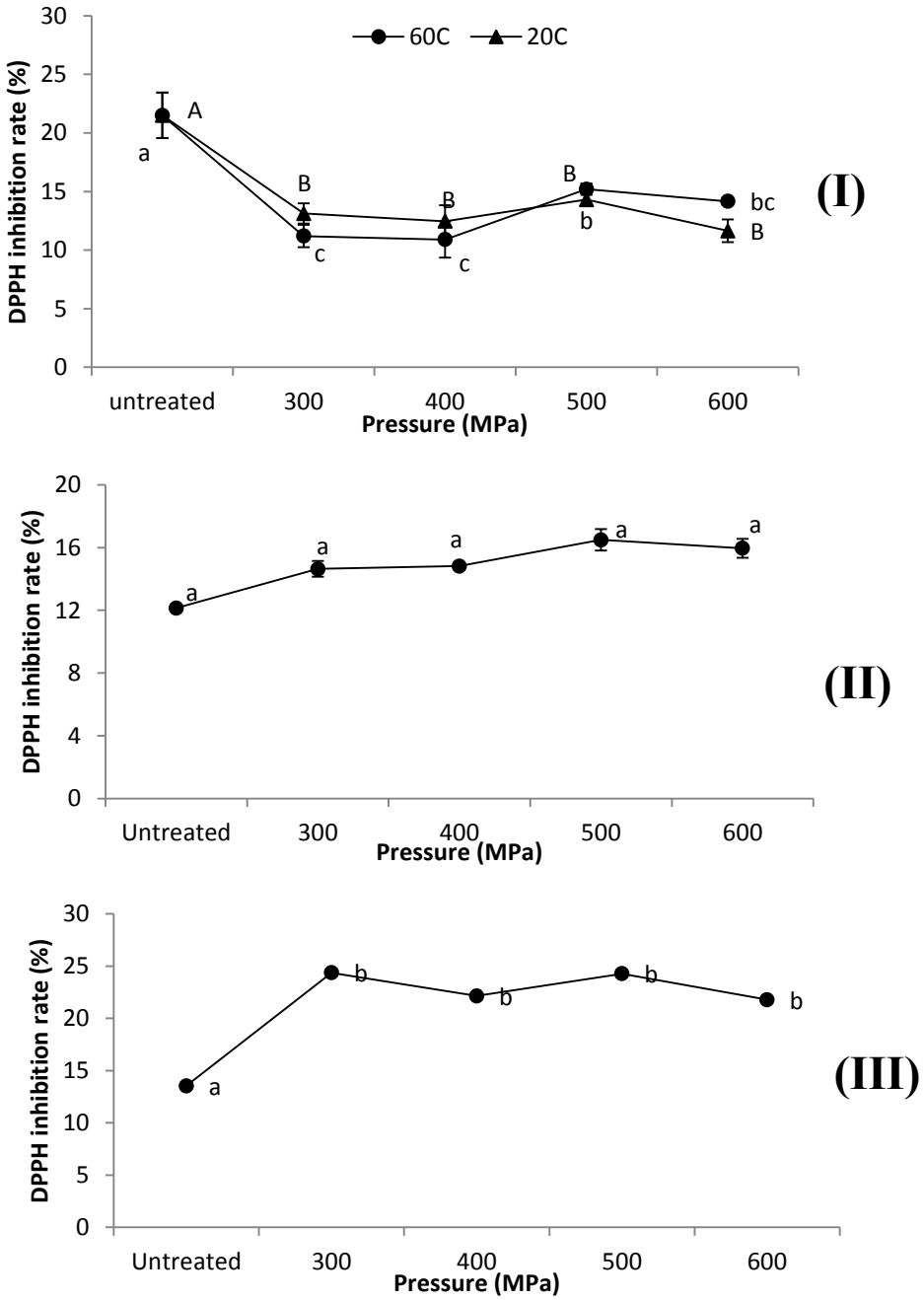


Figure 4-9 Inhibition rate of DPPH after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

4.7.2 FRAP in melon juice and melon milk

Due to the complexity of food matrices, TAC only measures the overall antioxidant capacity in foods rather than individual antioxidant compounds. Therefore, most studies use two methodologies for the determination of TAC. In this thesis, FRAP and DPPH assays were used.

FRAP of in untreated melon juice was 90.40±4.51 mg GAE/mL. HPP treatments at both 20 and 60 ºC for 5 min had no significant effects on FRAP in melon juice (Figure 4-10 (I), Appendix B.3), showing different trends compared with DPPH results (Fig. 4-9 (I)).

The TAC in MMS measured by FRAP (Fig. 4-10 (II)) had a similar trend to the DPPH results (Fig. 4-9 (II)). The initial FRAP value for untreated MMS was 99.12±5.03 mg GAE/mL and there was no significant change (79.76±1.78 - 95.26±7.73 mg GAE/mL) after all HPP treatments (Fig. 4-10 (II)).

