A COMPARISON AMONG DIFFERENT ALTERNATIVES OF PROCESSED GREEN JUICE BLENDS: EVALUATION OF ENZYME ACTIVITY, BIOACTIVE COMPONENTS, AND MICROFLORA

By

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To my parents who have worked so hard to provide me the best education, the most valuable gift that I have been given.
To Evee who inspired me to never give up chasing a dream.

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Health-conscious consumers associate drinking green juice blends with being healthy and lowering their sugar intake. The two primary objectives of this study were to examine commercial green juice blends alternatives at retail market, and to evaluate several product quality parameters of unprocessed and thermal pasteurized green juice blends. All products were evaluated for enzyme activities of polyphenol oxidase (PPO) and peroxidase (POD), levels of chlorophyll *a* and *b*, total carotenoids, total antioxidant capacity, total coliforms, *E. coli*, and total aerobic plate counts (APC).

For the first objective, three high-pressure processed (HPP) juices and one thermally processed juice were examined. Enzyme activities were higher in the HPP juices compared to the thermally processed juice. Chlorophyll levels appeared formula dependent. Levels of total carotenoids were similar among juices. Average levels of total coliforms were 2.56 ± 0.07 log CFU/ml for one HPP juice and lower than the detection limit for the remaining juices. Levels of *E. coli* were lower than the detection limit in all juices. APC, as a general quality indicator, varied widely among all juices.

For the second objective, a representative green juice blend was formulated, and then thermally treated in four independent trials. Thermal treatments applied to the juices were at 72 °C for 15 s, and at 90 °C for 30 s. In the control, average PPO and POD activities were 12.17 and 38.33 U/ml, respectively. After thermal processing, enzyme activities decreased and were significantly different among treatments ($P < 0.05$). PPO was more thermoresistant than POD. In the control, average levels of chlorophyll *a*, chlorophyll *b*, and total carotenoids were 56.04 ± 3.04, 25.36 ± 1.19, and 13.24 ± 0.80 µg/ml, respectively. After thermal processing, the levels of chlorophylls decreased significantly and differed among treatments ($P < 0.05$). Chlorophyll *a* was more affected than chlorophyll *b* during the mild thermal treatment. No significant differences existed between control and the milder treatment for the levels of total carotenoids ($P > 0.05$), but the levels decreased after the higher treatment. In the control, average antioxidant capacity was 0.67 ± 0.07 µmol of TE/ml, which increased after thermal treatments. No significant differences existed between treatments ($P > 0.05$). In the control, average levels of total coliforms and APC were 2.32 ± 0.62 and 5.44 ± 0.42 log CFU/ml, respectively. The level of *E. coli* was 1.00 log CFU/3 ml in only one of the four trials. After thermal processing, the levels of total coliforms and *E. coli* were lower than the detection limit. Average levels of APC after mild and higher treatments were 2.79 ± 0.30 and 2.09 ± 0.08 CFU/ml, respectively. Overall, results showed that thermal pasteurization reduced the enzyme activity of PPO and POD, levels of chlorophyll *a* and *b*, total coliforms, *E. coli*, and APC, especially at higher temperature. Furthermore, a milder thermal treatment increased the total antioxidant capacity and bioavailability of total carotenoids in green juice blends.

CHAPTER 1
INTRODUCTION

Health and wellness is a valuable market in the United States (US). In 2016, its division of food and beverages was expected to approximately account for $66 millions of industry revenue (IBISWorld 2016). Furthermore, health and wellness market also drives new food trends among consumers. A growing consumer segment is the health-conscious consumers who value food products with “natural” ingredients as well as those designated as sugar free, low salt, or made from fruit and vegetables (Nielsen 2015).

Drinking juices and beverages is a convenient means to consume fruit and vegetables. Juice production had a domestic demand of $12.8 billions and an industry revenue of $12.0 billions in 2016 (IBISWorld 2017). The fruit juice industry, however, has faced some concerns about the relation between obesity and juice consumption (PBH 2015). By contrast, vegetable juices are attractive to consumers because of their low sugar content (Mintel 2017). Drinking vegetable juice is considered among the acceptable means for achieving a healthy intake of vegetables according to the 2015-2020 Dietary Guidelines for Americans (HHS and USDA 2015).

Dark green leafy vegetables become popular among consumers because they are low calorie and great sources of nutrition. These vegetables contain high levels of vitamins (mainly vitamin A, C, E, and K) and minerals (iron, magnesium, potassium and calcium) as well as an abundance of carotenoids and antioxidants (Yan 2016). Therefore, green juice blends might be an attractive food product for the beverage industry and for consumers seeking to increase their vegetable intake.

The production of green juice blends, however, has challenges in meeting consumer expectations. One of the largest needs is to conserve the health benefits, both real and perceive, from vegetables and fruits, by minimizing the processing effects but without neglecting the product food safety. Since unpasteurized juice has limited shelf life and serious food safety implications, thermal pasteurization is the most reliable processing alternative for the juice industry (Sivapalasingam and others 2004; Vojdani and others 2008). Furthermore, non-thermal technologies become the popular processing alternative among consumers that are looking for minimally processed food (Huang and others 2017). Nevertheless, there is limited data reported about the effects of juice processing in green juice blends.

The overall goal of this study is to provide technical information about green juice blends that could potentially guide both processors and consumers in their choices. Specifically, this study aims to first examine a range of commercial green juice blends at retail market, and to then evaluate several product quality parameters of a representative treated green juice blend. All products were evaluated for enzyme activities of polyphenol oxidase (PPO) and peroxidase (POD), levels of chlorophyll *a* and chlorophyll *b*, total carotenoids, total antioxidant capacity, total coliforms, generic *Escherichia coli*, and total mesophilic aerobic plate counts (APC).

CHAPTER 2
LITERATURE REVIEW

Green Juice Blends

Vegetable juice is defined as the unfermented liquid (but fermentable product) obtained from the edible part of one or more vegetables, and preserved exclusively by physical means; it may be clear, turbid or pulpy (Bates and others 2001). Straight vegetable juice products, such as carrot, beet, and celery juices, generally have pH values higher than 6.0 (Danyluk and others 2012b). Wu and Shen (2011) categorize vegetables juices and beverages into four categories: 1) vegetable juice products from normally acidic produce, such as tomato juice; 2) acidic vegetable juice products that are mainly prepared with a low acid produce, but acidifying agent adjust product pH; 3) acidic vegetable juice products from fermented vegetables, such as sauerkraut juice; and 4) non-acidic vegetable juice products, such as carrot juice and asparagus juice.

Green juice blends are generally acidic vegetable juice products (pH < 4.6) characterized by a formulation containing different green vegetables and leafy greens, such as cucumber, celery lettuce, spinach, kale, parsley, and wheatgrass. Some fruits, mainly apple and citrus, may be added to enhance sweetness and flavors as well as to adjust product pH.

Green Juice Blends Market

In 2016, vegetable juice accounted for 1.2% of the US juice production, which had a total domestic demand of $12.8 billions and an industry revenue of $12.0 billions (IBISWorld 2017). Tomato, carrot, and red beet juices and beverages have been the most popular alternatives among vegetable juice products (Bates and others 2001; Grassin and Coutel 2010). Recently, health and wellness market drives growth of

vegetable juice products with green juices leading the new trends during the last five years (del Buono 2017). Consumers associate drinking green juice blends with being healthy and they are attracted to the low sugar content of those drinks (Mintel 2017). This perception and interest contrast with the reality about fruit juice consumption. It has steadily decreased during the last years (from 2004 to 2014) because of some concerns about the relation between obesity and juice consumption (PBH 2015).

There are many green juice blends on the market, which suggests consumer demand, but there is limited information available about the green juice blends market. Some US demographic findings regarding vegetable consumption suggest that adults over the age of 55 with annually incomes over $60 thousands consume the highest amounts of fruit and vegetables, and adults under the age of 40 are consuming more fruit and vegetables than their counterparts 10 years ago (PBH 2015). Nevertheless, the consumption of vegetable is still lower among mainstream consumers. In the US, less than 14% of adults in each state consumed the recommended amount of vegetables, which is 2−3 cups of vegetable daily (Moore and Thompson 2015).

Vegetable juice therefore might result attracted for a specific population segment and market, such as health-conscious consumers. Furthermore, consumers under 34 years old are most willing to pay a premium for health attributes, and those consumers also consider health attributes very important in food that they are purchasing (Nielsen 2015). Actually, the millennial generation is most interested in novel juice products that are both nutritious and delicious (IBISWorld 2017). Green juice blends market might be interested in those population segments.

Juice Processing

Pasteurization in juice processing traditionally refers to an application of heat sufficient for the destruction of pathogens or the reduction of spoilage organisms (Parish 1997). Pasteurization includes heating and cooling phases (come up and come down time), respectively, as well as a holding phase where food product is held at specific time and temperature to reduce its processing indicator, e.g., pathogen, enzyme, or spoilage organisms (Fellows 2009).

Refrigerated juices are usually processed by high-temperature-short-time (HTST) treatments with the intent to improve the retention of nutrients and fresh flavor profiles (McLellan and Padilla-Zakour 2005). Mazzotta (2001) concluded that a thermal processing at 71.1 °C for 3 s provide an effective treatment to achieve one log reduction of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in acidic juices. However, thermal pasteurization treatments targeting those vegetative cells are generally ineffective in the reduction of bacterial spores (Smelt and Brul 2014).

The spores of *Clostridium botulinum* are a highly concern in public health but its risk could be reduced lowering the product pH lesser than 4.6 (Bell and Kyriakides 2000). When vegetable juice products are low in acids (pH over 4.5), the juices require either pH reduction or severe thermal treatments at temperatures around 120 °C (Bates and others 2001). For example, a thermal processing for salty asparagus juice filled into cans requires 116 °C for 20 minutes (Wu and Shen 2011).

Nevertheless, thermal processing designed to destroy vegetative pathogens may not completely inactivate endogenous enzymes or spoilage bacteria that may cause quality loss during storage, and consequently shorten the product shelf life (Smelt and Brul 2014; Peng and others 2017). Juice processing treatments therefore may have

higher temperatures and longer holding times than processing parameters aiming to destroy the pertinent pathogen. For example, thermal processing treatments for orange juice are typically at 85 to 90 °C for 10-25 s, and treatments for clarified apple juice are at 85 °C for 15-120 s and at 95 °C for 10-30 s (Reyes-De-Corcuera and others 2014).

In addition to thermal treatments, non-thermal pasteurization technologies are promising alternatives to reduce the possible negative impact on thermal pasteurized food products, such as losses of thermolabile nutrients. High-pressure processing (HPP) is currently popular in juice industry. HPP juices increased from 20 companies marketing such juices in 2010 to more than 100 companies in 2015 (Huang and others 2017).

HPP treatments for acid fruit juices are generally at 400 MPa for 10 min at 20 °C to target pathogens such as *E. coli* O157:H7 as well as yeasts and molds (Daryaei and Balasubramaniam 2012). For instance, HPP treatments at 500 MPa for 10 min at 25 °C were used for processing of acidified broccoli-apple juice blends to achieve 5-log reduction of *E. coli* (Houška and others 2006), as well as for processing of tomato juices to inactive polygalacturonase (Hsu 2008). Furthermore, higher pressure treatments and shorter holding times (615 MPa for 2 min at 15 °C) achieved 6.4 log reduction of *E. coli* O157:H7 in carrot juice (Teo and others 2001).

Although HPP treatments result a good alternative to minimize processing impact on sensory attributes, incomplete enzyme inactivation and following chemical reactions result in some drawbacks for this technology during the product shelf life (Oey and others 2008). For instance, HPP treatment at 500 MPa for 10 min at 25 °C in tomato juice was limited to achieve the enzyme inactivation of pectin methylesterase (Hsu

2008). Furthermore, feasible pressure treatments cannot inactive most of bacterial endospores at ambient temperatures; thus, high-pressure treatment without thermal treatments is not an appropriate option for low-acid vegetable juice processing (Terefe and others 2014).

Additional non-thermal technologies have also been evaluated for vegetable juice processing. For example, ultrasound treatments for apple-carrot blends (Gao and Rupasinghe 2012), pulsed electric fields treatments for broccoli juice (Sánchez-Vega and others 2014) and orange-carrot blends (Rivas and others 2006), high pressure carbon dioxide processing for beetroot juice (Marszałek and others 2017), and gamma irradiation for kale and carrot juices (Song and others 2007). This denotes an especial interest by researches and industry to provide non-thermal treated juices.

Nevertheless, the juice matrix should be carefully evaluated as it significantly affects the efficacy of the potential processing treatment. For example, UV treatment of apple juice is feasible, while UV treatment of orange juice is not because of its cloudiness (Keklik and Demirci 2012). Finally, combination among thermal and non-thermal treatments and hurdle approaches have also been put forth as plausible alternatives for juice processing (Ross and others 2003). For instance, Evelyn and others (2017) found that 600 MPa at 70 or 75 °C achieved higher bacterial and mold spore inactivation (related to fruit spoilage) compared to thermal processing treatments at the same temperatures.

Consumer Perception

Current consumers are looking for healthy options and embracing new trends in food products. In addition to the so-called “free from chemicals and additives”, the term “minimally processed” is included into the new consumer preferences (Huang and

others 2017). Likewise, for the consumer segment who sacrifice costs and convenience for a high-quality product, the top three attributes perceived as healthy include “free from artificial ingredients or additives”, “high in healthy components”, and “minimally processed” (IFIC 2017b). Non-thermal processing technologies therefore have gained popularity among consumers and food industry. For instance, consumers perceive that HPP technology provides food products with better quality and food safety (Frewer and others 2011).

Nevertheless, it is imprecise whether consumer have a complete understanding of those perceived benefits or their thoughts are based on other consumer preferences (Asioli and others 2017). For instance, nearly double of consumers see bagged baby carrot as processed food compared to the same product, but organic; defining the term “processed” results arbitrary among consumers (IFIC 2017a). Perceived health benefits might not always correspond to reality. For example, ready-to-drink juice and juice drink purchases in the US (from 2008 to 2012) with low-sugar claims had the highest mean total sugar density compared to those juices with other claims or no claim (Taillie and others 2017).

Finally, findings regarding the economic aspects show that the mainstream consumer may have a different attitude to the minimally processed technology. For instance, they are concerned that non-thermal processing juice technologies could result in more expensive products and they generally are not willing to pay a premium for those technologies (Olsen and others 2010). Conversely, there is a growing consumer segment, the health-conscious consumers, that are most willing to pay a premium for health attributes (Nielsen 2015).

Food Safety Aspects of Juice

Several foodborne outbreaks have been associated with juice consumption. Many juices implicated in juice-associated outbreaks were reported as unpasteurized (Sivapalasingam and others 2004). In general, foodborne illness outbreaks due to bacterial pathogens and virus are the main concern because of their severe consequences at the public health level and the impact on the economy with loss of sales and trust (Sivapalasingam and others 2004; Van Boxstael and others 2013).

Danyluk and others (2012a) reviewed outbreaks of foodborne disease associated with fruit and vegetable juices between 1922 to 2010. Among the most recent cases, *Clostridium botulinum*, *Salmonella* Typhi, and *E. coli* O157:H7 were found in pasteurized carrot juice, frozen orange pulp, and unpasteurized apple juice, respectively. This contamination occurred in juices sold at retail market in the US. In 2006, refrigerated thermal treated carrot juices were the source of an international outbreak of botulism that caused one deceased (at day 90 after illness onset), and two ventilator dependents; the juices contained no chemical barriers to *C. botulinum* germination (Sheth and others 2008).

