INVESTIGATING NOVEL PROCESSING METHODS TO DEVELOP MINIMALLY PROCESSED, NUTRITIOUS CONCORD GRAPE PRODUCTS WITH EXTENDED SHELF-LIFE

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Yuanyuan Li

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INVESTIGATING NOVEL PROCESSING METHODS TO DEVELOP MINIMALLY PROCESSED, NUTRITIOUS CONCORD GRAPE PRODUCTS WITH EXTENDED SHELF-LIFE

Yuanyuan Li, Ph.D.

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Concord grape is an economically important cultivar in the United States, especially for New York state. However, Concord grape prices have been declining in recent years, caused by changes in consumers' preference towards fruit juices, in addition to an oversupply in certain years. Traditionally Concord grapes are used for juice, jam and jelly production. Concerns over the high-sugar and low-fiber content of these products have driven the food industries to apply new processing procedures. This dissertation investigated the feasibility of several processing methods and novel technologies to produce Concord grape products that meet consumers' demands. In the application of non-thermal technologies to produce a cold-pressed juice, the effect of combining high pressure processing and pulsed electric field was investigated and compared with the traditional thermal treatment. Another non-thermal technology, high pressure homogenization, was studied and compared with flash pasteurization in producing a hot-pressed juice. Furthermore, to overcome the shortage of fiber in carbohydrate-rich juice products, a feasible processing method was developed to incorporate seeds and skins in the whole Concord grape puree product; high pressure processing and thermal treatment were compared to determine the best preserving technology for this novel product. Apart from all aforementioned refrigerated products, a shelf-stable

whole grape snack was developed using the following three methods: freeze-drying, hot-air drying alone, or in combination with microwave-vacuum drying. Results from these research projects provide the food industry with possible directions and feasible parameters for utilizing novel processing technologies to produce fruit products that meet current consumers' demands.

BIOGRAPHICAL SKETCH

Yuanyuan Li was born and raised in China. In 2006, she enrolled for a Bachelor's Degree in Huazhong Agricultural University, where she was awarded twice with the National Fellowship for Undergraduate (top 1) by the Ministry of Education of China. In 2010, she graduated with outstanding graduate award and outstanding thesis award.

She pursued her degree of Master from 2010 to 2013 in China Agricultural University, Beijing, China. During that time, she performed her research under the supervision of Professor Qixin Sun and Professor Nizhong Fu. After getting her Master's degree, she worked at the China-Africa Joint Research Center, Chinese Academy of Sciences for three years. She assisted in kicking the program off the ground and was recognized as Excellent Employee in 2014. In helping building scientific collaborations and administrating education programs, she found real and meaningful changes, especially for what education brought to those African students. She really enjoyed the time of teaching and helping people to realize their career goals by education. Therefore, she quit the job and went to the United States to further her education.

She joined the Ph.D. Degree program at the Food Science Department of Cornell University in 2019. She was a IFT20 Product Development division poster winner (2^{nd} prize). She also assisted in the outreach programs at Cornell AgriTech, Geneva, NY. She is aiming to work in educational institutions or universities in the future.

DEDICATION

Dedicated to my beloved family members for their endless love and support.

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

Concord grape (*Vitis labruscana*) was introduced by Ephraim Wales Bull in the 1840's, after the productive vine had dominated the eastern United States for its hardy characteristics. Its origin has been proven to be *V. vinifera* (maternal donor) and *Vitis labrusca* (paternal donor) recently (1). It became an economically important variety after Thomas Welch processed it into juice in 1868. Nowadays, most of the Concord grapes are processed into juice, jelly and jam, and a smaller amount into candy and sweet wine. Today in the northern part of US, commercial purple grape juice is primarily made from Concord grapes (2). In New York State, Concord grape make up for 75% of all the grown grapes and has been mainly used for juice production. However, the utilized production of all grapes in the US has been decreasing since 2013, and the drop was consecutive from 2018 to 2020 at a ratio of 13–14% (3,4). One of the main reasons behind this decrease is attributed to the change in consumers' demand. Consumers are now more health-conscious, and thus cutting their intake of fruit juices because of the higher sugar content and lower, or nearly none, fiber content in juices compared to fruits in their intact forms. On the other hand, consumers are demanding more fresh-like and nutritious products that are minimally processed with clean label. Concord grape contains high levels of phenolic compounds that can exert beneficial effects, such as promoting cardiovascular health (5,6), combating inflammation (7), hindering tumorigenesis (8) and protecting neurocognitive signaling pathways (9). These health-promoting properties can attract a large number of consumers and thus, expanding its market value; however, the question is how to produce novel Concord grape products that meet the above-mentioned demands.