In Figure 4-10 (III), HPP treatments of 300-600 MPa/60 ºC/5 min improved the TAC in MMW measured by FRAP (131.15±2.09 to 138.69±1.16 mg GAE/mL) compared with the untreated MMW (114.46±1.07 mg GAE/mL).

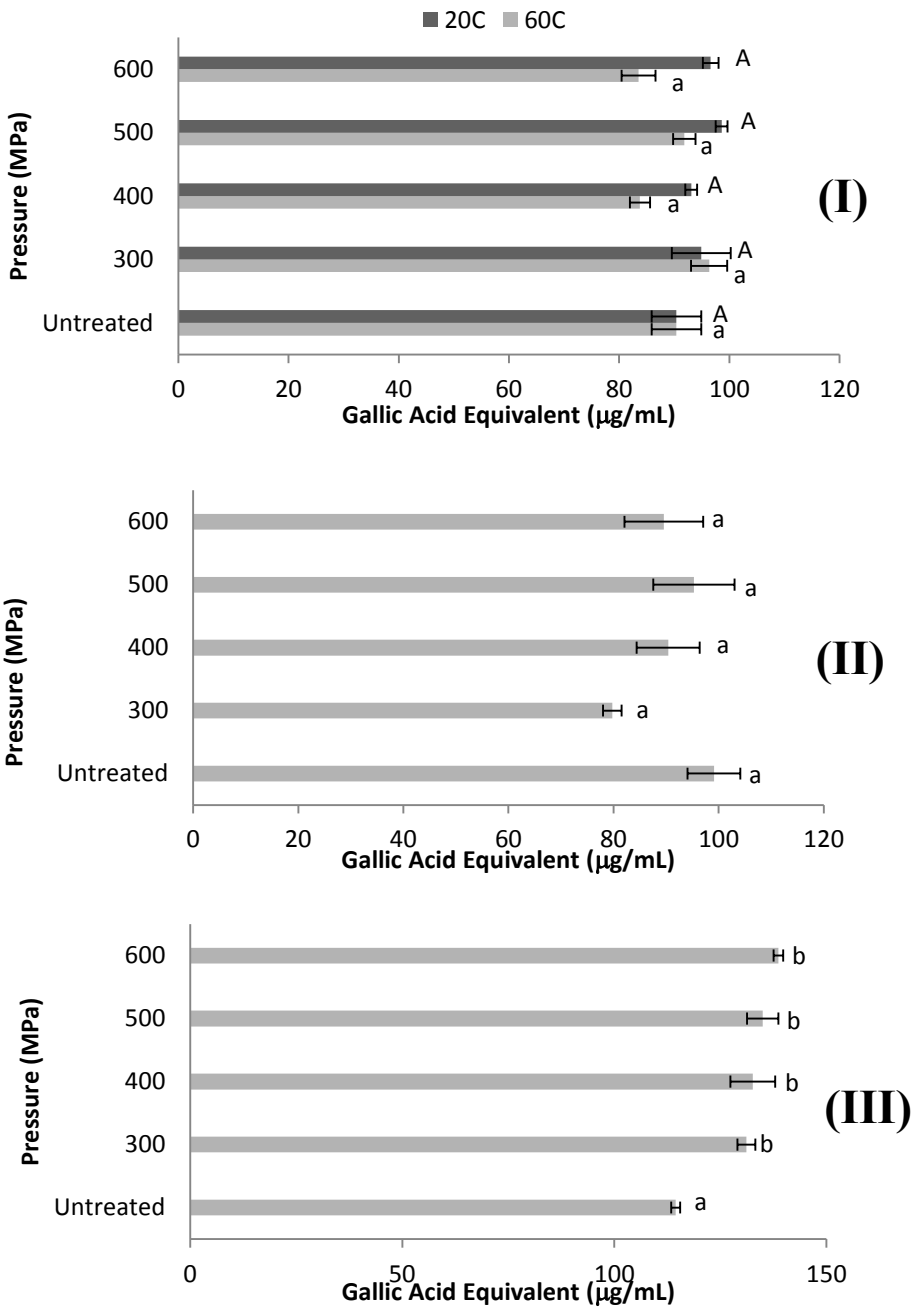


Figure 4-10 FRAP presented as gallic acid equivalent after 5 min HPP treatments of: (I) melon juice, (II) melon milk (semi-skim) and (III) melon milk (whole). Data that do not share the same lower case letters (a-c) mean significant difference($P<0.05$) between pressures at 60 °C, and data that do not share the same capital letters (A-C) mean significant difference ($P<0.05$) between pressures at 20 °C.