Up to the present, no outbreak reports of microbial foodborne illness have been connected to the consumption of green juice blends. However, numerous vegetables have been implicated in many foodborne illness outbreaks in the US. For example, one of the most significant event occurred in October 2006 when a multistate outbreak of *E. coli* O157:H7 linked to fresh spinach caused 199 persons infected, 102 hospitalizations, and 3 deaths (Sharapov and others 2016). The risk of contamination by foodborne pathogens can originate from the primary production, i.e., during growing and harvesting, through processing in the factory.

In an epidemiologic study from 1973 to 2012, Herman and others (2015) identified norovirus as the leading cause of the outbreaks related to contaminated leafy greens, caused by poor food safety practices in the field and in restaurants. In addition, Shiga toxin-producing *E. coli* (STEC) and *Salmonella* were identified as the common bacterial pathogens implicated. Foodborne illness due to STEC caused over 45% of hospitalizations and nearly half of the deaths. The number of confirmed outbreaks related to STEC contamination has increased in recent years.

Irrigation water and soil are considered risk factors for introduction of pathogens during the primary production where *E. coli* seems to be a suitable hygiene criterion for leafy greens (Castro-Ibáñez and others 2015; Holvoet and others 2014). Even low levels of *E. coli* O157:H7 on produce during the field production could result an outbreak because of the likelihood of cross-contamination during the post-harvest handling (Danyluk and Schaffner 2011). Furthermore, packinghouse practices might increase the risk of contamination with pathogens. In some instances, higher microbial concentrations on produce had been found in the final packing carton compared to the microbiological levels in the field (Ailes and others 2008). Schneider and others (2017) found similar results on tomatoes at different packinghouses, and they concluded that washing step at the packinghouse could result in a potential risk of cross-contamination when poor food safety practices are implemented.

Regulatory Aspects of Juice Production

Since 2001, the US Food and Drug Administration (FDA) mandates that the processing technology used in the juice production must be equivalent to a 5-log reduction of the pertinent microorganism, and that the juice be produced under a HACCP plan. The pertinent microorganism refers to the most resistant microorganism

of public health significance that is likely to occur in the juice (FDA 2004). Hazard Analysis Critical Control Point (HACCP) is a food safety system to address any risk of contamination with biological, chemical, or physical hazards (FDA 1997). In a review focusing on the years between1995 and 2005, it appears the federal mandate of HACCP for juice industry was associated with a reduction in (but not total elimination of) juice-associated outbreaks (Vojdani and others 2008).

Refrigerated green juice blends, if they are 100% juices, are cover by the juice HACCP rule. As defined by the federal law, juice refers to the aqueous liquid expressed or extracted from one or more fruits or vegetables (21 CFR Part 120). Therefore, the processing operations, i.e., thermal or non-thermal technology, of green juice blends requires a HACCP plan. The selection of the pertinent microorganism is of course critical for any processing regime for any green juice blends.

Because most vegetable juices have pH values higher than 4.6, they would be classified as low acid juices. The risk associated with spores of non-proteolytic and proteolytic strains of *Clostridium botulinum* must be considered as likely pertinent pathogens for low acid juice (FDA 2007). For acid vegetable juice (pH 3.7 to 4.6), e.g., green juice blends, there may be other designated pertinent pathogens, such as *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* (Mazzotta 2001).

Unpasteurized green juice blends may still be offered at retail establishments or businesses that make and sell juice directly to consumers, e.g., the novel juice bars, because they are exempt from the juice HACCP regulation (FDA 2004). However, the FDA (2015) also warns consumers about the risk of serious illnesses from drinking untreated juice, especially for the high-risk population segment, such as children, older

adults, pregnant women, and people with weakened immune systems. The FDA recommends a warning label for untreated juice that stating the likely presence of harmful bacteria. Proper refrigeration is critical for untreated juice, as well as juices susceptible to potential *C. botulinum* contamination (FDA 2007).

Quality Parameters of Green Juice Blends

Enzyme Activity

Polyphenol oxidase (PPO) and peroxidase (POD) have been detected in most known fruit and vegetables, including dark green vegetables and leafy greens. The enzyme activity of PPO and POD are mainly related to enzymatic browning (Vámos-Vigyázó and Haard 1981). PPO catalyzes hydroxylation and oxidation reactions that involve phenolic compounds; browning pigments result from condensations of the enzyme reaction products, e.g., *o*-quinones (Parkin 2008). Furthermore, POD utilizes hydrogen peroxide as an electron acceptor and oxidizes donor compounds; the reactions result in colored products (Hammer 1993).

The enzyme activity of PPO and POD in green vegetables and leafy greens can fluctuate through the plant components, e.g., roots, stalks, skin, pulp, seeds. For example, PPO activity in cucumber fruit is present only in the skin, but POD activity may also be located in pericarp and carpel tissues (Miller and others 1990). Furthermore, the enzyme activity of PPO and POD can also be affected by vegetable variety, maturity stage, pre-harvest conditions, and environmental factors (Golbeck and Cammarata 1981; Vámos-Vigyázó and Haard 1981; del Amor and others 2008; Korus 2011; Boo and others 2011).

Enzyme inactivation by thermal treatments is mainly related to destabilization of covalent and noncovalent interactions. The enzyme stability depends on matrix factors, such as pH, water content, and levels of salts and sugars (Ludikhuyze and others 2003). Therefore, such complexity makes difficult to extrapolate the thermal processing effects among different food systems. Furthermore, differences in enzyme systems in different plants (i.e. isozymes) make it difficult to precisely characterize enzymes. For example, overall thermal sensitivity of POD, among broccoli, asparagus and carrot, differed due to the relative proportions of the heat-labile and heat-resistant fractions in each vegetable (Morales-Blancas and others 2002).

Traditionally, POD is identified as a more thermoresistant enzyme compared to PPO in leafy greens vegetables. Inactivation of PPO requires only short exposures to temperatures between 70 to 90 °C; conversely, inactivation of POD requires several-minute exposures to temperatures between 80 to 100 °C (Vámos-Vigyázó and Haard 1981; Parkin 2008). However, contrary findings are also reported. For example, PPO appeared more thermoresistant than POD in blanched parsley pastes after blanching treatments at 80 °C for 1 to 10 min (Kaiser and others 2012).

Diverse results have been reported regarding the effects of non-thermal treatments in green vegetables and leafy greens. Terefe and others (2014) reviewed the effects of high-pressure processing in the enzyme activity of PPO and POD in some vegetables products. In general, PPO activity is extremely resistant to high pressure treatments and POD activity varies widely among products. But, some findings also suggested increase in the PPO and POD activities in products such as carrot, mushrooms, cloudy apple juice, tomato puree, and strawberry puree.

Finally, the complexity of enzymes is also extended to their assays. Selection of substrate and reaction conditions impacts the enzyme activity evaluation of PPO and POD; optimized conditions may vary among vegetables, especially blends. For example, Doğan and others (2005) found contrary thermal inactivation results of artichoke PPO after using different substrates in its activity assay. Thermal inactivation treatments indicated effectiveness with increasing temperature and time to inactivate artichoke PPO using 4-methylcatechol and pyrogallol substrates, but artichoke PPO activity appeared unaffected using catechol substrate. Nevertheless, Doğru and Erat (2012) identified catechol as the most suitable substrate for parsley PPO after examining the enzyme kinetics and substrate specificity. Therefore, individual characterization of enzymes from green vegetables and leafy greens provides a basis for evaluation reactions and conditions of enzyme activity of the juice blends.

Chlorophyll and Total Carotenoids

Chlorophylls and carotenoids are natural pigments found in vegetables. Chlorophylls mainly comprise two types: chlorophyll *a* and chlorophyll *b*. Both comprise a macrocyclic tetrapyrrole complex (porphyrin ring) with an esterified phytol group, a carbomethoxy group, a vinyl group, and an ethyl group; the form *a* also includes an ethyl group and the form *b* includes a formyl group (Schwartz and others 2008). Furthermore, chlorophyll ring complexes carry magnesium as the central metal ion in the tetrapyrrole center that ensures absorption of light in the blue to green-red spectrum (Roca and others 2016).

Carotenoids comprise several different molecules and derivatives. The basic structure includes a backbone of isoprene units where cyclic end groups might also be incorporated (Schwartz and others 2008). From the center of the molecule, carotenoids

are reversed resulting into two central side-chains with lateral methyl groups; this base structure can be modified by cyclization, hydrogenation and dehydrogenation, oxidation, or any combination of them (Kaczor and others 2016). The numerous conjugated double-bond structures serve as the light-absorbing chromophores that are responsible for the yellow to red color spectrum, a distinctive characteristic of carotenoids (Rodriguez-Amaya 2001).

Traditionally, carotenoids have been associated to health benefits because of their pro-vitamin A activities, e.g., β-carotene; eyes-protection benefits, e.g., lutein; and antioxidant capacity, e.g., lycopene (Calvo 2005; Saini and others 2015). To a smaller extent, chlorophylls have also been related to antioxidant capacity and potential health benefits, such as cancer preventative activity (Ferruzzi and Blakeslee 2007; Sözgen Başkan and others 2013). Nevertheless, as many health-related molecules, carotenoids could have different behaviors during the adsorption and metabolism. For instance, trans β-carotene have greater vitamin A value than their cis counterparts; conversely, cis forms of lycopene shows greater absorption than the trans configurations (Priyadarshani 2017).

Green vegetables and leafy greens are an excellent source of chlorophylls and carotenoids. They contain significant levels of chlorophyll *a* and chlorophyll *b*, violaxanthin, lutein, and β-carotene isomers (Heinonen and others 1989; Burns and others 2003). These types of vegetables are usually associated to high chlorophyll content, but carotenoids should not be ignored. There is a clear association with higher carotenoid levels and darker green vegetables because carotenoids are located into the chloroplasts and leaves have a high population of them (Britton and Khachik 2009).

Specific levels of carotenoids and chlorophylls might be affected by pre- and post-harvesting factors, such as cultivar genotype, ripening time, cultivation methods, farming practices, season, and climatic conditions (Kopsell and others 2004; Saini and others 2015; Walsh and others 2015). For instance, the levels of carotenoid and chlorophyll in kale differed through its maturity stages; the highest concentration of lutein occurred in 1-2-week old leaves but chlorophylls (*a* and *b*) and β-carotene reached their maximum concentrations at 2-3 weeks (Lefsrud and others 2007). These differences can be responsible for the range of values presented in the literature for these compounds.

Furthermore, during thermal processing treatments of green vegetables and leafy greens, pigments are also affected in different grade. Chlorophylls are severely affected by heat. Breakdown of chlorophylls results in the formation of pheophytins (displacement of magnesium ion) and pyropheophytins (displacement of carbomethoxy group), which occur in a lower extent for chlorophyll *b* because of its formyl group (Schwartz and others 2008). Conversely, carotenoids might remain unaffected by thermal treatments. Increases in apparent carotenoids levels could also occur because of carotenoids release from tissue cells (Walsh and others 2015). For example, thermal sterilization treatments severely reduced the concentration of carotenoids and chlorophylls but non-significant differences were found between high pressure treatments and cooking methods in the carotenoids levels among some xanthophyll-rich foods, such as spinach, kale, parsley, and dill (Arnold and others 2014).

In most of the schemes evaluated, thermal treatments reduced the levels of chlorophyll and consequently the color quality of the vegetables. Non-thermal

treatments might result a good processing alternative. For instance, chlorophyll concentrations were unaffected at extreme high-pressure treatments (800 MPa) combined with thermal treatments not higher than 50 °C in broccoli juice (Van Loey and others 1998). Furthermore, carotenoids are also pressure-resistant molecules. For example, no changes were found in the levels of β-carotene and lycopene in pressure-treated tomato juice at 600 MPa for 60 min (Butz and others 2002). Finally, HPP technology has a limited effect on the natural pigments levels (chlorophyll and carotenoids) in fruit and vegetables products; however, color degradation might still occur related to incomplete inactivation of enzymes (Oey and others 2008).

Antioxidant Capacity

Although consuming fruits is the most common means to intake antioxidant from plants, some vegetables have important levels of antioxidant compounds (Miller and others 2000; Chun and others 2005). Anti-oxidative and bioactive plant compounds generally include carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds (Liu 2004). After examining 56 vegetables, the main phenolic compounds with antioxidant activities were gallic acid, epicatechin, catechin, and quercetin (Deng and others 2013). Vitamin C also had a relatively high contribution to the antioxidant activities in cucumber and red pepper (Chu and others 2002). Likewise, the antioxidant capacity among *Brassica* vegetables varieties, such as broccoli, cauliflower, and kale, are mainly related to phenolic compounds and vitamin C, and in lesser extent to carotenoids and vitamin E (Podsędek 2007).

Processing of green vegetables and leafy green have an impact on their antioxidant capacity. Phytochemicals in vegetables, such as phenolic acid and flavonoids, might degrade or modify their structures during thermal and non-thermal

processing. New antioxidants compounds could be formed as well (Nayak and others 2015). Jiménez-Monreal and others (2009) identified four possibilities about the increase in their the antioxidant capacity. These included (1) the liberation of antioxidant components due to the cell wall disruptions; (2) production of stronger radical-scavenging capacity by thermal chemical reactions; (3) reduction of oxidation stress because of the inactivation of oxidative enzymes; and (4) production of new antioxidant components.

However, processing effects in the antioxidant activity of vegetables could result specific for each product and treatment. For example, the antioxidant activity capacity of spinach juices was significant reduced after thermal treatments (at 75 and 100 °C for 10 and 30 min each), but the antioxidant activity capacity of cabbage juices showed greater resistance to the same treatments (Roy and others 2007). Likewise, significant reductions of the antioxidant activity were found in garlic and zucchini juices but higher increases was found for tomato and yellow bell pepper juices (Gazzani and others 1998). Differences among products might be related to the differences in the bound and the free fractions of antioxidants among vegetables. For instance, free fraction of antioxidant in spinach was around 87%, but in cabbage was around 67% (Chu and others 2002).

Finally, numerus assays are reported to measure the antioxidant capacity for beverage and food products. Antioxidant assays might be an assay associated with lipid peroxidations, e.g., thiobarbituric acid assay (TBA), malonaldehyde/gas chromatography (MA/GC) assay, and β-carotene bleaching assay, or an assay associated with electron or radical scavenging, e.g., the 2,2-diphenyl-1-picrylhydrazyl

(DPPH) assay, 2,2′-azinobis(3-ethylben- zothiazoline-6-sulfonic acid) (ABTS) assay, and ferric reducing/antioxidant power (FRAP) assay (Moon and Shibamoto 2009). The DPPH assay is popular because of its simplicity and highly sensitivity. Reduction by antioxidant compounds is measured by the decrease in DPPH absorbance at 515 nm (Brand-Williams and others 1995). Nevertheless, beverages and food products might contain different antioxidant molecules, and different reactions might occur among antioxidant assays (Stratil and others 2006; Zulueta and others 2009). These differences can be responsible for the range of values presented in the literature.

Microbiological Quality Aspects

In addition to pathogens, spoilage microorganisms are also studied in vegetables products because they are associated to loss of the product quality and to off-flavors. Those organisms include several bacteria, yeast, and molds. The most common spoilage bacteria in vegetable products are lactic acid bacteria (LAB), *Erwinia* and *Pseudomonas* (Koo 2011). For instance, 46 isolated strains of pectolytic bacteria from three types of produce (green bell peppers, Romaine lettuce, and baby carrots) were identified as members of the genera of *Pseudomonas*, *Erwinia*, *Bacillus*, *Xanthomonas*, and *Flavobacterium* (Liao and Fett 2001).