Heat, enzymes and maceration (longer skin contact time) are commonly used by industries to destruct the cells in order to extract more juice, color pigments as well as nutrients. Traditionally, grape juice processors utilize hot-break/hot-press (heat and enzymes) to increase yield and enhance color intensity. In these procedures, grapes are heated to a moderate temperature, commonly 60 °C, and pectolytic enzymes are added for a time duration from 30 to 90 minutes (10). After that, the pressed grape juices are normally concentrated, using evaporation, resulting in the loss of volatile aromas along with the removal of water, even though some of them can be added back when diluting the juice back to original concentration. Pasteurization of the low pH grape juice is normally done by heating the juice to 85 to 88 °C for a minimum of 1 minute (10). However, applying heat can have negative effects on juice quality. Contrarily, cold press is also utilized for grape juice production, which can better preserve the fresh grape flavor (10). However, cold-pressed juice normally has a lighter color because of its less destructive effect on skin cell walls which trapped the pigments. It is of significance to develop novel processing methods to increase the diffusion of anthocyanins and other nutrients into the final juice while preserving the fresh flavor (16). Novel extraction methods to increase yield, enhance color intensity and extract polyphenols from grapes have been investigated, which include the applications of ultrasound, pulsed electric field (PEF), high voltage electric discharges (HVED) (11), high hydrostatic pressure (in combination with enzymes) (12), and supercritical carbon dioxide (13). These novel technologies exert advantages in shortening processing time, simplifying processes and being cost-effective (14).

To provide safe grape products, effective pasteurization must be applied. Thermal processing is a conventional and effective pasteurization method used for food processing. However, despite

its energy-intensive character, permanent and detrimental changes can occur after thermal processing. For instance, degradation of nutrients began at temperatures higher than 60 °C, and the rate increased with the temperature increment in production of Muscadine grape juice (15). Alternatively, non-thermal technologies have been thriven and widely studied to provide more nutritious products with fresher flavor.

Pulsed electric field (PEF) is a non-thermal processing technology that has many advantages compared to the conventional practice. PEF can inactivate microorganisms and ensure food safety at low temperature and thus, has minor impacts on organoleptic and sensory qualities. The principle for PEF is that applying short (μs) pulses under high voltage (kV) can cause electroporation. These pores can be reversible or irreversible, depending on variables such as cell type and energy input. The electric field strength, treatment time, food matrix and temperature all have impacts on the effectiveness of PEF treatment (16). Normally, much higher electric field strength is required for pathogenic and spoilage microorganism inactivation compared to that needed for generating pores on plant cells. For example, applying PEF (3 kV/cm) on grape by-product increase the extraction of total phenolics by 50% (17), while applying PEF (35 kV/cm) on grape juice was able to cause a 2-4 log reduction of spoilage microorganisms (18). Considering the energy and maintainence cost associated with grape juice processing, it is more cost effective to use PEF to increase the skin cell's permeability. Moreover, PEF is faster and more energy-efficient to disrupt cells in food processing compared to other methods, such as freezing-thawing, heating, enzymatic and mechanical techniques (19). In conclusion, it is advantageous to use PEF in increasing the permeability of grape skin compared to traditionally processes using enzyme and maceration, in which heat and much longer processing times are required.