The difference of the results obtained by the DPPH and FRAP assays in our study (Figure 4-9 and 4-10) is attributed to the different principles of those two methodologies. According to the reactions involved, TAC assays are classified into either Single Electron Transfer (SET) reaction or Hydrogen Atom Transfer (HAT) reaction (Huang et al., 2005). DPPH assay is based on HAT reaction with different mechanism. HAT-based assay uses peroxyl radicals from the reagent as probes to react with H-atoms from antioxidants. However, the probes in DPPH solution are long-lived and stable nitrogen radicals rather than highly reactive and transient peroxyl radicals (Huang et al., 2005). Therefore, some antioxidants that can react fast with peroxyl radicals react more slowly or are even inactive to DPPH radicals. As a result, the TAC values might be under estimated. Different from the DPPH assay, the FRAP assay relies on SET reaction (Equation 4.1).

Probe (oxidant) + eletron (from antioxidant) → reduced probe + oxidized antioxidant (4.1)

The oxidant in reactive solution draws an electron from the antioxidant, leading to color changes of the oxidant, which is proportional to the antioxidant concentration. This mechanism is similar to a traditional redox titration analysis. The results can be related to TAC, assuming that TAC is equal to reducing capacity (Benzie & Strain, 1999)

Recently, Torres-Ossandón et al. (2015) used the DPPH and ORAC assays to evaluate the effect of HPP treatments of 300-500 MPa/20 °C/5 min on cape gooseberry juice. They found that all treatments significantly increased the DPPH scavenging capacity whereas treatments of 300

and 400 MPa decreased ORAC results, which were attributed to the degradation of anthocyanins. Tadapaneni et al. (2012) showed that HPP treatments of 600-800 MPa at room temperature (18-22 ºC) for 1 min reduced FRAP values in strawberry juice.

4.8 Correlation between TAC, total phenolics content and ascorbic acid

Our study showed a similar trend between TAC measured by the FRAP assay and total phenolics content. Other studies revealed that the total phenolics content was highly related to TAC in non-citrus fruits, including nectarines, peaches and plums (Gil, Tomas-Barberan, Hess-Pierce & Kader, 2002) or fruit juices (apple juice and pineapple juice) (Gardner, White, McPhail & Duthie, 2000), knowing that phenolics are the most abundant secondary metabolites in fruits. By investigating the relationship between TAC and total phenolics content, ascorbic acid, carotenoids in different cultivars of nectarines, peaches and plums, Gil et al. (2002) found a high correlation between total phenolics content and TAC measured by the DPPH and FRAP assays. Tomas-Barberan et al. (2001) reported that hydroxycinnamic acid derivatives and flavan-3-ols were responsible for the TAC in nectarines and peaches while the TAC in plums was mainly attributed to the presence of flavan-3-ols rather than hydroxycinnamates. Since flavonols and anthocyanins are mainly in the peels, they do not contribute to the TAC in those fruits. Gil et al. (2002) also reported the ascorbic acid contents in nectarines (48-132 μg/g), peaches (36-126 μg/g) and plums (25-102 μg/g), which was not the main antioxidant compound in those fruits. This finding is in agreement with previous studies for strawberry, raspberry and other berries (Kalt, Forney, Martin & Prior, 1999). Gardner et al. (2000) revealed that ascorbic acid was more

likely to be the major contributor to the TAC in high ascorbic acid foods, like orange (1.008-1.385 μM/mL). The same was found in guava extract, which had a high ascorbic acid content (1.742-3.967 mg/g) and that resulted in a high content of TAC measured by DPPH, ABTS and FRAP assays (Thaipong et al., 2006). However, this result is different from the study of 27 selected kinds of fruits, which showed that the contribution of ascorbic acid to the TAC measured by ABTS scavenging capacity varied from 25.5% in orange to 70% in rambutan king (Leong & Shui, 2002).

4.9 HPP effects of cell rupture

The increase of bioactive compounds after HPP treatments is mainly attributed to the rupture of the plant cells in melon pulp caused by compression effects of high pressure, leading to the release of cytosol into the extracellular space. By measuring the conductivity of the water surrounding cells in raw broccoli during the treatments at 100-400 MPa/10-60 ºC/4-90 min, Eylen, Oey, Hendrickx & Loey (2008) stated that increasing pressure-temperature combinations caused more severe cell damage while temperature above 60 ºC had no effects on cell damage. However, cell damage in broccoli was less likely to occur under thermal treatments. High pressure (100-800 MPa) caused permeabilization of plant cells which facilitated the leakage of vitamins into intercellular space (Dornenburg & Knorr, 1993). High pressure would increase the extraction yields since it deprotonates charged groups and disrupts salt bridges and hydrophobic bonds in cell membranes.