LAB cause spoilage in fruits and vegetables products during storage at refrigerated temperatures; these bacteria lower pH and produce acetylmethylcarbinol and diacetyl, which provide off-flavors to food products (Barth and others 2009). Nevertheless, its occurrence could differ among vegetables products. For example, the average levels of LAB ranged 3.0 to 5.9 log CFU/g in fresh-cut arugula, 4.3 to 7.6 log CFU/g in fresh-cut carrot, 3.7 to 6.9 log CFU/g in fresh-cut spinach, and 1.7 to 6.3 log CFU/g in fresh-cut lettuce (Abadias and others 2008).

In general, yeast and molds are usually the main concerns after juice thermal processing. A large spectrum of fungi species is also related to loss of product quality in vegetables, e.g., *Cladosporium*, *Alternaria*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizopus*, *Rhizoctonia*, and some mildews (Tournas 2005). Furthermore, some spore-forming species related to spoilage are able to growth even at acidic conditions; traditionally, spores are not a main concern in acidic product. These thermally resisted organisms include *Bacillus coagulans*, *B. macerans*, *B. subtilis*, and *Alicyclobacillus* spp. (Worobo and Splittstoesser 2005).

Finally, a plate count of aerobic mesophilic microorganisms (APC) has been used as quality indicator for fresh produce by several studies (Liao and Fett 2001; Johnston and others 2006; Abadias and others 2008; Ailes and others 2008). This indicator is for microorganisms that grow aerobically at mesophilic temperatures (typically between 20 and 45 °C) on a food product, but it does not differentiate types of bacteria (Swanson and others 1992). Although lower levels of APC could be associated with unspoiled products, higher counts of APC do not always correlate with spoilage and they cannot be used only to predict the product shelf life (Barth and others 2009).

CHAPTER 3
MATERIALS AND METHODS

Retail Screening of Commercial Juices

The first section of this study examined commercial green juice blends found at the retail market. Generally, options of green juice blends include different green vegetables and leafy greens, such as cucumber, celery lettuce, spinach, kale, parsley, and wheatgrass. Refrigerated green juice blends offered at retail are usually high pressure treated or thermally treated. For this work, three high pressure processed (HPP) juices and one thermally processed juice were comprehensively evaluated. The HPP juices included Suja Über Greens™ (Suja Life, LLC, San Diego, CA), Naked Pressed™ Bright Greens™ (Naked Juice Co., Monrovia, CA), and Evolution Fresh™ Essential Greens with Lime (Evolution Fresh, Inc., Rancho Cucamonga, CA). The thermally processed juice was Naked® Kale Blazer® (Naked Juice Co., Monrovia, CA). The label information of these commercial juices is summarized in Table 3-1.

All products were evaluated for enzyme activities of polyphenol oxidase (PPO) and peroxidase (POD) as well as the levels of chlorophyll *a*, chlorophyll *b*, total carotenoids, total antioxidant capacity, total coliforms, generic *Escherichia coli*, and total aerobic plate counts (APC). The pH and °Brix were also measured. For this purpose, three bottles of each juice brand were pooled to complete one liter of juice. Bottles of juices were purchased in a local supermarket and kept unopened in refrigeration prior to analysis. The shelf life of the products was unknown, however the remaining shelf life, i.e. days to the enjoy by or best by date of the commercial juices, ranged from 12 to 43 days when the assays were performed.

Sample Preparation of Model Juice

The second section of this study examined a formulated, representative green blended fresh juice and two thermal treatments of that same juice. This study mainly focused on the quality attributes of vegetable juices; thus, fruit juices (with the exception of lemon juice) were excluded from the juice formulation. A representative green juice blend was formulated and prepared according to the ingredients stated in a commercial juice available at the retail market. Evolution Fresh™ Green Devotion is a green juice blend that fulfilled the requirements of readily available ingredients that were observed as ingredients in other commercial green juice blends.

Three different formulas were prepared and tasted in comparison to the commercial alternative as a reference. The final formula included 55% (v/v) celery juice, 20% (v/v) cucumber juice, 8% (v/v) spinach juice, 5% (v/v) kale juice, 5% (v/v) Romaine lettuce juice, 4% (v/v) lemon juice, and 3% (v/v) flat-leaf parsley juice. The model product was a medium-acid juice with an average pH value of 4.3 and 4.0 °Brix.

Ingredients, fresh and unpacked produce, were purchased in a local supermarket before 24 h to complete experiments and kept in refrigeration prior to use. Each ingredient was individually cleaned under running tap water and the excess water was drained off. A masticating juice maker (Omega J8006, Omega Products, Inc., Harrisburg, PA) individually extracted juices from minor to major ingredients. Then, juices were immediately cooled in ice-water bath to prevent variations of enzyme activity. A freshly batch of one liter was prepared pooling each juice according to the formula and the pH and °Brix were measured. The juice maker was cleaned flowing a standard cleaning procedure detailed in Appendix A. Finally, the final blend was subdivided in three batches: untreated juice (Control), Treatment 1, and Treatment 2.

Thermal Processing Treatments

Pasteurization treatments for juices are often specific for each juice product and process, especially in validation studies associated with a 5-log reduction of the pertinent microorganism (Peng and others 2017). In this document, literature reviews were focused on thermal processing treatments of acidic green juice blends. Selection of the thermal processing treatments were based on typical commercial conditions for juice pasteurization. Treatment 1 represented a mild thermal treatment (representative of a label terminology of lightly pasteurized or gourmet pasteurized) at 72 °C for 15 s. This standard treatment could accomplish a 5-log reduction of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in acidic juices (Mazzotta 2001). Treatment 2 represented a typical thermal treatment of many juices at 90 °C for 30 s. These parameters are commonly used in industry for enzyme inactivation and spoilage microorganism control (Reyes-De-Corcuera and others 2014).

The thermal processes were performed using a water bath at three degrees higher than the processing temperatures, i.e. 75 and 93 ± 1 °C, to minimize come-up times. Eight 15 ml glass tubes were filled with 10 ml of juice, closed with plastic caps, and submerged into a water bath (Precision GP 05, Thermo Fisher Scientific, Waltham, MA). The water level completely exceeded the level of the juice aliquots. An additional glass tube containing the same volume of juice was used to record the temperature. After reaching the processing temperatures, tubes were kept submerged into the water bath until the holding time for each treatment was reached. Then, tubes were removed from the water bath and immediately cooled until 35 to 45 °C in an ice-water bath in order to avoid cooling lags.

Enzyme Activity of Polyphenol Oxidase and Peroxidase

Enzyme extractions and assay of the enzyme activities were completed as described by Terefe and others (2010) with some modifications. The activities of PPO and POD were analyzed in triplicate for all samples. To extract the enzymes, 4.5 ml of juice were mixed with 4.5 ml of extraction solution and agitated using a vortex mixer for 30 s. The extraction solution in 0.2 M sodium phosphate buffer (pH 6.5) included 4% (w/v) poly(vinylpolypyrrolidone) (PVPP), 1% (v/v) 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (Triton^{TM} X-100), and 1 M NaCl. Mixtures were centrifuged for 30 min at 4500 rpm (5250 x g) and 4 °C (Allegra X-15R, Beckman Coulter, Inc., Brea, CA), with the supernatants comprising the enzyme extracts.

For measurement of the PPO activity, 200 µL of enzyme extract were mixed with 3 ml of 0.07 M catechol. Catechol solution was prepared before assays in 0.05 M sodium phosphate buffer (pH 6.5). For blank readings, 0.1 M sodium phosphate buffer (pH 6.5) solution were used instead of enzyme extract at the same volume. Enzyme reactions were measured in kinetic/time mode at 420 nm and ambient temperature for 5 min using an UV-visible spectrophotometer (DU 730, Beckman Coulter, Inc., Brea, CA). One PPO activity unit (U) was expressed as an increase of 0.1 unit in absorbance per min. Results were expressed as U/ml of juice.

For measurement of the POD activity, enzyme extracts were mixed with 1.5 ml of 0.05 M sodium phosphate buffer (pH 6.5), 0.2 ml of *p*-phenylenediamine diluted at 1% in 0.05 M sodium phosphate buffer (pH 6.5), and 0.2 ml of 1.5% hydrogen peroxide. It was used different amounts of enzyme extract to obtain accurate absorbance readings. It was used 7.5, 75 and 200 µL of enzyme extract for the assays of Control, Treatment 1, and Treatment 2, respectively. For the high pressure processed commercial juices, it

was used from 5 to 10 µL of enzyme extract. For the thermally pasteurized commercial juice, it was used 200 µL of enzyme extract. Enzyme reactions were measured in kinetic/time mode at 485 nm and ambient temperature for 10 min using an UV-visible spectrophotometer (DU 730, Beckman Coulter, Inc., Brea, CA). One POD activity unit (U) was expressed as an increase of 0.1 unit in absorbance per min. Results were expressed as U/ml of juice.

Measurement of Chlorophylls and Total Carotenoids

Extraction procedures were completed as described by Lee and Castle (2001) with some modifications. Analysis was completed in triplicated for each treatment. A stock extraction solution was prepared containing 50% (v/v) hexane, 25% (v/v) acetone, and 25% (v/v) ethanol. An aliquot of 2.5 ml of juice was mixed with 5 ml of extraction solution and agitated using a vortex mixer for 30 s. Then, mixtures were centrifuged at 4500 rpm (3260 x G) for 5 min (Sorval ST 8, Thermo Fisher Scientific, Waltham, MA). One ml of the supernatants was collected and diluted with acetone until absorbance readings were accurate. Absorbance was measure at 662, 645 and 470 nm using an UV-visible spectrophotometer (DU 730, Beckman Coulter, Inc., Brea, CA). Levels of chlorophyll *a*, chlorophyll *b*, and total carotenoids were estimated using mathematical models established by Lichtenthaler (1987). Results were expressed as µg per ml of juice (µg/ml). Model are described below:

$$\text{Chlorophyll } a \text{ (Ch } a\text{)} = 11.24\ A_{662} - 2.04\ A_{645} \quad (3\text{-}1)$$

$$\text{Chlorophyll } b \text{ (Ch } b\text{)} = 20.13\ A_{645} - 4.19\ A_{662} \quad (3\text{-}2)$$

$$\text{Total Carotenoids} = (1000\ A_{470} - 1.90\ \text{Ch } a - 63.14\ \text{Ch } b)/214 \quad (3\text{-}3)$$

Measurement of the Total Antioxidant Capacity

Total antioxidant capacity of juice treatments were analyzed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Martínez-Flores and others (2015) with some modifications. Analysis was completed in triplicated for each treatment. One milliliter of juice was mixed with 5 ml of methanol. Mixtures were agitated using a vortex mixer for 30 s and kept in darkness for 90 min at ambient temperature. Then, mixtures were centrifuged at 4500 rpm (3260 x g) for 10 min (Sorval ST 8, Thermo Fisher Scientific, Waltham, MA). The supernatants were used to quantify the antioxidant capacity of samples.

A stock solution of 1 mM DPPH was prepared with methanol and kept at room temperature and darkness for no longer than one month. The working solution of 0.2 mM DPPH was prepared only before assays. In addition, a standard curve assay was needed for the assay. Thus, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) solutions from 0.05 mM to 0.80 mM in methanol were prepared prior assays. Two hundred microliters of each sample extract, Trolox solutions, and methanol (acting as a blank) were mixed with 3.8 ml of 0.2 mM DPPH solution. All mixtures were kept at room temperature in darkness for 45 min when the reaction reached a constant level. The decrease in absorbance was measured at 515 nm using an UV-visible spectrophotometer (DU 730, Beckman Coulter, Inc., Brea, CA). Results were expressed as µmoles of Trolox equivalent (TE) per ml of juice (µmoles of TE/ml).

Microbiological Analysis

Ten milliliters were sampled by triplicate from each treatment and commercial juice. Samples were kept in refrigeration prior to analysis. One milliliter of each sample was diluted with 9 ml of sterile buffered peptone water (Oxoid, Thermo Fisher Scientific,

Waltham, MA). Following serial dilutions were prepared with 1 to 9 ml of sterile buffered peptone water. To determine total mesophilic aerobic plate counts (APC), one hundred microliters of each dilution were spread plated in duplicate onto standard methods agar (Remel, Thermo Fisher Scientific, Waltham, MA). All of the dishes were incubated for 48 48 h at 35 °C. To determine total coliforms, one ml of each dilution was plated by duplicate onto center of bottom 3M Petrifilm™ *E. coli* / Coliform Count (EC) (3M Company, Maplewood, MN). All petrifilms were incubated for 24 h at 35 °C. Results of generic *E. coli* required additional incubation of 24 hours at 35 °C.

When sample counts resulted below the assay limit of detection (10 CFU/ml) for total coliforms and APC, the data point was assigned 5 CFU/ml (0.70 log CFU/ml); halfway value between 0 and the detection limit (Ailes and others 2008). Because of the low loads of *E. coli*, that results were reported as log CFU per 3 ml of juice (the total amount of juice analyzed per sample).

Statistical Design and Analysis

Nine quality attributes were evaluated among the commercial green juice blends at retail market as well as for the representative, formulated treated green juice blend. The product quality attributes included enzyme activity of PPO and POD as well as the levels of chlorophyll *a*, chlorophyll *b*, total carotenoids, total antioxidant capacity, total coliforms, generic *E. coli*, and APC. All of the assays were performed in triplicate starting with the enzyme activity assay and ending with the microbiological analysis. The assays were completed in less than 12 h to minimize the storage effects in the samples. For each quality attribute, mean and standard deviation were calculated and reported.

For the second part of the study, it was analyzed an unprocessed juice (Control) and two thermal treatments. Treatment 1 represented a standard mild pasteurization at 72 °C for 15 s. Treatment 2 represented a higher thermal treatment at 90 °C for 30 s. The experiment was completed in four independent occasions during August 2017. Each occasion was reported as Trial 1, Trial 2, Trial 3, and Trial 4. The same treatments were performed in each trial.

Results were individually analyzed per trial. To analyze overall results between treatments, mean and standard deviation among the four trials were also calculated and reported. To compare thermal treatments, results were normalized to relative values by dividing the results in treated juices over the results in untreated juices for each quality attribute. Furthermore, microbial reductions, i.e., the initial count minus the final count, were estimated to examine significant differences between treatments.

Data analysis was performed using RStudio (RStudio, Inc., Boston, MA). The rejection criteria of null hypothesis in favor of the alternative hypothesis was p-value less than 0.05 ($P < 0.05$). Analysis of variance was performed to compare treatments for each trial set as well as to compare the overall normalized results between treatments. When differences existed among treatments, multiple comparisons were performed by Tukey-Kramer method (Bower 2009). Finally, *t*-student test was performed to compare microbial reductions.

Table 3-1. Label information of commercial green juice blends found at the retail market and evaluated in this study.

	Size	Processing Treatments	Ingredients[a]	Shelf Life Remaining[b]
Suja Über Greens™	12 fl. oz. (354 ml)	High Pressure Processed	**Cucumber** Juice, **Celery** Juice, Grapefruit Juice, **Green Chard** Juice, **Green Leaf Lettuce** Juice, Lemon Juice, **Kale** Juice, **Spinach** Juice, **Parsley** Juice, Tea (Water, Peppermint Tea Leaf, Spearmint Leaf)	31 days
Naked Pressed™ Bright Greens™	12 fl. oz. (354 ml)	Cold Pressed[c]	Apple Juice, **Cucumber** Juice, **Celery** Juice, **Romaine Lettuce** Juice, Lemon Juice, **Wheatgrass** Juice, Lemon Oil	12 days
Evolution Fresh™ Essential Greens with Lime	15.2 fl. oz. (450 ml)	High Pressure Processed	**Celery** Juice, **Cucumber** Juice, **Spinach** Juice, **Romaine Lettuce** Juice, **Kale** Juice, Lime Juice, **Parsley** Juice	16 days
Naked® Kale Blazer®	15.2 fl. oz. (450 ml)	Pasteurized[d]	Orange Juice, **Kale** Puree, Apple Juice, **Cucumber** Juice, **Spinach** Juice, **Celery** Puree, Ginger Juice, Lemon Juice, Natural Flavors	43 days

[a]The bold ingredients are green vegetables or leafy greens.
[b]The remaining days to the enjoy by or best by date labeled on the bottles.
[c]Cold pressed usually refers to a high-pressure processing treatment, however that was not stated on the label. Company's website does indicate that all juices are pasteurized.
[d]Pasteurized usually refers to a thermal processing treatment.