High pressure processing (HPP), on the other hand, is one of the most widely implemented non-thermal technology in food industries. Since the first industrial application of HPP on treating foods in 1990, its commercial application categories and marketplace have been expanding. High pressure can destruct the vegetative cells of pathogens and spoilage microorganisms at low temperature. Increasing temperature during HPP due to adiabatic heating can be eliminated by controlling the system temperature, such as using cooling water. When foodstuff is delivered into the vessel, high pressure is instantaneously and uniformly applied, regardless of the geometry and size. HPP utilizes high pressure (100-600 MPa) for a short time duration to reduce microorganisms by causing cell morphology changes, such as cell membrane rupture (>200 MPa); inhibiting metabolic reactions, such as partial protein denaturation (> 100 MPa); while having no effect on molecular bonds which are commonly found in nutrients and flavor compounds (20, 21). Moreover, HPP can be applied to packed food, eliminating post process contamination during packaging. HPP has been successfully applied for the preservation of many commercial fruit products, and for shelf-life extension without negatively affecting the products' organoleptic properties (22).

Similar to HPP, High pressure homogenization (HPH), can also be utilized as a non-thermal pasteurization method. HPH has been used in dairy and food emulsions since its introduction to the food industry around 1900 (23). Normally, the pressure level used in HPH is lower than HPP, ranging from 100-400 MPa, with the pressure range of 300-400 MPa classified as ultra-high pressure homogenization (UHPH). HPH destructs the microorganisms by exposing them to high pressure, fluid-mechanic force, such as cavitation, turbulence, shear stress, impingement, as well as increment of valve/orifice temperature (24). Despite the design of the valve/orifice, the physicochemical properties of the pumped fluid, such as viscosity, also greatly impact the HPH performance. HPH can also cause enzyme inactivation, which may be attributed to the

aggregation of unfolded proteins. Increase of temperature during HPH, because of the friction at the orifice, can be minimized by including a cooling device, after which the duration of the liquid exposing to high temperature is negligible. HPH has been studied in milk and fruit juices for inactivation of microorganisms and extension of shelf-life without degrading their qualities (25).

Despite the abovementioned processing methods, drying is another important processing method for grape processing, especially for geographical areas with short harvest season and large quantity of grapes for transportation. Dried grapes, or raisins, are mainly processed using seedless grape varieties, such as "Thompson Seedless", which accounts for 95% of California raisin production (26). The pale greenish skin of Thompson seedless grape contains significantly less antioxidants than the dark blue-black Concord grape, as a result of gene mutation which is responsible to generate anthocyanins.

Traditional production of raisins normally consists of pretreatment, drying and post-drying steps. In pretreatment, chemicals or physical abrasion are often used to destroy the waxy layer of grape skin. The problems with these procedures include food safety issues caused by using chemical aids, color loss during abrasion and environmental impact of the dipping solution. Solar drying and hot air drying are traditionally used in raisin production. However, excessive exposure to high temperature during hot air drying can cause severe volumetric changes (shrinkage, collapse) and loss of nutrients (27).

Freeze drying, or lyophilization, which utilizes refrigeration and vacuum system to remove the water by sublimation, has been praised highly for preserving the quality of dehydrated products. However, these systems require high energy input, and the freezing and drying cycles are extremely time-consuming, making it less affordable and efficient in fruit processing. Freeze drying is more often utilized in high-value products (28).

Alternatively, microwave-vacuum drying (MVD) has been studied recently for its efficient heating as well as drying at low temperature. Unlike the low efficiency of heating by conduction and diffusion of water/vapor by pressure in hot air drying, MVD is a volumetric heating method in which the electromagnetic energy is directly transferred into heat in the foodstuff by causing molecular agitation. Moreover, the vacuum pulled in the system lowers the water boiling point and increases the drying rate by removing surface water and generating a vapor pressure gradient. Therefore, MVD not only increases the drying efficiency by using less energy and drying time, but also preserves the nutrients, color and texture compared to the conventional hot-air drying (29).