Chapter 5. Conclusions and Recommendations

5.1 Conclusions

This study is the first to blend two types of milk with honeydew melon juice. The results showed the effects of HPP on honeydew melon juice and melon juice milk. Due to the food matrix effect, different effects of HPP were found on the contents of bioactive compounds in melon juice, MMS and MMW.

HPP treatments at 300 – 600 MPa/20 and 60 ºC/5 min significantly ($p<0.05$) increased vitamin C content by 53.2-223.6% due to the increased cell permeability induced by HPP. Other bioactive compounds including total phenolics content, vitamin B3 and folic acid were retained in melon juice after all HPP treatments. The significant decrease ($p<0.05$) of total flavonoids content after HPP treatments at 20 ºC might be related to enzymes activity that was activitated by HPP.

In MMS, HPP treatments of 300 – 600 MPa/60 ºC/5 min significantly ($p<0.05$) increased folic acid content while total phenolics content and total flavonoids decreased after 300 - 400 MPa/60 ºC/5 min. All HPP treatments retained ascorbic acid, niacin, α-tocopherol and TAC.

Significant ($p<0.05$) increases of total phenolics content, ascorbic acid and α-tocopherol were observed in MMW, indicating increased cell wall permeability caused by HPP treatments. There was a reduction of folic acid content after 300-500 MPa/60 ºC/5 min and significant ($p<0.05$) changes of color parameters. Niacin content in MMW showed complex results after different HPP treatments.

Other conclusions and limitations are: pH of both melon juice and melon milk increased after HPP treatments, but it is unclear if this is due to pressurization or depressurization. The different results between MMS and MMW after HPP treatments might be related to the fat content (semi skim milk: 2% M.F. and whole milk: 3.87% M.F.) in the two samples. The increase in greenness (a*) yellowness (b*) and lightness (L*) of melon juice after all HPP treatments indicated the release of cytosol and chlorophyll compounds from melon cells induced by high pressure. However, these compounds were not analyzed. The increase in redness (a*) and lightness (L*) and decrease in yellowness (b*) of MMW indicated the release of β-carotene from milk fat globules induced by high pressure. However, β-carotene was not detected in this thesis so the relationship between redness and β-carotene content could not be demontrasted. The contents of total phenolics, total flavonoids and ascorbic acid in untreated MMS and MMW are much higher than that in untreated melon juice, which may be due to the blending effect which caused cell damages and released those compounds into the samples. This can be proved by measuring total phenolics content, total flavonoids content and ascorbic acid content in melon milk before and after blending.

5.2 Recommendations

The following recommendations are offered for future studies to better understand the effects of HPP on melon juice and melon milk:

- Analysis should be conducted regarding the microstructure of milk protein in honeydew melon by using HPLC, calorimetric titration and NMR scattering, before and after mixing

melon juice and milk, in order to understand the interaction between phenolic compounds and proteins.

- Flavonoids like quercetin and hesperidin, should be determined by HPLC to better understand the effects of HPP on total phenolics content and total flavonoids content.
- Cell permeability should be investigated by detecting the conductivity of melon juice or juice milk to prove the extend of release of intracellular bioactive compounds from plant cells induced by HPP.
- Given that pH changes are affected by pressurization and depressurization, measurements should be taken during HPP treatments.
- Chlorophyll compounds content should be determined by spectrometry or HPLC before and after HPP treatments to explain the change of greenness and yellowness in samples. The content of β−carotenoids should be determined by HPLC to explain the change of redness in MMW.
- Dehydroascorbic acid should be measured using HPLC to understand any transformation or oxidation of ascorbic acid in the HPP treated samples.
- Isomers of folic acid should be investigated by HPLC to understand the increase of folic acid in MMS and decrease of folic acid in MMW after HPP treatments.
- Detection of NADP using spectroscopy is recommended to understand the change of free niacin in melon juice and MMW after certain HPP treatments.

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Appendix A: Calibration curves

A.1. Standards and calibration for total phenolics determination

Table A.1. Concentration and absorbance of standard gallic acid solutions in Total Phenolics assay using spectrometry.