CHAPTER 4
RESULTS AND DISCUSSION

Product Quality Parameters in Commercial Green Juice Blends

The results of nine product quality parameters examined in representative commercial green juice blends are summarized in Table 4-1. The examined juices included three high pressure treated (HPP) juices (Suja Über Greens™, Naked Pressed™ Bright Greens™, and Evolution Fresh™ Essential Greens with Lime) and one thermally treated, i.e. pasteurized, juice (Naked® Kale Blazer®). The product quality parameters included the enzyme activity of polyphenol oxidase (PPO) and peroxidase (POD) as well as the levels of chlorophyll *a*, chlorophyll *b*, total carotenoids, total antioxidant capacity, total coliforms, generic *Escherichia coli*, and total mesophilic aerobic plate counts (APC). The pH values and °Brix were reported as well.

All juices were called “green” because of their content of dark vegetables and leafy greens, even though some products included fruit juices as their first ingredient. The vegetables used for green blend juices often included leafy green options, such as kale, spinach, chard, lettuce, wheatgrass, and parsley. Green vegetables, such as cucumber and celery, were commonly used as well. The average °Brix values for Naked® Kale Blazer® and Naked Pressed™ Bright were higher (9.4 ± 0.0 and 8.0 ± 0.0) than Suja Über Greens™ and Evolution Fresh™ Essential Greens with Lime (4.2 ± 0.0 and 3.8 ± 0.0). Higher values could be related to the apple juices used for those products. Furthermore, all juices were also acidic vegetable juices with pH values from 3.76 ± 0.03 to 4.29 ± 0.02. The use of citrus (mainly lemon) juices likely contributed acidifying the blends.

The enzyme acitivity of PPO and POD was generally higher in the HPP juices compared to the thermal treated juice. Findings suggested that HPP might be less effective to reduce the enzyme activity of POD compared to the thermally treated juice. The commercial HPP juices had POD activities ranged from 139.61 ± 9.89 to 63.28 ± 0.39 U/ml; conversely, the POD was inactive (0.05 ± 0.01 U/ml) in the thermal treated juice Naked® Kale Blazer®. The enzyme activity of PPO ranged from 7.91 to 9.89 U/ml among the commercial juices. The PPO activity was quite similar among the commercial juices despite the different formulations, but the thermally treated juice had lower PPO activity in general.

In general, PPO and POD are resistant to HPP treatments. Seyderhelm and others (1996) concluded that POD and PPO had the highest barostability in Tris buffer at 600 and 700 MPa compared to other enzymes (catalase, phosphatase, lipase, pectin esterase, lactoperoxidase, and lipoxygenase). Furthermore, Hernández and Cano (1998) found that HPP treatments in tomato puree from 50 to 500 MPa did not show any significant effect in PPO activity and a small reduction in POD activity. Likewise, findings by Park and others (2002) showed that PPO activity inactivation in pressure-treated carrot juices was around 20% and 40% after HPP treatments for 10 min at 200 and 400 MPa, respectively. Keenan and others (2012) examined HPP treatments for a fruit beverage that mainly contained strawberry and apple. Treatments at 450 MPa for 5 min achieved small reduction (35%) of PPO activity, but severe treatments at 600 MPa for 10 min did achieved 83% reduction of PPO activity.

The levels of chlorophylls (*a* and *b*) among juices appeared formula-dependent. For instance, the average level of chlorophyll *a* in the thermally treated Naked® Kale

Blazer® was 18.55 ± 9.89 µg/ml, but the average levels of chlorophyll *a* in the high pressure processed Suja Über Greens™ and Evolution Fresh™ Essential Greens were 7.57 ± 0.70 and 35.22 ± 0.77, respectively. There was not a trend among the average of chlorophyll *a* in the juices evaluated. Nevertheless, the levels of chlorophyll *a* were higher than the levels of chlorophyll *b* in all of the commercial juices examined.

The processing effect in the chlorophyll levels might be marginal among the pressure treated commercial juices. For example, Zhao and others (2013) found non-significant differences in the levels of chlorophyll *a* and *b* among pressure-treated cucumber drinks (400 MPa for 4 min and 500 MPa for 2 min) and control. Similarly, Wang and others (2012) observed non-significant differences in the levels of chlorophyll *a* and *b* in spinach puree after HPP treatments at different pressures (200, 400, and 600 MPa) and processing time (5, 15, and 25 min). Van Loey and others (1998) described a high stability of chlorophyll in broccoli juice after severe HPP treatments at 800 MPa with temperatures not exceeding 50 °C. Conversely, differences existed among HPP and thermal treatments. Butz and others (2002) compared the pressure effect at 600 MPa in the chlorophylls levels of minced broccoli at 25 and 75 °C during 10, 20 and 40 min. Significant reductions were observed for those treatments at 75 °C.

Contrary to the processing effects in chlorophylls, the levels of total carotenoids and total antioxidant capacity might be superior in thermally treated juices compared to pressure treated juices. For instance, in the thermally treated Naked® Kale Blazer®, the average level of total carotenoids and total antioxidant capacity were 7.35 ± 0.16 µg/ml and 1.80 ± 0.10 µmol of TE/ml. respectively. Conversely, among the HPP juices, the average total carotenoids ranged 2.30 ± 0.20 to 5.47 ± 0.16 µg/ml and the total

antioxidant capacity ranged 0.32 ± 0.07 to 0.40 ± 0.09 µmol of TE/ml. The formula effect, however, should not be ignored.

Thermal treatments might enhance the bioavailability of antioxidant molecules, such as carotenoids and phenolic compounds. For example, Chen and others (2015) found that the total antioxidant activity in asparagus juice was significant higher in the thermally treated samples (121 °C for 3 min) compared to the control and the pressure treated samples at different pressures (200, 400, and 600 MPa) and treatment times (10 and 20 min). Conversely, additional findings suggested non-significant differences among treatments. For a vegetable beverage that included celery and cucumber, Barba and others (2010) found non-significant differences in the total antioxidant capacity among the thermally treated samples (90 °C for 15 s and 98 °C for 21 s) and the pressured treated samples at 400 MPa for 5 to 9 min. Significant reductions, however, were found for treatments from 100 to 300 MPa for 3 to 9 min. Finally, Andrés and others (2016) supported the previous findings after examining HPP treatments for a carrot beverage that contained some fruit juices. Their work showed non-significant differences among the thermally-treated samples (°80 C for 3 min) and the pressure-treated samples at different pressures (450 and 600 MPa) for 3 min.

Carotenoids molecules usually have high stability with respect to processing technologies. Butz and others (2002) observed non-significant differences in the levels of lycopene and β-carotene in tomato homogenates after pressure treatments at 600 MPa for 60 min as well as after thermal treatments at 95 °C for 60 min. Furthermore, Barba and others (2010) also found non-significant differences in the total carotenoids for a thermally treated vegetable beverages (90 °C for 15 s and 98 °C for 21 s),

however significant reductions were observed among the pressure treated beverages (from 100 to 400 MPa for 2 to 9 min). Findings by Andrés and others (2016) also supported the insignificant effects by thermal treatments (°80 C for 3 min) in a carrot beverage that contained some fruit juices, but their work found higher antioxidant capacity after HPP treatments for 3 min at 450 MPa and 600 MPa.

Finally, microbiological quality parameters showed that, as was expected, the average levels of generic *E. coli* were lower than the detection limit in all of the commercial juices examined. The average levels of total coliforms also were lower than the detection limit except in the pressure treated Suja Über Greens™ (2.56 ± 0.07 log CFU/ml). Lastly, the average levels of APC among the high pressure processed juice ranged from 2.93 to 4.20 log CFU/ml, but it was lower than the detection limit in the thermally treated juice. Findings by Zhao and others (2013) found that, after HPP treatment at 500 MPa for 2 min, the initial levels of APC in a cucumber drink was around 3.0 log CFU/ml and it increased less than 1-log after 50 days at 4 °C; however, HPP treatment at 400 MPa for 4 min and thermal treatment at 85 °C for 15 s reached APC levels around 3.5 log CFU/ml and then increased around 1.5 log after storage at 4 °C for 50 days. Nevertheless, all of the examined commercial juices had different shelf life periods as well as the elaboration dates and processing parameters were unknown.

Enzyme Activity of PPO and POD in Untreated and Thermally Treated Formulated Green Juice Blends

The overall enzyme activities of PPO and POD in untreated and thermally treated green juice blends are summarized in Table 4-2. This model, formulated green juice blend (pH 4.27 ± 0.03 and °Brix 3.8 ± 0.3) was prepared and then thermally treated at 72 °C for 15 s (Treatment 1), and at 90 °C for 30 s (Treatment 2). As was expected, the

activities of PPO and POD were higher in the untreated juices compared to the thermally treated juices. The average enzyme activity of PPO in untreated green juice blends was 12.17 ± 0.30 U/ml among four independent trials. After the milder and the higher thermal treatments, the PPO activity significantly decreased to 8.35 ± 0.28 and 7.48 ± 0.33 U/ml, respectively. Significant differences were found among treatments and the control ($P < 0.05$).

Furthermore, the average enzyme activity of POD in untreated green juice blends was 38.33 ± 7.05 U/ml among four independent trials. POD activity ranged from 28.37 to 43.77 U/ml (summarized in Appendix B). After the milder and the higher thermal treatments, the POD activity significantly decreased to 8.59 ± 1.60 and 1.79 ± 0.73 U/ml, respectively. Significant differences were found between the treatments and the control ($P < 0.05$), but not between the thermal treatments ($P > 0.05$).

The enzyme activity of POD had higher variation in untreated green juice blends. Differences in the enzyme activity of vegetables could be related to maturity stage or growing conditions, such as weather and farming procedures (Vámos-Vigyázó and Haard 1981). For instance, Korus (2011) found significant differences in the POD activity among the maturity stages in three different cultivars of kale in two different years. POD activity in kale ranged approximately 5 to 15.0 ΔA/min/g of fresh matter of sample; higher levels generally occurred at the last maturity stage. Likewise, Boo and others (2011) found differences on the POD activities in lettuce at various combinations of day/night temperatures. The POD activity in samples was highest at 25/20 °C, but the lowest activity was found for samples at lower temperatures (10/13 °C). Nevertheless, the growing conditions for the ingredients used in the green juice blends were unknown.

To evaluate the effects between treatments, a more suitable approach is to compare the decrease of enzyme activity on a relative basis, i.e., reporting final enzyme activity over initial enzyme activity. Reporting absolute values can make comparisons between data problematic. The remaining fractions of enzyme activity after treatments are summarized in Table 4-3.

Comparisons in terms of the relative enzyme activity suggested that PPO was more thermoresistant than POD in green juice blends. The remained factions of PPO activities were 0.69 ± 0.02 and 0.61 ± 0.02 after thermal treatments at 72 °C for 15 s and at 90 °C for 30 s, respectively. Conversely, the remained fractions of POD activities were 0.23 ± 0.04 and 0.05 ± 0.01 after thermal treatments at 72 °C for 15 s and 90 °C for 30 s, respectively. Significant differences were found among treatments for both enzymes ($P < 0.05$).

These findings were in agreement with those reported by Kaiser and others (2012). PPO was more thermoresistant than POD in blanched parsley paste during treatments from 1 to 10 min at 80 °C. Non-significant differences were actually found in PPO activity between the control (unprocessed sample) and the treatment at 80 °C for 1 min. However, when the study treated an un-blanched parsley paste at the same temperature and time, i.e., 80 °C for 1 min, completely inactivation of PPO and POD were achieved. Likewise, Park and Fricker (1977) found that spinach POD extract at pH 6.0 was reduced 90% of its activity after treatments at 80 °C for 40 s and at 90 °C for 5.8 s. The sensitivity of POD to thermal treatments increased when the extract pH's were reduced. The same reduction at 80 °C was completed in 20 s for the POD extract

at pH 4.0. The average pH in the green juice blends was 4.27 ± 0.03. The milder treatment at 72 °C for 15 s reduced almost 80% of POD activity.

Among the thermal treatments evaluated for the representative green juice blend, it seemed that the holding times did not suffice to achieve the PPO inactivation. For instance, Heimdal and others (1994) found that isolated lettuce PPO was stable in treatments to 70 °C for 5 min but steadily decreased at 80 and 90 °C for 5 min. Likewise, after thermal treatments to isolated enzymes from cucumber, Miller and others (1990) found that the sensitivity of PPO and POD to thermal treatments similarly increased after 70 °C for 10 min, completing the enzyme inactivation after thermal treatments at 90 to 100 °C for 10 min.

Moreover, different substrates used during the assays of PPO activity could yield diverse results for thermally treated samples. For instance, significant inactivation of POD activity (more than 50%) was reported at 75 °C for 30 min using pyrogallol and 4-methylcatechol in the assays of artichoke PPO, but it appeared unaffected when catechol was used in the assays (Doğan and others 2005). Likewise, the normalized activity of PPO in celery roots after thermal treatment at 80 °C was approximately 0.75 for L-dopa substrate, but it was less than 0.10 for catechol and pyrogallol substrates (Yagar 2004). Nevertheless, catechol was the best substrate for parsley PPO after comparing its enzyme kinetic parameters among 4-methylcatechol, pyrogallol, L-dopa, dopamine, and catechin (Doğru and Erat 2012). Limited evidence exists about the most suitable substrate to evaluate the enzyme activity of PPO and POD in green juice blends.

Levels of Chlorophylls and Total Carotenoids in Untreated and Thermally Treated Formulated Green Juice Blends

The levels of chlorophyll *a*, chlorophyll *b*, and total carotenoids were examined in untreated and thermally treated green juice blends. Overall results are summarized in Table 4-2. This model, formulated green juice blend (pH 4.27 ± 0.03 and °Brix 3.8 ± 0.3) was prepared and then thermally treated at 72 °C for 15 s (Treatment 1), and at 90 °C for 30 s (Treatment 2). Furthermore, reporting absolute values can make comparisons between data problematic. To evaluate the effects between treatments, a more suitable approach is to compare the decrease of the levels of chlorophylls and total carotenoids on a relative base, i.e., final concentrations over initial concentrations. The normalized results of the levels of chlorophylls and total carotenoids after the thermal treatments are summarized in Table 4-3.

Chlorophylls

The average levels of chlorophyll *a* and chlorophyll *b* in untreated green juice blends were 56.04 ± 3.04 and 25.36 ± 1.19 µg/ml, respectively. After the milder thermal treatments, the average levels of chlorophyll *a* and chlorophyll *b* significantly decreased to 38.17 ± 2.63 and 20.49 ± 1.29 µg/ml, respectively. Likewise, after the higher thermal treatments, the average levels of chlorophyll *a* and chlorophyll *b* significantly decreased to 32.68 ± 2.63 and 14.66 ± 1.03 µg/ml, respectively. Significant differences were found in the absolute levels of chlorophyll *a* between the control and the treatments ($P < 0.05$), but non-significant differences existed between the treatments ($P > 0.05$). Conversely, significant differences were found in the absolute levels of chlorophyll *b* among the control and the treatments ($P < 0.05$).