Despite the differences in drying technology, the intrinsic properties of fruit products also greatly impact the time, energy, and final texture of the dehydrated product. Drying berry purees is difficult as they are juicy, sugary, viscous, sticky and sensitive to heat. Foam mat drying, which involves adding foaming agent and stabilizers, is a good option for drying high-sugar fruit juice and puree, which would normally result in a powdery product. With the increasing demand for less additives and clean label, using simple ingredients to develop nutritious fruit products is preferred. In hot air drying, normally there are two stages as the drying process progressed: firstly, the rapid drying stage, in which the drying rate is high when removing free water; secondly the drying rate is much lower to remove the bound water, which takes about two thirds of the time to remove one third of the moisture (29). On the other hand, directly drying berry puree by MVD causes splashing due to the violent escape of water vapor inside the puree. Therefore, it is feasible and efficient to use hot-air drying to decrease the moisture content in the first place, and use MVD in the second stage to rapidly dry the puree. Moreover, this combination could be used to produce puffed products with intact structure.

Most of the grape products do not utilize the whole fruit, especially the skin and seeds, which are rich in bioactive compounds. Grape pomace waste or by-products from the wine and juice industry constituted about 13.5-14.5% of the total processed grape weight, leading to a production waste as high as 20% (30). Amongst these wastes, grape skin, the main reservoir for anthocyanins, can be utilized as FDA approved natural colorants; grape seeds contain high level of phenolic compounds and unsaturated fatty acids, among which linoleic acid is an essential fatty acid. Grape seeds contain 10-17% oil, and among them about 72-76% (w/w) is linoleic acid, the content of which is higher than that in safflower oil and sunflower oil (31). As a result, grape seed oil is a favorable salad dressing oil for its nutritional value and distinct aroma. Flavonoids, such as gallic acid, catechin, epicatechin and procyanidin, are the most abundant polyphenols in grape seeds. Proanthocyanidins, one of the main phenols in grape seeds, exhibit 50 times higher antioxidant capacity than vitamin C and 20 times higher than vitamin E (32). These antioxidants are highly praised for their free radical scavenging capacities which promote cardiovascular and brain health, combat aging, cancer and tumor that arose by the production of free radicals. These health benefits prompt the sale of grape seed extracts. The grape seed extracts are commonly produced by solid-liquid extraction which involves organic solvents which are not environmentally friendly (33). Moreover, these grape skin or grape seeds products are sold as dietary supplement. Developing novel whole Concord grape products, including skin and seeds, can meet costumers' demands and eliminate waste. Additionally, whole Concord grape products will be a heathier choice than juice as they have not only more nutrients, but also a balanced food matrix that contains protein, fiber, fatty acids and carbohydrates.

In summary, the investigation of novel processing methods to develop more nutritious and fresh-like Concord grape products is crucial to meet consumers' demands, create market value and boost the Concord grape business, especially in New York State which represents the oldest and largest Concord grape growing belt in the Lake Erie area.

Thus, the main objectives of this research project are as follows:

Objective 1: Determine the feasibility of using HPH to pasteurize hot-pressed Concord grape juice: validation of reduction of pathogenic and spoilage microorganism, changes of microbial, physicochemical and nutritional properties during refrigerated storage;

Objective 2: Evaluate the combination of pulsed electric field and high pressure processing in producing a cold-pressed Concord grape juice: sensorial attributes, changes of microbial, physicochemical, enzymatic and nutritional properties during refrigerated storage;

Objective 3: Develop feasible methods to create a whole Concord grape puree that include seeds and skin; assess the quality of whole Concord grape puree processed by high pressure processing versus thermal processing: sensorial attributes, changes of microbial, physicochemical, enzymatic and nutritional properties during refrigerated storage;

Objective 4: Determine the proper processing methods in producing a shelf-stable dehydrated whole Concord grape snack dried by freeze-drying, hot-air drying alone or in combination with microwave vacuum drying: efficiency and qualities changes during storage.