Samplec code	**Dilution**	**Concentration /Y (g/L)**	**Absorbance /X**						
Blank			0.005						
Stock solution	1	5	A1	A2	A3	A1-Blank	A2-Blank	A3-Blank	$A_{average}$
GA40	40	0.13	0.071	0.071	0.073	0.066	0.066	0.068	0.067
GA20	20	0.25	0.198	0.204	0.197	0.193	0.199	0.192	0.195
GA10	10	0.50	0.483	0.47	0.476	0.478	0.465	0.471	0.471
GA5	5	1.00	0.958	0.935	0.929	0.953	0.930	0.924	0.936

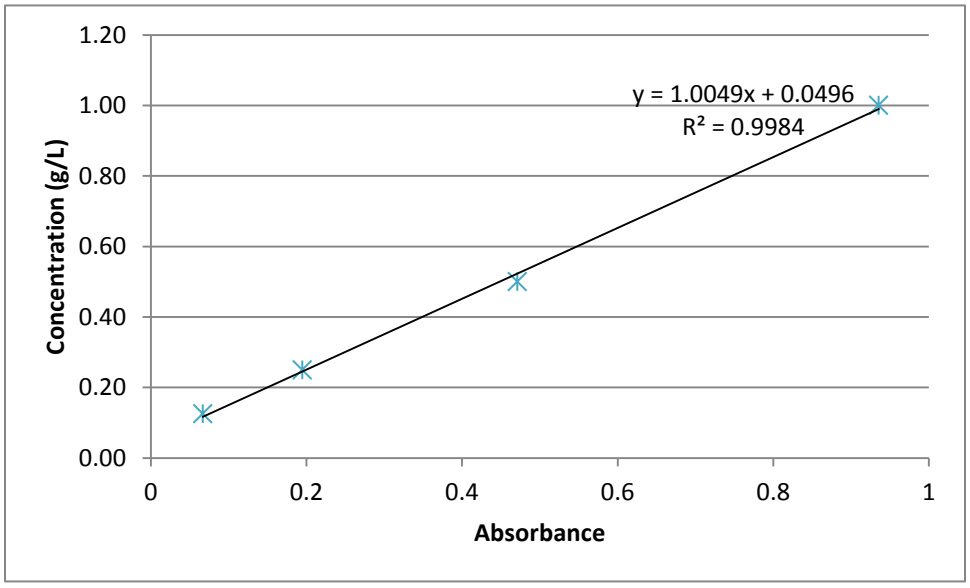

Figure A.1. Calibration curve of gallic acid solution in total phenolics assay.

A.2. Standards and calibration for total flavonoidss determination

Table A.2. Concentration and absorbance of standard quercetin solutions in Total Flavonoids content assay using spectrometry.

Sample code	Dilution	Concentration /Y(mg/mL)	Absorbance			After blank			$A_{average}$
Blank			0.355	0.36	0.357				0.357
Stock solution	1	0.5							
S30	30	0.017	0.667	0.678	0.659	0.3095	0.3205	0.3015	0.3105
S50	50	0.01	0.527	0.538	0.528	0.1695	0.1805	0.1705	0.1735
S100	100	0.005	0.409	0.413	0.409	0.0515	0.0555	0.0515	0.0528
S150	150	0.003	0.381	0.379	0.383	0.0235	0.0215	0.0255	0.0235

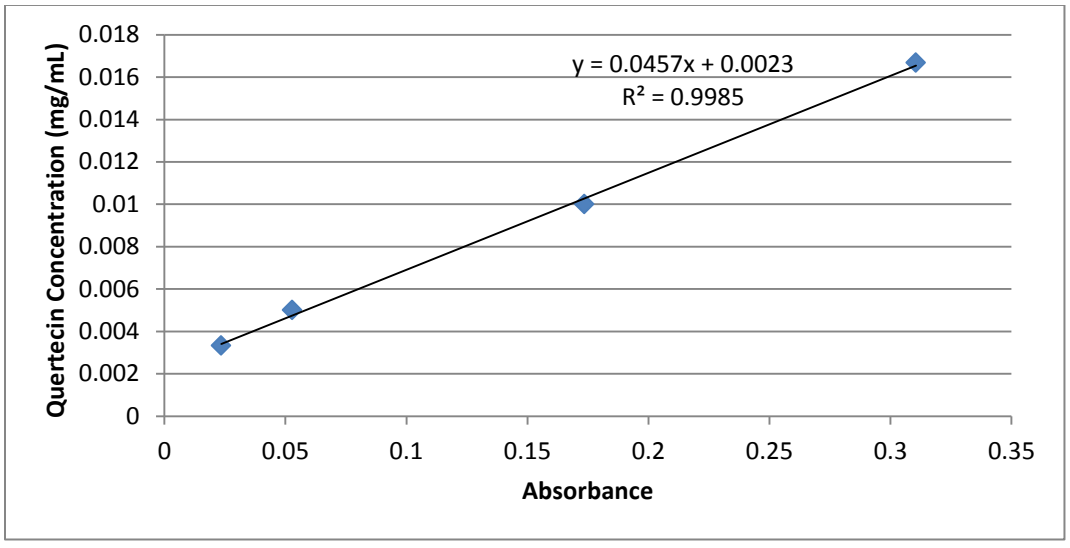

Figure A.2. Calibration curve of quercetin solution in total flavonoid content assay.