The average level of chlorophyll *a* was twofold the level of chlorophyll *b* in untreated green juice blends. The levels of chlorophyll *a* and *b* in the control ranged from 52.94 to 59.94 µg/ml and from 23.85 to 26.47 µg/ml, respectively (summarized in Appendix B). The overall *a*:*b* ratio of chlorophylls in higher plants is usually 3 (Schwartz and others 2008). It was likely that some levels of chlorophylls were retained in the pomace after juicing process.

Comparison in terms of the normalized levels of chlorophylls suggested that chlorophyll *a* was more thermolabile than chlorophyll *b* in green juice blend after a mild thermal treatment at 72 °C for 15 s. The relative levels of chlorophyll *a* and chlorophyll *b* after the milder treatment were 0.68 ± 0.05 and 0.81 ± 0.04, respectively. The higher thermal sensitivity of chlorophyll *a* compared to chlorophyll *b* has been reported as well in thermally treated broccoli juices (Van Loey and others 1998; Weemaes and others 1999), thermally treated parsley paste (Kaiser and others 2012), thermally treated cilantro puree (Rudra and others 2008), and thermally treated cucumber drinks (Zhao and others 2013). Schwartz and others (2008) explained that higher stability of chlorophyll *b* is associated with electron-withdrawing effect of its formyl group.

Nevertheless, chlorophyll *a* and chlorophyll *b* resulted affect at the same extent after the high thermal treatment at 90 °C for 30 s in green juice blends. The relative levels of chlorophyll *a* and chlorophyll *b* after the higher treatment were 0.58 ± 0.03 and 0.58 ± 0.01, respectively. Significant differences were found among the control and the treatments for the relative levels of chlorophyll *a* and *b* ($P < 0.05$). Kaiser and others (2012) evaluated the levels of chlorophyll *a* and chlorophyll *b* in thermally treated parsley pastes at 80 to 100 °C for 1 to 10 min. Although overall results indicated

significant reductions of the chlorophyll levels at higher temperatures, some increases in the chlorophyll levels occurred at 5 and 7 min at 80 °C compared to treatments at 1 and 10 min. Those variations could be result of cell disruptions and then release of chlorophyll contents.

Furthermore, after cooking kale leaves, Korus (2013) found reduction of 23% in the levels of chlorophyll *b*, and 17% in the levels of chlorophyll *a*. Those values were lower than those reported in this study. Reduction in the levels of chlorophyll *a* and chlorophyll *b* in green juice blends was 42% for both after the high thermal treatment at 90 °C for 30 s. A higher reduction in the chlorophyll levels could be explained by the pH effect; the average pH value in the representative green juice blend was 4.27 ± 0.03. Acidic conditions enhance the conversion of chlorophyll to pheophytin (Gunawan and Barringer 2000; Koca and others 2007). For instance, Rudra and others (2008) suggested that chlorophylls were less heat stable in cilantro purees at pH 5.5 than cilantro purees at pH 7.5 and pH 8.5.

Finally, Zhao and others (2013) studied the thermal treatments effects on the chlorophylls levels in a cucumber drink (pH 6.60 and 3.0 °Brix) during 50 days at 4 °C. Although some peaks in the levels of both chlorophylls were reported in the middle of that period, the levels of chlorophylls were lower than the initial concentrations after the thermal treatment at 85 °C for 15 s. Conversely, Castillejo and others (2016a) found that chlorophylls were unaffected in acidic vegetable beverages (tomato, red pepper, broccoli, and spices) during 58 days at 5 °C.

Total Carotenoids

The average level of total carotenoids in untreated green juice blends was 13.24 ± 0.80 µg/ml among four independent trials. The total carotenoids in untreated juices

ranged from 12.51 to 14.37 µg/ml. After the mild thermal treatment at 72 °C for 15 s and the high thermal treatment at 90 °C for 30 s, the levels of total carotenoids were 14.04 ± 0.77 and 11.83 ± 0.90 µg/ml, respectively. Significant differences in the absolute levels of total carotenoids were found between the thermal treatments ($P < 0.05$), but any treatment significantly differed with the control ($P > 0.05$).

Among the different carotenoids, it was likely that lutein and β-carotene were the most representative in the untreated green juice blends. Heinonen and others (1989) found that lutein and β-carotene were the highest carotenoids found in spinach, lettuce, celery, parsley, and cucumber. Likewise, Arnold and others (2014) found lutein and β-carotene as the main carotenoids for kale and parsley.

Comparisons in terms of the relative levels of total carotenoids suggested that carotenoids were unaffected by the milder thermal treatment. The relative level of total carotenoids was 1.06 ± 0.06. Non-significant difference existed between the control and the milder treatment ($P > 0.05$). Conversely, the higher thermal treatment significantly differed with the control ($P < 0.05$). The relative level of total carotenoids was 0.89 ± 0.05 after the higher treatment.

In vegetable beverages that included celery and cucumber, Barba and others (2010) found that the levels of total carotenoids resulted unaffected after thermal treatments at 90 and 98 °C for 15 to 21 min. However, those comparisons were in absolute values. Treated green juice blends also resulted unaffected compared to the control in terms of absolute values. Likewise, Korus (2013) found non-significant differences in the levels of total carotenoids and β-carotene in kale after cooking procedures. Conversely, Lessin and others (1997) found that the levels of β-carotene

significantly increased in boiled broccoli, canned collard, and canned spinach. The increases of carotenoids levels were related to increase of the disruptions of carotenoprotein complexes.

In more severe thermal treatments, Arnold and others (2014) found non-significant differences in the levels of β-carotene between untreated and thermally treated kale jars at 121 °C for 5 to 20 min. But, significant differences in the levels of β-carotene were found for parsley jars at the same treatments. The levels of lutein were also reduced in both products at the same treatments. Conversely, Updike and Schwartz (2003) found that the levels of lutein increased in canned kale and spinach after thermal treatments. However, those increments in the lutein levels were mainly in the cis configuration levels; actually, the levels of trans configuration decreased in both products after the thermal treatments. Isomerization of β-carotene was also reported by Lessin and others (1997). A significant increase in total β-carotene was found in canned collard. The total β-carotene (trans and cis) increased from 273.9 to 409.7 µg/g of dry tissue, but it was mainly related to an increase of the cis configuration.

Nevertheless, researchers still question whether cis isomers of carotenoids molecules could have the same health benefits as that proposed for their all-trans counterparts. Findings could differ among carotenoid structures. For instance, trans β-carotene have better absorption than their cis counterparts; conversely, cis lycopene shows greater absorption than their trans configurations (Priyadarshani 2017).

Total Antioxidant Capacity in Untreated and Thermally Treated Formulated Green Juice Blends

The overall total antioxidant capacity in untreated and thermally treated green juice blends are summarized in Table 4-2. This model, formulated green juice blend (pH

4.27 ± 0.03 and °Brix 3.8 ± 0.3) was prepared and then thermally treated at 72 °C for 15 s (Treatment 1), and at 90 °C for 30 s (Treatment 2). The average total antioxidant capacity in untreated green juice blends was 0.67 ± 0.07 µmol of TE/ml. After the milder thermal treatment, the average total antioxidant capacity was 0.82 ± 0.10 µmol of TE/ml. There were no significant differences between that treatment and the control ($P > 0.05$). Conversely, the average total antioxidant capacity was significantly increased after the higher treatment ($P < 0.05$). The average total antioxidant capacity was 0.85 ± 0.09 µmol of TE/ml. Non-significant differences existed between the thermal treatments ($P > 0.05$).

Nevertheless, reporting absolute values can make comparisons between data problematic. To evaluate the effects between treatments, a more suitable approach is to compare to compare the decrease of antioxidant capacity on a relative base, i.e., final total antioxidant capacity over their initial levels. The normalized total antioxidant capacity after treatments are summarized in Table 4-3. Examining these results, the total antioxidant capacity resulted significantly higher in any thermal treatment compared to the control ($P < 0.05$). Non-significant were found between the thermal treatments ($P > 0.05$). After the milder and the higher treatments, the relative values of total antioxidant capacity were 1.23 ± 0.05 and 1.27 ± 0.03, respectively.

In general, antioxidant losses during processing in fruit and vegetables are related to the high thermal-sensitivity of vitamin C, and in a lesser extent due to carotenoids degradation (Kalt 2005). In thermally treated green juice blends, the total antioxidant capacity significantly increased after the highest thermal treatment. However, total carotenoids decreased after the highest thermal treatment as it was

described in the last section. Furthermore, chlorophyll *a* and *b* have also been related to antioxidant activity in vegetables (Sözgen Başkan and others 2013), but they were severed affected after thermal treatments in green juice blends. Therefore, it was likely that the main role in the antioxidant capacity in the treated green juice blends was by the effect of phenolic and flavonoid contents.

Bunea and others (2008) found that increase in the antioxidant capacity in boiled spinach occurred because simple phenolic compounds released after the breakdown of more complex structures containing them, i.e., cellulose networks. Chu and others (2002) compared the free and bound fractions of phenolic compounds in celery, cucumber, lettuce, and spinach. They all had bound fractions of phenolics between 12.5 to 28.5%. Although thermal treatments seem to enhance the release of phenolic compounds, longer treatments at high temperatures could produce a negative impact. Kaiser and others (2012) found that thermally treated parsley pastes at 90 °C for 7 to 10 min decreased the phenolic contents and the antioxidant capacity. Results after treatments at 80 °C did not differ with the untreated pastes.

Additionally, Sun and others (2007) suggested that flavonoids were among the mainly antioxidants related to the antioxidant capacity of asparagus and broccoli juices. These included rutin, quercetin, and kaempferol sophorosides. Flavonoid compounds could result exceptionally thermoresistant. Hostetler and others (2012) studied the flavonoid profiles in parsley and Chinese celery juices. Non-significant differences existed in thermally treated parsley and celery juices after thermal treatments at 100 to 121 °C for up to 30 min. In addition, the pH effect was also evaluated in celery juices. Non-significant differences existed at pH 3.0 to 5.0 after thermal processing at 121 °C

for 15 to 60 min. Nevertheless, the study found that thermal treatments did effect the conversion of apiin to apigenin 7-*O*-glucoside which could improve the absorption.

Finally, differences in the assays to evaluate the total antioxidant capacity could yield mixed results. For instance, Barba and others (2010) compared the total antioxidant capacity in a vegetable beverage that included celery and cucumber after thermal treatments at 90°C for 15 to 21 min. Non-significant differences existed among the control and the thermal treatments by ABTS assay; conversely, significant differences existed among them by ORAC assay. Sun and others (2007) suggested the use of more than one method to evaluated the antioxidant activity.

Natural Microflora in Untreated and Thermally Treated Formulated Green Juice Blends

The levels of total coliforms, generic *E. coli*, and APC were examined in untreated and thermally treated green juice blends. This model, formulated green juice blend (pH 4.27 ± 0.03 and °Brix 3.8 ± 0.3) was prepared and then thermal treated at 72 °C for 15 s (Treatment 1), and at 90 °C for 30 s (Treatment 2). Results are summarized in Table 4-4. The average level of total coliforms in untreated green juice blends was 2.32 ± 0.62 log CFU/ml. The levels ranged from 1.51 to 2.90 log CFU/ml in four independent trials. After the milder and the higher thermal treatments, the average levels of total coliforms were lower than the detection limit (1.00 log CFU/ml). Significant differences were found between the control and the thermal treatments ($P < 0.05$), but non-significant differences existed between the treatments ($P > 0.05$).

E. coli resulted unusual but still possible in untreated green juice blends. The level of *E. coli* was 1.00 log CFU/3 ml in only one of the four trials, which 1.00 log CFU/ml was found in one of three samples (1 ml) of juice analyzed. Therefore, it

resulted more accurate to report the levels of *E. coli* by total volume of juice analyzed. Moreover, the milder thermal treatment sufficed to reduce that level of *E. coli* in green juice blends. After the milder and the higher thermal treatments, the levels of *E. coli* were lower than the detection limit (1.00 log CFU/ml).

Furthermore, the average level of APC in untreated green juice blends was 5.44 ± 0.42 log CFU/ml. The levels ranged from 4.91 to 5.85 log CFU/ml in four independent trials. After the milder and the higher thermal treatments, the average levels of APC were 2.79 ± 0.30 and 2.09 ± 0.08 log CFU/ml, respectively. Significant differences were found among the control and the thermal treatments ($P < 0.05$). To compare the effects of the thermal treatments on the APC levels, the log reductions are summarized in Table 4-5. Significant differences existed between thermal treatments ($P < 0.05$). The milder thermal treatment at 72 °C for 15 s achieved a log reduction of 2.65 ± 0.30 log CFU/ml and the higher thermal treatment 90 °C for 30 s achieved a log reduction of 3.35 ± 0.37 log CFU/ml.

Overall findings in the untreated green juice blends were in agreement with the microflora found in fresh produce. For instance, Johnston and others (2006) evaluated fresh produce in final boxes for distribution. The levels of total coliforms, *E. coli*, and APC were 2.54, 1.27, and 6.50 log CFU/g, respectively. Furthermore, Valentin-Bon and others (2008) found that the total bacterial count in bagged cut spinach and lettuce mixes ranged from 4.0 to 8.3 log CFU/g and from 4.5 to 7.9 log CFU/g, respectively. Ailes and others (2008) examined the occurrence and levels of APC, total coliforms and *E. coli* on several leafy greens and vegetables, including most of the ingredients used for the green juice blend. The total average of APC was 6.0 ± 0.04 log CFU/g; the

highest loads were found in cantaloupe, cilantro, mustard greens, and cilantro. The total average of total coliforms was 2.2 ± 0.05 log CFU/g; the highest loads were found in arugula, cantaloupe, and parsley. Lastly, the total average of *E. coli* was 1.0 ± 0.03 log CFU/g; cantaloupe, cabbage, and cilantro had the highest percentages of occurrence (29 to 25%). Therefore, it was likely that parsley contributed with the most load of APC and total coliforms among the ingredients used for the green juice blend. In addition, the lower incidence of *E. coli* were also reported in several vegetables, such as spinach (Ilic and others 2008) and lettuce (Holvoet and others 2014). Among 2,531 fresh produce commodities, Most Probable Number (MPN) values of 1,000 *E. coli* cells/g were found in only six samples and MPN values < 10 *E. coli* cells/g (detection limit) were found in 2,442 samples (USDA 2011).

Moreover, similar levels of APC were also found in untreated juice and beverages. For instance, Song and others (2007) found the average levels of APC in untreated kale juice was 6.80 log CFU/ml, but total coliforms were higher than those levels previously reported on fresh produce. The average levels of total coliforms in kale juice was 6.86 log CFU/ml. After storage at 10 °C for three days, APC and total coliforms increased to levels around 10 log CFU/ml. Findings in untreated green juice blends were in agreement with those reported by Zhao and others (2013). In a cucumber drink (3.0 °Brix and 6.6 pH), the initial level of APC was 5.28 log CFU/ml. Those levels decreased to approximately 3.50 log CFU/ml after a thermal treatment at 85 °C for 15 s which resulted higher than those reported after the milder thermal treatment in green juice blends at 72 °C for 15 s. After 50 days of storage at 4 °C, the average levels of APC in cucumber drink increased by 1.60 log CFU/ml.

Finally, Castillejo and others (2016b) found lower levels of APC in an acidified vegetable drink (4.3 °Brix and 4.5 pH) that contained cucumber, spinach, and broccoli. The level of APC was 4.4 log CFU/g in untreated samples. After thermal treatments, the levels of APC reduced to 1.7 to 1.8 log CFU/g. Non-significant differences existed between the thermal treatments at 80 °C for 3 min and 90 °C for 45 s. Findings were contrary to the treatments used for the green juice blends; however, Castillejo and others (2016b) used 75 mg/l NaClO to sanitize their ingredients prior to the drink preparation.

Comparison among the Thermally Treated Formulated Green Juice Blends and the Commercial Alternatives

Comparisons among the thermally treated formulated green juice blends and the commercial juice alternatives at retail market sowed that the enzyme activity of POD and PPO in the thermally treated juices were lower than the activities found in the commercial high pressure processed juices. The PPO activity in the thermally treated juices was similar with the activity found in the commercial thermally treated juice. Nevertheless, the commercial thermal treatment appeared to be a more severe treatment than those used in this study (72 °C for 15 s and 90 °C for 30 s) because the enzyme activity of POD in the commercial thermally treated juice was completely inactive (0.05 ± 0.01 U/ml).

The levels of chlorophyll did not show any trend among the juice alternatives, i.e. the formulated, representative juice and the commercial juices. The levels of chlorophylls therefore appeared formula dependent. Conversely, the total carotenoids among the commercial high pressure processed juices showed similar figures but the levels of total carotenoids were superior in the thermally treated juices. Likewise, the

antioxidant capacity was higher in the thermally treated juices compared to the commercial high pressure processed alternatives. The commercial thermally treated juice showed the highest total antioxidant capacity (1.80 µmol of TE/ml) among the juice alternatives. Although that could be explained because of its fruit juice content, some of the HPP juices also included fruit in their ingredients and their antioxidant capacity were lower. Thermal treatment therefore appeared to increase the antioxidant capacity in green juice blends.

Finally, microbiological quality parameters showed that the commercial green juice blends had lower levels of total coliforms. Similarly, the evaluated thermal treatments for the formulated, representative green juice blend effectively reduced the initial levels of total coliforms. Conversely, the levels of APC differed among all juices. The commercial thermal treatment appeared to be a more severe treatment than those used in this study. The shelf life differences among the products, however, limited the comparison among them. Lastly, generic *E. coli* was not reported in any juice alternative as was expected. Current commercially-available juices all are apparently appropriately processed.

Table 4-1. Summary of product quality parameters in some commercial green juice blends found at the retail market and evaluated in this study.

	Suja[a,e]	Naked Pressed[b,e]	Evolution Fresh[c,e]	Naked[d,e]
pH	4.26 ± 0.02	3.76 ± 0.03	4.29 ± 0.02	3.91 ± 0.03
°Brix	4.2 ± 0.0	8.0 ± 0.0	3.8 ± 0.0	9.4 ± 0.0
PPO (U/ml)[f]	9.89 ± 0.42	9.67 ± 0.07	10.22 ± 0.90	7.91 ± 1.13
POD (U/ml)[f]	139.61 ± 9.89	34.58 ± 1.47	63.29 ± 0.39	0.05 ± 0.01
Chlorophyll *a* (µg/ml)	7.57 ± 0.70	11.14 ± 0.18	35.22 ± 0.77	18.55 ± 0.23
Chlorophyll *b* (µg/ml)	5.45 ± 0.56	6.00 ± 0.11	18.71 ± 0.43	2.69 ± 0.06
Total Carotenoids (µg/ml)	2.30 ± 0.20	2.39 ± 0.04	5.47 ± 0.16	7.35 ± 0.16
T. Antioxidant Capacity (µmol of TE/ml)	0.32 ± 0.07	0.40 ± 0.09	0.40 ± 0.03	1.80 ± 0.10
Total Coliforms (log CFU/ml)	2.56 ± 0.07	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00
APC (log CFU/ml)	4.20 ± 0.20	2.93 ± 0.13	3.19 ± 0.20	0.70 ± 0.00

[a]Suja Über Greens™; 31 days to the enjoy by or best by date.
[b]Naked Pressed™ Bright Greens™; 12 days to the enjoy by or best by date.
[c]Evolution Fresh™ Essential Greens with Lime; 16 days to the enjoy by or best by date.
[d]Naked® Kale Blazer®; 43 days to the enjoy by or best by date.
[e]Values given are the means (± standard deviation) for one independent experiment.
[f]1 U = 0.1 Δ absorbance / min

Table 4-2. Overall results of the enzyme activity, chlorophylls & total carotenoids levels, and total antioxidant capacity among treatments.

	PPO (U/ml)[d,e,f]	POD (U/ml)[d,e,f]	Chlorophyll *a* (µg/ml)[d,e]	Chlorophyll *b* (µg/ml)[d,e]	Total Carotenoids (µg/ml)[d,e]	Antioxidant Capacity (µmol of TE/ml)[d,e]
Control[a]	12.17 ± 0.30 a	38.33 ± 7.05 a	56.04 ± 3.04 a	25.36 ± 1.19 a	13.24 ± 0.80 a,b	0.67 ± 0.07 b
Treatment 1[b]	8.35 ± 0.28 b	8.59 ± 1.60 b	38.17 ± 2.63 b	20.49 ± 1.29 b	14.04 ± 0.77 a	0.82 ± 0.10 a,b
Treatment 2[c]	7.48 ± 0.33 c	1.79 ± 0.73 b	32.68 ± 2.82 b	14.66 ± 1.03 c	11.83 ± 0.90 b	0.85 ± 0.09 a

[a]Control refers to untreated green juice blends.
[b]Treatment 1 was completed at 72 °C for 15 s.
[c]Treatment 2 was completed at 90 °C for 30 s.
[d]Values given are the means (± standard deviation) for four independent experiments.
[e]Significant differences ($P < 0.05$) existed among treatments with different letters within the same column.
[f]1 U = 0.1 Δ absorbance / min

Table 4-3. Overall normalized results of the enzyme activity, chlorophylls & total carotenoids levels, and total antioxidant capacity among treatments.

	PPO	POD	Chlorophyll *a*	Chlorophyll *b*	Total Carotenoids	Antioxidant Capacity
Control[a]	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 b
Treatment 1[b]	0.69 ± 0.02 b	0.23 ± 0.04 b	0.68 ± 0.05 b	0.81 ± 0.04 b	1.06 ± 0.06 a	1.23 ± 0.05 a
Treatment 2[c]	0.61 ± 0.02 c	0.05 ± 0.01 c	0.58 ± 0.03 c	0.58 ± 0.01 c	0.89 ± 0.05 b	1.27 ± 0.03 a

[a]Control refers to untreated green juice blends.
[b]Treatment 1 was completed at 72 °C for 15 s.
[c]Treatment 2 was completed at 90 °C for 30 s.
[d]Values given are the means (± standard deviation) for four independent experiments.
[e]Significant differences ($P < 0.05$) existed among treatments with different letters within the same column.

Table 4-4. Summary of natural microflora in untreated green juice blends and thermally treated green juice blends.

	Treatments	Total Coliforms (log CFU/ml)[e,f]	*E. coli* (log CFU/3 ml)	APC (log CFU/ml)[e,f]
Trial 1[a]	Control[b]	2.68 ± 0.15 a	< 1.00	5.85 ± 0.38 a
	Treatment 1[c]	0.70 ± 0.00 b	< 1.00	3.07 ± 0.09 b
	Treatment 2[d]	0.70 ± 0.00 b	< 1.00	2.16 ± 0.15 c
Trial 2	Control	2.90 ± 0.05 a	1.00	5.70 ± 0.14 a
	Treatment 1	0.70 ± 0.00 b	< 1.00	2.71 ± 0.20 b
	Treatment 2	0.70 ± 0.00 b	< 1.00	2.16 ± 0.15 c
Trial 3	Control	1.51 ± 0.29 a	< 1.00	5.31 ± 0.28 a
	Treatment 1	0.70 ± 0.00 b	< 1.00	3.00 ± 0.07 b
	Treatment 2	0.70 ± 0.00 b	< 1.00	2.00 ± 0.30 c
Trial 4	Control	2.17 ± 0.21 a	< 1.00	4.91 ± 0.13 a
	Treatment 1	0.70 ± 0.00 b	< 1.00	2.40 ± 0.17 b
	Treatment 2	0.70 ± 0.00 b	< 1.00	2.06 ± 0.10 b
Overall	Control	2.32 ± 0.62 a	n/a	5.44 ± 0.42 a
	Treatment 1	0.70 ± 0.00 b	n/a	2.79 ± 0.30 b
	Treatment 2	0.70 ± 0.00 b	n/a	2.09 ± 0.08 c

[a]Trials were independent repetitions of the experiment completed during August 2016.
[b]Control refers to untreated green juice blends.
[c]Treatment 1 was at 72 °C for 15 s.
[d]Treatment 2 was at 90 °C for 30 s.
[e]Values given are the means (± standard deviation) per trial set.
[f]Significant differences ($P < 0.05$) existed between treatments with different letters within each trial set and overall results.

Table 4-5. Overall log reductions of APC in thermally treated green juice blends.

	Log Reduction of APC (log CFU/ml)[c,d]
Treatment 1[a]	2.65 ± 0.30 b
Treatment 2[b]	3.35 ± 0.37 a

[a]Treatment 1 was completed at 72 °C for 15 s.
[b]Treatment 2 was completed at 90 °C for 30 s.
[c]Values given are the means (± standard deviation) for four independent experiments.
[d]Significant differences ($P < 0.05$) existed between treatments with different letters within the same column.

CHAPTER 5
CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

In general, results showed that the traditional thermal pasteurization used by the juice industry effectively reduced the enzyme activity of peroxidase (POD) in green juice blends, and to a lesser extent, the activity of polyphenol oxidase (PPO). Juice processors could consider the inactivation of PPO as suitable criteria for thermal processing efficacy for green juice blends. Consumers who are seeking minimally processed food may decide that thermal pasteurization is not desirable due to this enzyme inactivation. Chlorophyll levels in green juice blends were negatively affected by thermal treatments. High-pressure processing, however, seems to these effects; thus, HPP (or cold pressed) seems more attractive to consumers because of the perceived benefits.

Conversely, juice processor could highlight that thermally treated green juice blends provide higher antioxidants and similar levels of carotenoids compared to fresh, unprocessed juices. Health benefits from carotenoids are extensively well described in the literature. Gourmet pasteurization might be a good alternative for juice processor of green juice blends for some consumers. Furthermore, considering that thermally treated juices are low-priced than the high-pressure processed juices (due to presumably lower processing costs), thermal treatment could represent good alternative for consumer whose main concern is to obtain the health benefits related to vegetables at an intermediate price.

More studies, however, are needed to validate thermal treatments in compliance with the Juice HACCP Regulation. Shelf life studies would also provide more information to establish the suitable pasteurization treatments that minimize the

processing effects without neglecting product food safety and quality. In these types of studies, *Escherichia coli* O157:H7 would reasonably be considered the pertinent microorganism for thermal processing designs of green juice blends. Potential contamination of *E. coli* in unpasteurized green juice blends might cause consumers (specially the vulnerable groups) who perceive unpasteurized juices healthier than pasteurized juices to reconsider their position. Additional work could also include a comprehensive juice processing study, examining thermal and non-thermal alternatives, for the same representative formula which would provide accurate results to conclude benefits and drawbacks from each technology for the green juice blend processing.

Finally, future work could also be performed on the sensory aspects of green juice blends, such as color degradation. Chlorophyll is readily associated with the green color of the juice blends; however, it can degrade by processing effects, the product pH, or environmental conditions, e.g., light exposure. Understanding the effect of color on juice blend acceptability is an interesting area of further study. From a flavor perspective juice processors might consider the use of fruit juices to enhance flavor and sweetness. Different formulas could be evaluated among potential consumers, although health-conscious consumers may be less likely to buy these blends due to the sugar content from fruits. In summary, there are several areas of inquiry that are possible for green juice blends that help more fully understand the risks, and potential benefits (both real and perceived) to the health-conscious consumer.

APPENDIX A
SSOP OF THE JUICING MACHINE

A sanitation standard operating procedure to clean was elaborated for the masticating juice machine after its use. The procedure was developed according to manufacture recommendation. After concluding each trial, the procedure was followed as it is described below:

1. Unplug the equipment.
2. Remove the drum set from the housing part and place it in the sink.
3. Take the removal parts off the drum set: the funnel, the auger, the juicing screen, and the end cap.
4. Remove chunk residues from the juicer parts under running tap water.
5. Soak items in hot tap water.
6. Rub each juicer part including sieve and collection bowls with scrubber sponges and dishwashing liquid soap.
7. Brush the juicing screen using a cleaning brush and dishwashing liquid soap.
8. Rinse items in running hot tap water until the suds are gone.
9. Place items upside down. Allow all surface to air-dry.
10. Put the unit back together.

A validation of the cleaning procedure was completed during the preliminaries studies. The juicing screen, the most demanding part of the juice maker for cleaning, was swabbed using a wet swab with sterile peptone water over 10 cm^2. APC was from 2 to 4 CFU/10 cm^2.

APPENDIX B
SUMMARY OF QUALITY PARAMETERS OF FORMULATED GREEN JUICE BLENDS AFTER FOUR INDEPENDENT TRIALS

Summary of the Enzyme Activity (PPO and POD) in Untreated and Thermally Treated Formulated Green Juice Blends

Trials[a]	Treatments	PPO (U/ml)[e,f,g]	POD (U/ml)[e,f,g]
1	Control[b]	11.96 ± 1.09 a	28.37 ± 1.15 a
	Treatment 1[c]	8.20 ± 0.50 b	6.85 ± 0.30 b
	Treatment 2[d]	7.22 ± 0.63 b	1.00 ± 0.09 c
2	Control	12.44 ± 1.66 a	38.32 ± 2.39 a
	Treatment 1	8.22 ± 0.76 b	10.68 ± 1.05 b
	Treatment 2	7.31 ± 0.40 b	1.38 ± 0.18 c
3	Control	12.40 ± 0.46 a	43.77 ± 1.01 a
	Treatment 1	8.77 ± 0.99 b	8.07 ± 0.50 b
	Treatment 2	7.96 ± 0.51 b	2.60 ± 0.09 c
4	Control	11.87 ± 1.04 a	42.85 ± 0.50 a
	Treatment 1	8.22 ± 0.80 b	8.75 ± 0.18 b
	Treatment 2	7.42 ± 0.78 b	2.17 ± 0.03 c

[a]Trials were independent repetitions of the experiment completed during August 2016.
[b]Control refers to untreated green juice blends.
[c]Treatment 1 was at 72 °C for 15 s.
[d]Treatment 2 was at 90 °C for 30 s.
[e]Values given are the means (± standard deviation) per trial set.
[f]Significant differences ($P < 0.05$) existed between treatments with different letters within each trial set.
[g]1 U = 0.1 Δ absorbance / min

Summary of the Levels of Chlorophylls and Total Carotenoids in Untreated and Thermally Treated Formulated Green Juice Blends

Trials[a]	Treatments	Chlorophyll *a* (µg/ml)[e,f]	Chlorophyll *b* (µg/ml) [e,f]	Total Carotenoids (µg/ml) [e,f]
1	Control[b]	56.79 ± 1.33 a	26.13 ± 0.38 a	13.18 ± 0.48 a,b
	Treatment 1[c]	37.98 ± 1.75 b	20.94 ± 1.10 b	13.59 ± 0.76 a
	Treatment 2[d]	32.46 ± 1.27 c	15.21 ± 0.39 c	11.93 ± 0.13 b
2	Control	52.94 ± 2.53 a	23.85 ± 0.89 a	12.51 ± 0.63 a
	Treatment 1	34.64 ± 0.54 b	18.70 ± 0.41 b	13.18 ± 0.17 a
	Treatment 2	28.96 ± 1.70 c	13.26 ± 0.63 c	10.52 ± 0.43 b
3	Control	59.94 ± 0.73 a	26.47 ± 0.15 a	14.37 ± 0.18 a
	Treatment 1	39.25 ± 1.37 b	20.58 ± 0.42 b	14.66 ± 0.66 a
	Treatment 2	35.71 ± 1.62 c	14.58 ± 0.60 c	12.43 ± 0.44 b
4	Control	54.51 ± 0.37 a	25.01 ± 0.21 a	12.89 ± 0.64 b
	Treatment 1	40.82 ± 0.29 b	21.74 ± 0.11 b	14.71 ± 0.33 a
	Treatment 2	33.59 ± 0.98 c	15.60 ± 0.17 c	12.43 ± 0.47 b

[a]Trials were independent repetitions of the experiment completed during August 2016.
[b]Control refers to untreated green juice blends.
[c]Treatment 1 was at 72 °C for 15 s.
[d]Treatment 2 was at 90 °C for 30 s.
[e]Values represent mean ± standard deviation per trial set.
[f]Significant differences ($P < 0.05$) existed between treatments with different letters within each trial set.