A.3. Standards and calibration for vitamin C determination

Table A.3. Concentration and peak area of standard L-ascorbic acid solutions in Vitamin C measurement using HPLC.

Sample Code	Stock solution	S2	S3	S4	S6	S8
Dilution	1	20	30	40	60	80
Concentration/ Y(mg/mL)	4	0.2	0.133	0.1	0.067	0.05
Peak area (after blank)/X		2472811	1670660	1377269	978015	733597

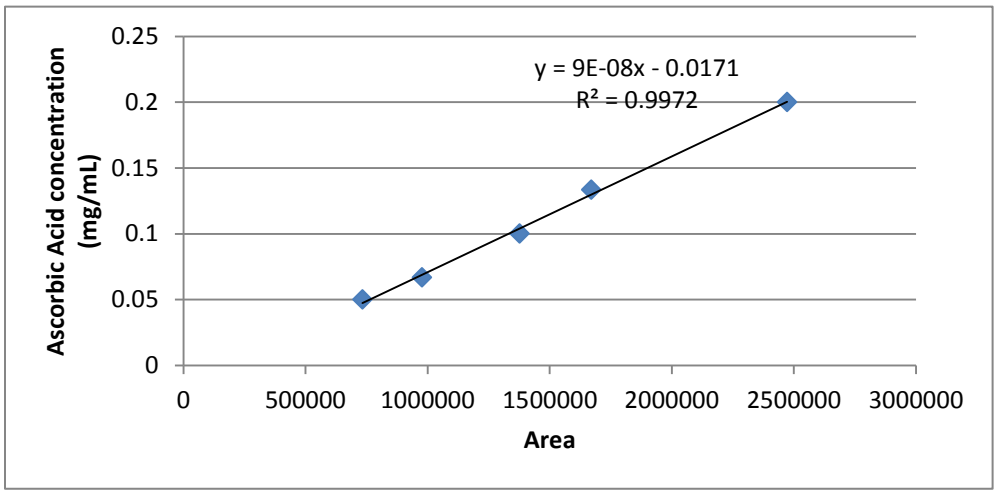

Figure A.3. Calibration curve of L-ascorbic acid solution using HPLC.

A.4. Standards and calibration in vitamin B3 determination

Table A.4. Concentration and peak area of standard niacin solutions in Vitamin B3 measurement using HPLC.

Sample Code	Stock solution	S3	S4	S6	S8	S10
Dilution	1	30	40	60	80	100
Concentration/ Y(mg/mL)	4.484	0.149	0.1121	0.075	0.056	0.045
Peak area (after blank)		2140073	1587971	1185152	887036	752176

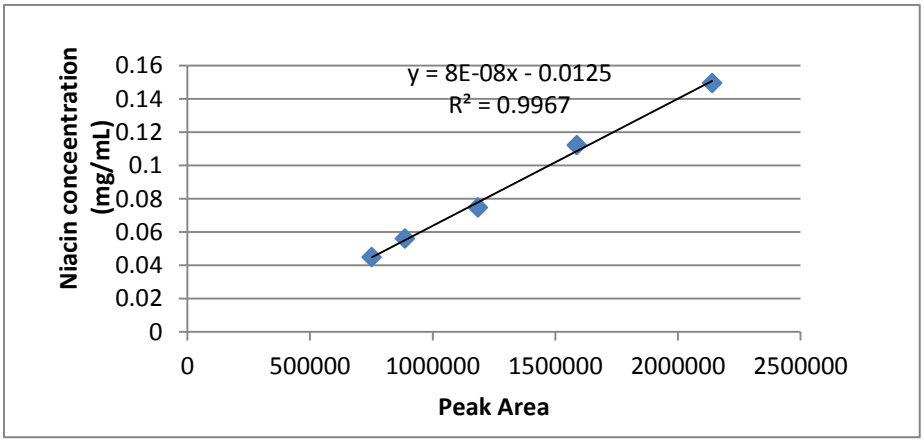

Figure A.4. Calibration curve of niacin solution using HPLC.

A.5. Standards and Calibration in folic acid determination

Table A.5. Concentration and peak area of standard folic acid solutions for Folic Acid measurement using HPLC.