Summary of the Total Antioxidant Capacity in in Untreated and Thermally Treated Formulated Green Juice Blends

Trials[a]	Treatments	Total Antioxidant Capacity (µmol of TE/ml)[e,f]
1	Control[b]	0.58 ± 0.03 b
	Treatment 1[c]	0.74 ± 0.03 a
	Treatment 2[d]	0.74 ± 0.03 a
2	Control	0.68 ± 0.09 a
	Treatment 1	0.72 ± 0.06 a
	Treatment 2	0.90 ± 0.12 a
3	Control	0.74 ± 0.03 b
	Treatment 1	0.94 ± 0.07 a
	Treatment 2	0.94 ± 0.07 a
4	Control	0.66 ± 0.06 b
	Treatment 1	0.86 ± 0.03 a
	Treatment 2	0.80 ± 0.03 a

[a]Trials were independent repetitions of the experiment completed during August 2016.
[b]Control refers to untreated green juice blends.
[c]Treatment 1 was at 72 °C for 15 s.
[d]Treatment 2 was at 90 °C for 30 s.
[e]Values represent mean ± standard deviation per trial set
[f]Significant differences ($P < 0.05$) existed between treatments with different letters within each trial set.

LIST OF REFERENCES

Abadias M, Usall J, Anguera M, Solsona C, Viñas I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. International Journal of Food Microbiology 123(1):121-9.

Ailes EC, Leon JS, Jaykus L-A, Johnston LM, Clayton HA, Blanding S, Kleinbaum DG, Backer LC, Moe CL. 2008. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. Journal of Food Protection 71(12):2389-97.

Andrés V, Villanueva MJ, Tenorio MD. 2016. The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. Food Chemistry 192:328-35.

Arnold C, Schwarzenbolz U, Böhm V. 2014. Carotenoids and chlorophylls in processed xanthophyll-rich food. LWT - Food Science and Technology 57(1):442-5.

Asioli D, Aschemann-Witzel J, Caputo V, Vecchio R, Annunziata A, Næs T, Varela P. 2017. Making sense of the “clean label” trends: a review of consumer food choice behavior and discussion of industry implications. Food Research International 99(Part 1):58-71.

Barba FJ, Esteve MJ, Frigola A. 2010. Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. Journal of Agricultural and Food Chemistry 58(18):10070-5.

Barth M, Hankinson TR, Zhuang H, Breidt F. 2009. Microbiological spoilage of fruits and vegetables. In: Sperber W, Doyle M, editors. Compendium of the microbiological spoilage of foods and beverages. New York, NY: Springer New York. p. 135-83.

Bates RP, Morris jR, Crandall PG. 2001. Principles and practices of small- and medium-scale fruit juice processing. In: FAO, editor. Rome, Italy.

Bell C, Kyriakides A. 2000. *Clostridium Botulinum*. Oxford, UK: Blackwell Science.

Boo H-O, Heo B-G, Gorinstein S, Chon S-U. 2011. Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. Plant Science 181(4):479-84.

Bower JA. 2009. Statistical methods for food science. West Sussex, UK: Blackwell Publishing Ltd.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1):25-30.

Britton G, Khachik F. 2009. Carotenoids in Food. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids. Birkhäuser Verlag, Basel. p. 47-9.

Bunea A, Andjelkovic M, Socaciu C, Bobis O, Neacsu M, Verhé R, Camp JV. 2008. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). Food Chemistry 108(2):649-56.

Burns J, Fraser PD, Bramley PM. 2003. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. Phytochemistry 62(6):939-47.

Butz P, Edenharder R, García AF, Fister H, Merkel C, Tauscher B. 2002. Changes in functional properties of vegetables induced by high pressure treatment. Food Research International 35(2):295-300.

Calvo MM. 2005. Lutein: a valuable ingredient of fruit and vegetables. Critical Reviews in Food Science and Nutrition 45(7-8):671-96.

Castillejo N, Martínez-Hernández GB, Gómez PA, Artés F, Artés-Hernández F. 2016a. Red fresh vegetables smoothies with extended shelf life as an innovative source of health-promoting compounds. Journal of Food Science and Technology 53(3):1475-86.

Castillejo N, Martínez-Hernández GB, Monaco K, Gómez PA, Aguayo E, Artés F, Artés-Hernández F. 2016b. Preservation of bioactive compounds of a green vegetable smoothie using short time–high temperature mild thermal treatment. Food Science and Technology International 23(1):46-60.

Castro-Ibáñez I, Gil MI, Tudela JA, Ivanek R, Allende A. 2015. Assessment of microbial risk factors and impact of meteorological conditions during production of baby spinach in the southeast of Spain. Food Microbiology 49(Supplement C):173-81.

Chen X, Qin W, Ma L, Xu F, Jin P, Zheng Y. 2015. Effect of high pressure processing and thermal treatment on physicochemical parameters, antioxidant activity and volatile compounds of green asparagus juice. LWT - Food Science and Technology 62(1, Part 2):927-33.

Chu Y-F, Sun J, Wu X, Liu RH. 2002. Antioxidant and antiproliferative activities of common vegetables. Journal of Agricultural and Food Chemistry 50(23):6910-6.

Chun OK, Kim D-O, Smith N, Schroeder D, Han JT, Lee CY. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. Journal of the Science of Food and Agriculture 85(10):1715-24.

Danyluk MD, Goodrich-Schneider RM, Schneider KR, Harris LJ, Worobo RW. 2012a. Outbreaks of foodborne disease associated with fruit and vegetable juices, 1922–2010. EDIS - University of Florida IFAS Extension (FSHN 12-04):1-7.

Danyluk MD, Parish ME, Goodrich-Schneider RM, Worobo RW. 2012b. Microbial decontamination of juices. In: Demirci A, Ngadi MO, editors. Microbial decontamination in the food industry: novel methods and applications. Sawston, Cambridge, UK: Woodhead Publishing Limited. p 163-89.

Danyluk MD, Schaffner DW. 2011. Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. Journal of Food Protection 74(5):700-8.

Daryaei H, Balasubramaniam VM. 2012. Microbial decontamination of food by high pressure processing. In: Demirci A, Ngadi MO, editors. Microbial decontamination in the food industry: novel methods and applications. Sawston, Cambridge, UK: Woodhead Publishing Limited. p. 370-406.

del Amor FM, Serrano-Martínez A, Fortea I, Núñez-Delicado E. 2008. Differential effect of organic cultivation on the levels of phenolics, peroxidase and capsidiol in sweet peppers. Journal of the Science of Food and Agriculture 88(5):770-7.

del Buono A. 2017 Jan 11. Health and wellness drives growth of vegetable juices, juice smoothies. Beverage Industry Magazine. Available from: https://www.bevindustry.com/articles/89923-health-and-wellness-drives-growth-of-vegetable-juices-juice-smoothies?. Accessed 2017 October 18.

Deng G-F, Lin X, Xu X-R, Gao L-L, Xie J-F, Li H-B. 2013. Antioxidant capacities and total phenolic contents of 56 vegetables. Journal of Functional Foods 5(1):260-6.

Doğru YZ, Erat M. 2012. Investigation of some kinetic properties of polyphenol oxidase from parsley (*Petroselinum crispum*, Apiaceae). Food Research International 49(1):411-5.

Doğan S, Turan Y, Ertürk H, Arslan O. 2005. Characterization and purification of polyphenol oxidase from artichoke (*Cynara scolymus* L.). Journal of Agricultural and Food Chemistry 53(3):776-85.

Evelyn, Milani E, Silva FVM. 2017. Comparing high pressure thermal processing and thermosonication with thermal processing for the inactivation of bacteria, moulds, and yeasts spores in foods. Journal of Food Engineering 214(Supplement C):90-6.

Fellows PJ. 2009. Food processing technology: principles and practice. Third ed. Cambridge, UK: Woodhead Publishing Limited.

Ferruzzi MG, Blakeslee J. 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutrition Research 27(1):1-12.

Food and Drug Administation (FDA). 1997. HACCP principles & application guidelines. Available from: https://www.fda.gov/Food/GuidanceRegulation/HACCP/ucm2006801.htm. Accessed 2017 September 30.

Food and Drug Administation (FDA). 2004. Guidance for industry: juice HACCP hazards and controls guidance first edition. Available from: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm072557.htm. Accessed 2017 September 30.

Food and Drug Administration (FDA). 2007. Guidance for industry: refrigerated carrot juice and other refrigerated low-acid juices. Available from: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm072481.htm. Accessed 2017 September 30.

Food and Drug Administration (FDA). 2015. Talking about juice safety: what you need to know. Available from: https://www.fda.gov/food/resourcesforyou/consumers/ucm110526.htm. Accessed 2017 September 30.

Frewer LJ, Bergmann K, Brennan M, Lion R, Meertens R, Rowe G, Siegrist M, Vereijken C. 2011. Consumer response to novel agri-food technologies: implications for predicting consumer acceptance of emerging food technologies. Trends in Food Science & Technology 22(8):442-56.

Gao J, Rupasinghe HPV. 2012. Nutritional, physicochemical and microbial quality of ultrasound-treated apple-carrot juice blends. Food and Nutrition Sciences 3(2):212-8.

Gazzani G, Papetti A, Massolini G, Daglia M. 1998. Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. Journal of Agricultural and Food Chemistry 46(10):4118-22.

Golbeck JH, Cammarata KV. 1981. Spinach thylakoid polyphenol oxidase. Plant Physiology 67(5):977.

Grassin C, Coutel Y. 2010. Enzymes in fruit and vegetable processing and juice extraction. In: Whitehurst R, van Oort M, editors. Enzymes in Food Technology. Second ed. West Sussex, UK: John Wiley & Sons, Ltd. p. 236-63.

Gunawan MI, Barringer SA. 2000. Green color degradation of blanched broccoli (*Brassica oleracea*) due to acid and microbial growth. Journal of Food Processing and Preservation 24(3):253-63.

Hammer F. 1993. Oxidoreductases. In: Nagodawithana T, Reed G, editors. Enzymes in food processing. Third ed. San Diego, CA: Academic Press, Inc. p 221-77.

Heimdal H, Larsen LM, Poll L. 1994. Characterization of polyphenol oxidase from photosynthetic and vascular lettuce tissues (*Lactuca sativa*). Journal of Agricultural and Food Chemistry 42(7):1428-33.

Heinonen MI, Ollilainen V, Linkola EK, Varo PT, Koivistoinen PE. 1989. Carotenoids in finnish foods: vegetables, fruits, and berries. Journal of Agricultural and Food Chemistry 37(3):655-9.

Herman KM, Hall AJ, Gould LH. 2015. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiology and Infection 143(14):3011-21.

Hernández A, Cano MP. 1998. High-pressure and temperature effects on enzyme inactivation in tomato puree. Journal of Agricultural and Food Chemistry 46(1):266-70.

Holvoet K, Sampers I, Seynnaeve M, Uyttendaele M. 2014. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. International Journal of Food Microbiology 171(Supplement C):21-31.

Hostetler GL, Riedl KM, Schwartz SJ. 2012. Endogenous enzymes, heat, and pH affect flavone profiles in parsley (*Petroselinum crispum* var. *neapolitanum*) and celery (*Apium graveolens*) during juice processing. Journal of Agricultural and Food Chemistry 60(1):202-8.

Houška M, Strohalm J, Kocurová K, Totušek J, Lefnerová D, Tříska J, Vrchotová N, Fiedrleová V, Holasova M, Gabrovská D, Paulíčková I. 2006. High pressure and foods—fruit/vegetable juices. Journal of Food Engineering 77(3):386-98.

Hsu K-C. 2008. Evaluation of processing qualities of tomato juice induced by thermal and pressure processing. LWT - Food Science and Technology 41(3):450-9.

Huang H-W, Wu S-J, Lu J-K, Shyu Y-T, Wang C-Y. 2017. Current status and future trends of high-pressure processing in food industry. Food Control 72(Part A):1-8.

IBISWorld. 2016. Health Food & Supplement Store Franchises in the US. Available from: http://clients1.ibisworld.com/reports/us/industry/default.aspx?entid=6010. Accessed 2017 October 18.

IBISWorld. 2017. Juice production in the US. Available from: http://clients1.ibisworld.com/reports/us/industry/default.aspx?entid=287. Accessed 2017 October 18.

Ilic S, Odomeru J, LeJeune JT. 2008. Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. Journal of Food Protection 71(12):2398-403.

International Food Information Council Foundation (IFIC). 2017a. Food and Health Survey: "A healthy perspective: understanding American food values". Available from: http://www.foodinsight.org/2017-food-and-health-survey. Accessed 2017 October 18.

International Food Information Council Foundation (IFIC). 2017b. "Anatomy of a foodie": group profiles from the 2017 Food and Health Survey. Available from: http://www.foodinsight.org/articles/anatomy-foodie-group-profiles-2017-food-and-health-survey. Accessed 2017 October 18.

Jiménez-Monreal AM, García-Diz L, Martínez-Tomé M, Mariscal M, Murcia MA. 2009. Influence of cooking methods on antioxidant activity of vegetables. Journal of Food Science 74(3):H97-H103.

Johnston LM, Jaykus L-A, Moll D, Anciso J, Mora B, Moe CL. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. International Journal of Food Microbiology 112(2):83-95.

Kaczor A, Baranska M, Czamara K. 2016. Carotenoids: overview of nomenclature, structures, occurrences, and functions. In: Baranska M, Kaczor A, editors. Carotenoids: nutrition, analysis, and technology. First ed. West Sussex, UK: John Wiley & Sons, Ltd. p 1-14.

Kaiser A, Brinkmann M, Carle R, Kammerer DR. 2012. Influence of thermal treatment on color, enzyme activities, and antioxidant capacity of innovative pastelike parsley products. Journal of Agricultural and Food Chemistry 60(12):3291-301.

Kalt W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. Journal of Food Science 70(1):R11-R9.

Keenan DF, Rößle C, Gormley R, Butler F, Brunton NP. 2012. Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. LWT - Food Science and Technology 45:50-7.

Keklik NM, Demirci A. 2012. Microbial decontamination of food by ultraviolet (UV) and pulsed UV light. In: Demirci A, Ngadi MO, editors. Microbial decontamination in the food industry: novel methods and applications. Cambridge, UK: Woodhead Publishing Limited. p 344-69.

Koca N, Karadeniz F, Burdurlu HS. 2007. Effect of pH on chlorophyll degradation and colour loss in blanched green peas. Food Chemistry 100(2):609-15.