Sample Code	Stock solution	S1	S3	S4	S6	S8
Dilution	1	10	30	40	60	80
Concentration/ Y(mg/mL)	3.919	0.3919	0.1306	0.0980	0.0653	0.0490
Peak area (after blank)/X		7136672	2209390	2498645	1831366	1477607

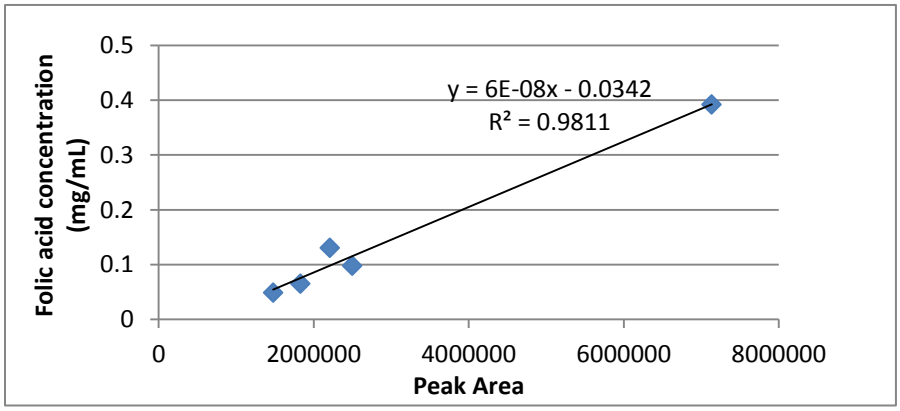

Figure A.5. Calibration curve of folic acid solution using chromatography.

A.6. Standards and calibration in α-tocopherol determination

Table A.6. Concentration and peak area of standard folic acid solutions in Folic Acid measurement using chromatography.

Sample Code	Stock solution	S4	S8	S20	S100
Dilution	1	4	8	20	100
Concentration/ Y(μg/mL)	6.353	1.588	0.794	0.318	0.064
Peak area (after blank)/X		25337	13699	4854	1289

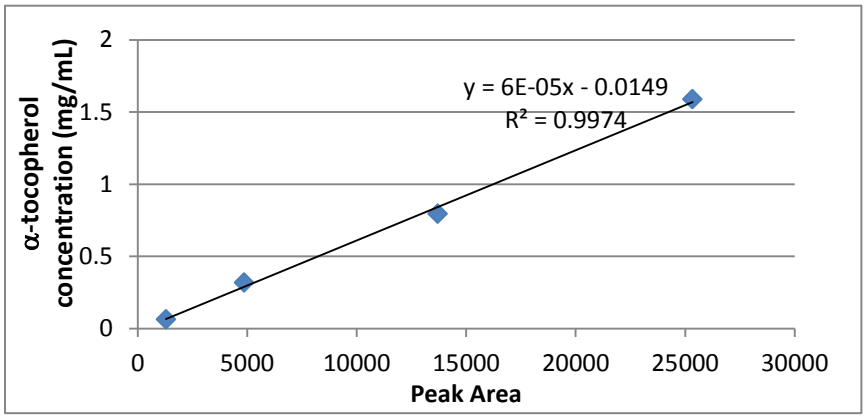

Figure A.6. Calibration curve of folic acid solution using chromatography.

Appendix B: Bioactive compounds

B.1. Total phenolics and total flavonoids contents

Table B.1. Values of total phenolics and total flavonoids content in melon juice and melon milk after HPP treatments.

Treatments (5 min)	Total phenolics content (μg GAE/mL)	Total flavonoids content (μg QE/mL)
Honeydew melon juice		
Untreated	333.7±1.1B	193.0±5.5A
300MPa/20ºC	332.5±4.0B	169.9±4.5B
400MPa/20ºC	333.1±10.1B	178.15±5.8B
500MPa/20ºC	336.9±3.8B	168.8±7.4B
600MPa/20ºC	362.9±9.6A	180.0±1.9B
Untreated	333.7± 1.1a	193.0±5.5a
300MPa/60ºC	333.7±2.2ab	180±6.5a
400MPa/60ºC	343.9±6.1ab	201.2±2.8a
500MPa/60ºC	361.0±6.1a	219.4±23.6a
600MPa/60ºC	359.7±13.7a	187.1±8.6a
Melon Milk (semi-skim) (MMS)		
Untreated	690.3±4.0a	355.9±14.9a
300MPa/60ºC	585.9±3.9c	230.6±2.9d
400MPa/60ºC	614.0±5.6bc	250.7±5.1bc
500MPa/60ºC	669.2±11.4ab	265.4±5.6b
600MPa/60ºC	733.5±7.9a	231.8±7.9cd
Melon Milk (whole) (MMW)		
Untreated	773.4±18.3c	294.5±1.0a
300MPa/60ºC	968.2±45.5b	298.8±3.0a
400MPa/60ºC	1062.1±24.1a	297.9±6.6a
500MPa/60ºC	974.2±22.8b	281.0±14.2a
600MPa/60ºC	1014.0±11.9ab	285.4±12.6a

Mean±standard deviation (n=3) within each row with different letters (a-c) are significantly ($P<0.05$) different.
Mean±standard deviation within each column with different letter (A-E) are significantly ($P<0.05$) different.
GAE: gallic acid equivalent; QE: quercetin equivalent.