Koo J. 2011. Microbial safety of fresh and processed vegetables. In: Sinha NK, editor. Handbook of vegetales and vegetables processing. Ames, IA: Blackwell Publishing Ltd. p 483-503.

Kopsell DA, Kopsell DE, Lefsrud MG, Curran-Celentano J, Dukach LE. 2004. Variation in lutein, β-carotene, and chlorophyll concentrations among *Brassica oleracea* cultigens and seasons. Hort Science 39(2):361-4.

Korus A. 2011. Level of vitamin C, polyphenols, and antioxidant and enzymatic activity in three varieties of kale (*Brassica oleracea* L. var. *acephala*) at different stages of maturity. International Journal of Food Properties 14(5):1069-80.

Korus A. 2013. Effect of preliminary and technological treatments on the content of chlorophylls and carotenoids in kale (*Brassica oleracea* L. var. *acephala*). Journal of Food Processing and Preservation 37(4):335-44.

Lee HS, Castle WS. 2001. Seasonal changes of carotenoid pigments and color in hamlin, earlygold, and budd blood orange juices. Journal of Agricultural and Food Chemistry 49(2):877-82.

Lefsrud M, Kopsell D, Wenzel A, Sheehan J. 2007. Changes in kale (*Brassica oleracea* L. var. *acephala*) carotenoid and chlorophyll pigment concentrations during leaf ontogeny. Scientia Horticulturae 112(2):136-41.

Lessin WJ, Catigani GL, Schwartz SJ. 1997. Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. Journal of Agricultural and Food Chemistry 45(10):3728-32.

Liao C-H, Fett WF. 2001. Analysis of native microflora and selection of strains antagonistic to human pathogens on fresh produce. Journal of Food Protection 64(8):1110-5.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148:350-82.

Liu RH. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of Nutrition 134(12):3479S-85S.

Ludikhuyze L, Van Loey A, Indrawati, Smout C, Hendrickx M. 2003. Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: from kinetic information to process engineering aspects. Critical Reviews in Food Science and Nutrition 43(5):527-86.

Marszałek K, Krzyżanowska J, Woźniak Ł, Skąpska S. 2017. Kinetic modelling of polyphenol oxidase, peroxidase, pectin esterase, polygalacturonase, degradation of the main pigments and polyphenols in beetroot juice during high pressure carbon dioxide treatment. LWT - Food Science and Technology 85(Part B):412-7.

Martínez-Flores HE, Garnica-Romo MG, Bermúdez-Aguirre D, Pokhrel PR, Barbosa-Cánovas GV. 2015. Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. Food Chemistry 172:650-6.

Mazzotta AS. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. Journal of Food Protection 64(3):315-20.

McLellan MR, Padilla-Zakour OI. 2005. Juice processing. In: Barrett DM, Somogyi L, Ramaswamy H, editors. Processing fruits: science and technology. Second ed. Boca Raton, FL: CRC Press LLC. p 73-95.

Miller AR, Kelley TJ, Mujer CV. 1990. Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit: tissue and substrate specificity. Phytochemistry 29(3):705-9.

Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. 2000. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. Journal of the American College of Nutrition 19(sup3):312S-9S.

Mintel. 2017. Juice and Juice Drinks. Available from: http://academic.mintel.com/display/793199/. Accessed 2017 September 28.

Moon J-K, Shibamoto T. 2009. Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry 57(5):1655-66.

Moore LV, Thompson FE. 2015. Adults meeting fruit and vegetable intake recommendations - United States, 2013. In: CDC, editor. MMWR. Atlanta, GA: U.S. Department of Health and Human Services (HHS). p 709-13.

Morales-Blancas EF, Chandia VE, Cisneros-Zevallos L. 2002. Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. Journal of Food Science 67(1):146-54.

Nayak B, Liu RH, Tang J. 2015. Effect of processing on phenolic antioxidants of fruits, vegetables, and grains—a review. Critical Reviews in Food Science and Nutrition 55(7):887-918.

Nielsen. 2015. We are what we eat: healthy eating trends around the world. Available from: https://www.nielsen.com/content/dam/nielsenglobal/eu/nielseninsights/pdfs/Nielsen Global Health and Wellness Report - January 2015.pdf. Accessed 2017 September 28.

Oey I, Lille M, Van Loey A, Hendrickx M. 2008. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. Trends in Food Science & Technology 19(6):320-8.

Olsen NV, Grunert KG, Sonne A-M. 2010. Consumer acceptance of high-pressure processing and pulsed-electric field: a review. Trends in Food Science & Technology 21(9):464-72.

Parish ME. 1997. Public health and nonpasteurized fruit juices. Critical Reviews in Microbiology 23(2):109-19.

Park KH, Fricker A. 1977. Thermische inaktivierung und lagerungsverhalten technologisch wichtiger enzyme. Zeitschrift für Ernährungswissenschaft 16(2):81-91.

Park S-J, Lee J-I, Parke J. 2002. Effects of a combined process of high-pressure carbon dioxide and high hydrostatic pressure on the quality of carrot juice. Journal of Food Science 67(5):1827-34.

Parkin KL. 2008. Enzymes. In: Damodaran S, Parkin KL, Fennema OR, editors. Fennema's Food Chemistry. Fourth ed. Boca Raton, FL: CRC Press. p 331-435.

Peng J, Tang J, Barrett DM, Sablani SS, Anderson N, Powers JR. 2017. Thermal pasteurization of ready-to-eat foods and vegetables: critical factors for process design and effects on quality. Critical Reviews in Food Science and Nutrition 57(14):2970-95.

Podsędek A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT - Food Science and Technology 40(1):1-11.

Priyadarshani AMB. 2017. A review on factors influencing bioaccessibility and bioefficacy of carotenoids. Critical Reviews in Food Science and Nutrition 57(8):1710-7.

Produce for Better Health Foundation (PBH). 2015. State of the plate: 2015 study on America's consumption of fruit & vegetables. Available from: https://www.pbhfoundation.org/pdfs/about/res/pbh_res/State_of_the_Plate_2015_WEB_Bookmarked.pdf. Accessed 2017 September 28.

Reyes-De-Corcuera JI, Goodrich-Schneider RM, Barringer SA, Landeros-Urbina MA. 2014. Processing of fruit and vegetables beverages. In: Clark S, Jung S, Lamsal B, editors. Food Processing: principles and applications. Second ed. West Sussex, UK: John Wiley & Sons, Ltd. p 339-62.

Rivas A, Rodrigo D, Martínez A, Barbosa-Cánovas GV, Rodrigo M. 2006. Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. LWT - Food Science and Technology 39(10):1163-70.

Roca M, Chen K, Pérez-Gálvez A. 2016. Chlorophylls. In: Carle R, Schweiggert RM, editors. Handbook on natural pigments in food and beverages: industrial applications for improving food color. Duxford, UK: Elsevier Ltd. p 125-58.

Rodriguez-Amaya DB. 2001. Carotenoid analysis in foods. Washington, DC: OMNI Research.

Ross AIV, Griffiths MW, Mittal GS, Deeth HC. 2003. Combining nonthermal technologies to control foodborne microorganisms. International Journal of Food Microbiology 89(2–3):125-38.

Roy MK, Takenaka M, Isobe S, Tsushida T. 2007. Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: effects of thermal treatment. Food Chemistry 103(1):106-14.

Rudra SG, Sarkar BC, Shivhare US. 2008. Thermal degradation kinetics of chlorophyll in pureed coriander leaves. Food and Bioprocess Technology 1(1):91-9.

Saini RK, Nile SH, Park SW. 2015. Carotenoids from fruits and vegetables: chemistry, analysis, occurrence, bioavailability and biological activities. Food Research International 76(Part 3):735-50.

Sánchez-Vega R, Elez-Martínez P, Martín-Belloso O. 2014. Effects of high-intensity pulsed electric fields processing parameters on the chlorophyll content and its degradation compounds in broccoli juice. Food and Bioprocess Technology 7(4):1137-48.

Schneider KR, De J, Li Y, Sreedharan A, Goodrich Schneider R, Danyluk MD, Pahl DM, Walsh CS, Todd-Searle J, Schaffner DW, Kline W, Buchanan RL. 2017. Microbial evaluation of pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control 73, Part B:511-7.

Schwartz SJ, von Elbe JH, Giusti MM. 2008. Colorants. In: Damodaran S, Parkin KL, Fennema OR, editors. Fennema's Food Chemistry. Fourth ed. Boca Raton, FL: CRC Press. p. 571-99.

Seyderhelm I, Boguslawski S, Michaelis G, Knorr D. 1996. Pressure induced inactivation of selected food enzymes. Journal of Food Science 61(2):308-10.

Sharapov UM, Wendel AM, Davis JP, Keene WE, Farrar J, Sodha S, Hyytia-Trees E, Leeper M, Gerner-Smidt P, Griffin PM, Braden C. 2016. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of fresh spinach: United States, 2006. Journal of Food Protection 79(12):2024-30.

Sheth AN, Wiersma P, Atrubin D, Dubey V, Zink D, Skinner G, Doerr F, Juliao P, Gonzalez G, Burnett C, Drenzek C, Shuler C, Austin J, Ellis A, Maslanka S, Sobel J. 2008. International outbreak of severe botulism with prolonged toxemia caused by commercial carrot juice. Clin Infect Dis 47(10):1245-51.

Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. 2004. Fresh Produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection 67(10):2342-53.

Smelt JPPM, Brul S. 2014. Thermal inactivation of microorganisms. Critical Reviews in Food Science and Nutrition 54(10):1371-85.

Song H-P, Byun M-W, Jo C, Lee C-H, Kim K-S, Kim D-H. 2007. Effects of gamma irradiation on the microbiological, nutritional, and sensory properties of fresh vegetable juice. Food Control 18(1):5-10.

Sözgen Başkan K, Tütem E, Özer N, Apak R. 2013. Spectrophotometric and chromatographic assessment of contributions of carotenoids and chlorophylls to the total antioxidant capacities of plant foods. Journal of Agricultural and Food Chemistry 61(47):11371-81.

Stratil P, Klejdus B, Kubáň V. 2006. Determination of total content of phenolic compounds and their antioxidant activity in vegetables–evaluation of spectrophotometric methods. Journal of Agricultural and Food Chemistry 54(3):607-16.

Sun T, Powers JR, Tang J. 2007. Evaluation of the antioxidant activity of asparagus, broccoli and their juices. Food Chemistry 105(1):101-6.

Swanson KM, Busta FF, Peterson EH, Johnson MG. 1992. Colony count methods. In: Vanderzant C, Splittstoesser DF, editors. Compendium of methods for the microbiological examination of foods. Third ed. Washington, DC: American Public Health Association. p. 75-95.

Taillie LS, Ng SW, Xue Y, Busey E, Harding M. 2017. No fat, no sugar, no salt… No problem? Prevalence of “low-content” nutrient claims and their associations with the nutritional profile of food and beverage purchases in the United States. Journal of the Academy of Nutrition and Dietetics 117(9):1366-74.e6.

Teo AY-L, Ravishankar S, Sizer CE. 2001. Effect of low-temperature, high-pressure treatment on the survival of *Escherichia coli* O157:H7 and *Salmonella* in unpasteurized fruit juices. Journal of Food Protection 64(8):1122-7.

Terefe NS, Buckow R, Versteeg C. 2014. Quality-related enzymes in fruit and vegetable products: effects of novel food processing technologies, Part 1: High-Pressure Processing. Critical Reviews in Food Science and Nutrition 54(1):24-63.

Terefe NS, Yang YH, Knoerzer K, Buckow R, Versteeg C. 2010. High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. Innovative Food Science & Emerging Technologies 11(1):52-60.

Tournas VH. 2005. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Critical Reviews in Microbiology 31(1):33-44.

Updike AA, Schwartz SJ. 2003. Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. Journal of Agricultural and Food Chemistry 51(21):6184-90.

US Department of Health & Human Services (HHS), US Department of Agricultural (USDA). 2015. 2015-2020 Dietary Guidelines for Americans. 8th ed

US Department of Agricultural (USDA). 2011. Microbiological Data Program: progress update and 2009 data summary. Washington, DC.

Valentin-Bon I, Jacobson A, Monday SR, Feng PCH. 2008. Microbiological quality of bagged cut spinach and lettuce mixes. Appl. Environ. Microbiol. 74(4):1240-2.

Vámos-Vigyázó L, Haard NF. 1981. Polyphenol oxidases and peroxidases in fruits and vegetables. Critical Reviews in Food Science and Nutrition 15(1):49-127.

Van Boxstael S, Habib I, Jacxsens L, De Vocht M, Baert L, Van De Perre E, Rajkovic A, Lopez-Galvez F, Sampers I, Spanoghe P, De Meulenaer B, Uyttendaele M. 2013. Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. Food Control 32(1):190-7.

Van Loey A, Ooms V, Weemaes C, Van den Broeck I, Ludikhuyze L, Indrawati, Denys S, Hendrickx M. 1998. Thermal and pressure−temperature degradation of chlorophyll in broccoli (*Brassica oleracea* L. *italica*) juice: a kinetic study. Journal of Agricultural and Food Chemistry 46(12):5289-94.

Vojdani JD, Beuchat LR, Tauxe RV. 2008. Juice-associated outbreaks of human illness in the United States, 1995 through 2005. Journal of Food Protection 71(2):356-64.

Walsh RP, Bartlett H, Eperjesi F. 2015. Variation in carotenoid content of kale and other vegetables: a review of pre- and post-harvest effects. Journal of Agricultural and Food Chemistry 63(44):9677-82.

Wang R, Wang T, Zheng Q, Hu X, Zhang Y, Liao X. 2012. Effects of high hydrostatic pressure on color of spinach purée and related properties. Journal of the Science of Food and Agriculture 92(7):1417-23.

Weemaes CA, Ooms V, Van Loey AM, Hendrickx ME. 1999. Kinetics of chlorophyll degradation and color loss in heated broccoli juice. Journal of Agricultural and Food Chemistry 47(6):2404-9.

Worobo R, Splittstoesser DF. 2005. Microbiology of fruit products. In: Barrett DM, Somogyi L, Ramaswamy H, editors. Processing fruits: science and technology. Second ed. Boca Raton, FL: CRC Press. p 261-84.

Wu JSB, Shen S-C. 2011. Processing of vegetable juice and blends. In: Sinha NK, editor. Handbook of vegetable and vegetable processing. First ed. Ames, IA: Blackwell Publishing Ltd. p 335-50.

Yagar H. 2004. Some biochemical properties of polyphenol oxidase from celery. Preparative Biochemistry and Biotechnology 34(4):387-97.

Yan L. 2016. Dark green leafy vegetables. USDA - Agricultural Research Service. Available from: https://www.ars.usda.gov/plains-area/gfnd/gfhnrc/docs/news-2013/dark-green-leafy-vegetables/. Accessed 2017 October 18.

Zhao L, Wang S, Liu F, Dong P, Huang W, Xiong L, Liao X. 2013. Comparing the effects of high hydrostatic pressure and thermal pasteurization combined with nisin on the quality of cucumber juice drinks. Innovative Food Science & Emerging Technologies 17(Supplement C):27-36.

Zulueta A, Esteve MJ, Frígola A. 2009. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. Food Chemistry 114(1):310-6.

BIOGRAPHICAL SKETCH

Galo W. Chuchuca Morán was born in Machala, Ecuador. Galo earned the degree of food engineering from the Escuela Superior Politénica del Litoral (ESPOL) in February 2013. He worked in the food industry for a few years, and then decided to return to school. In August 2015, he moved to Gainesville, FL to work towards his degree of Master of Science in food science and human nutrition from the University of Florida. He graduated in the winter of 2017 and plans to pursue a career in academia in May 2018.