B.2. Vitamins content

Table B.2. Values of bioactive compounds (μg/mL) in melon juice and melon milk after HPP treatments.

Treatment (5 min)	Ascorbic Acid	Niacin	Folic Acid	α-tocopherol
Honeydew melon juice				
Untreated	109.21±34.06C	5.736±0.055BC	1.037±0.041A	0.04±0.01
300MPa/20ºC	261.91±1.76B	7.259±0.897A	1.138±0.051A	ND
400MPa/20ºC	261.20±9.48B	6.535±0.234AB	1.257±0.148A	ND
500MPa/20ºC	283.57±0.00AB	5.050±0.127C	1.133±0.060A	ND
600 MPa/20ºC	327.93±0.21A	6.980±0.432AB	1.266±0.021A	ND
Untreated	109.21±34.06c	5.736±0.055a	1.037±0.041a	0.04±0.01
300MPa/60ºC	167.32±26.99b	6.723±1.447a	1.121±0.118a	ND
400MPa/60ºC	274.69±55.57ab	5.925±0.283a	1.133±0.016a	ND
500MPa/60ºC	301.36±24.82ab	7.161±0.654a	1.016±0.223a	ND
600MPa/60ºC	353.39±2.68a	6.402±0.953a	1.136±0.30a	ND
Melon Milk (semi-skim) (MMS)				
Untreated	139.63±22.73a	5.436±0.862a	0.820±0.149b	0.18±0.03a
300MPa/60ºC	95.19±0.05a	7.372±0.880a	1.173±0.000b	0.20±0.01a
400MPa/60ºC	118.91±0.000a	6.887±1.725a	1435±0.059a	0.23±0.02a
500MPa/60ºC	96.11±17.92a	5.632±1.713a	1.253±0.000ab	0.22±0.00
600MPa/60ºC	133.85±0.51a	6.310±1.472a	1.350±0.007a	0.20±0.00a
Melon Milk (whole) (MMW)				
Untreated	268.5±1.81b	1.195±0.091b	0.721±0.021a	0.45±0.01b
300MPa/60ºC	295.16±8.57a	0.817±0.014c	0.407±0.000b	0.67±0.05a
400MPa/60ºC	295.52±6.82a	1.154±0.037b	0.439±0.052b	0.69±0.01a
500MPa/60ºC	268.95±1.45b	1.624±0.067a	0.468±0.039b	0.60±0.02ab
600MPa/60ºC	264.22±9.22b	1.348±0.097ab	0.975±0.000a	0.69±0.01a

Mean±standard deviation (n=2) within each row with different letters (a-c) are significantly (P<0.05) different.
Mean±standard deviation within each column with different letter (A-E) are significantly (P<0.05) different.
ND: not detected.

B.3. Total Antioxidant Capacity

Table B.3. Values of Total Antioxidant Capacity (TAC) measured by FRAP and DPPH in melon juice and melon milk after HPP treatments.

Treatments (5 min)	FRAP (mg GAE/mL)	DPPH inhibiition rate
Honeydew melon juice		
Untreated	90.40±4.51A	21.51±1.94%A
300MPa/20°C	94.93±3.27A	13.13±0.86%B
400MPa/20°C	93.09±1.81A	12.46±1.38%B
500MPa/20°C	98.62±2.01A	14.31±0.54%B
600MPa/20°C	96.61±3.06A	11.64±0.96%B
Untreated	90.40±4.51a	21.51±1.94%a
300MPa/60°C	96.35±5.34a	11.20±0.96%c
400MPa/60°C	83.78±1.07a	10.90±1.54%c
500MPa/60°C	91.83±1.07a	15.20±0.48%b
600MPa/60°C	83.53±1.42a	14.16±0.21%bc
Melon Milk (2% M.F.) (MM2)		
Untreated	99.12±5.03a	12.14±1.26%a
300MPa/60°C	79.76±1.78a	14.64±0.51%a
400MPa/60°C	90.40±6.00a	14.82±0.25%a
500MPa/60°C	95.26±7.73	16.49±0.68%a
600MPa/60°C	89.56±7.51	15.95±0.61%a
Melon Milk (whole) (MMW)		
Untreated	114.46±1.07a	13.48±1.33%a
300MPa/60°C	131.15±2.09b	24.38±0.57%b
400MPa/60°C	132.65±5.28b	22.14±0.36%b
500MPa/60°C	135.00±3.71b	24.29±0.09%b
600MPa/60°C	138.69±1.16b	21.79±0.51%b

Mean±standard deviation (n=2) within each row with different letters (a-c) are significantly ($P<0.05$) different.
Mean±standard deviation within each column with different letter (A-E) are significantly ($P<0.05$) different.