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CHAPTER 2

EVALUATION OF HIGH PRESSURE HOMOGENIZATION ON PROCESSING CONCORD GRAPE JUICE IN COMPARISON WITH THERMAL TREATMENT: SATETY, QUALITY AND REFRIGERATED SHELF-LIFE

ABSTRACT

High pressure homogenization can be used as a non-thermal technology to process fruit juices. This work evaluated the effect of high pressure homogenization (150/200/250/300 MPa, single pass, 10 °C) on inactivation of *Escherichia coli* (*E. coli* ATCC 25922) and spoilage microorganism (*Saccharomyces cerevisiae*) in Concord grape juice, as well as changes of physicochemical properties, bioactive compounds, antioxidant activity, polyphenoloxidase (PPO) and shelf-life under refrigeration in comparison with flash pasteurization (71.1 °C, 3 seconds). A pressure level of no less than 250 MPa in HPH treatment was able to achieve a greater than 5-log reduction of *E. coli*; while HPH-300 MPa was able to achieve a greater than 4-log reduction of *Saccharomyces cerevisiae*. No significant changes of physicochemical properties, total phenolics, total anthocyanins, antioxidant activity and residual PPO enzyme activity were observed between HPH-treated and heat-treated samples ($p<0.05$). HPH-300 MPa was able to extend the shelf-life under refrigeration to a minimum of 15 weeks with comparable qualities to the heat-treated juice.

1. INTRODUCTION

Concord grape juice, in addition to its palatable flavor, is a rich source of antioxidants which has been praised highly for its health-promoting properties. Benefits of consumption of Concord grape juice including: improving cognitive and motor function (1–3); preventing atherosclerosis (4); inhibiting tumorigenesis (5), etc. These benefits are attributed to the high level of antioxidants, which reduce the oxidative stress (6). Traditionally, grape juices are processed by applying heat for extraction, enzyme maceration and pasteurization. However, heat can negatively affect the juice quality, especially in sensorial and nutritional aspects (7,8). Alternatively, non-thermal technologies can be used to preserve foodstuff with little quality degradation, which meet the ever-growing consumer demand for fresh-like products (9).

High pressure homogenization (HPH) is a promising non-thermal technology to continuously process liquid foods, such as milk and fruit juices. A high pressure homogenizer utilizes a pump and a valve to change the pressure and speed of the fluid passing through the orifice, consequently causing cavitation and shear stress (10). HPH disrupts microorganism cells, and alters food components, by high pressure and velocity gradients which cause cavitation, shear stress and impingement (11). The friction in the homogenizer also leads to temperature increases (15-20 °C/100 MPa), which affects microbial reduction, however, is thought to be subtle in comparison with the mechanical forces (11). For example, it is reported that impingement plays a major role in disrupting yeast cells (12). The efficacy of HPH in ensuring food safety largely depends on pressure level, valve geometry, number of passes as well as fluid temperature (13). On the other hand, food matrix and microorganism strains also have great impact on the efficacy

of HPH as a preservation method. It was supported by evidence that gram-positive bacteria were more resistant to high pressure homogenization than gram-negative bacteria, mainly due to the higher structural strength in peptidoglycan, consisting of about 40 layers in gram-positive bacteria compared to 1-5 layers in gram-negative bacteria (13). With larger size and thicker structure, yeast resistance to HPH was generally found between gram-positive and gram-negative bacteria (14).

In fruit juice processing, the Food and Drug Administration (FDA) requires a 5-log reduction in pertinent pathogens (15). *E. coli* O157:H7 is one of the two targeted microorganisms suggested by the National Advisory Committee on Microbiological criteria in Foods (NACMCF) to be considered as pertinent pathogens if no strain association was identified in the food. It is also reported that *E. coli* O157:H7 caused fruit juice outbreaks in US (16). Moreover, in evaluating food processing operations, it is advantageous to use non-pathogenic surrogates, such as *E. coli* ATCC 25922, instead of pathogens (*E. coli* O157:H7) (17). In orange juice, a 5-log reduction of *E. coli* O157:H7 was achieved by HPH (200 MPa, 3 passes, 25 °C) (18). In apple juice, the inactivation of *E. coli* O157:H7 increased with the pressure increment, and a 5-log reduction was achieved with pressures higher than 250 MPa (19). Despite pathogen studies, HPH was also able to inactivate spoilage microorganisms and extend shelf-life, either alone or with other hurdles (antimicrobials, heat and ultrasound), such as in mango juice (20), apple juice (21), apricot and carrot juices (22), banana juice (23), peach juice (24) and tomato juice (25). For example, *Saccharomyces cerevisiae* was one of the mostly commonly seen and studied yeast that was responsible for juice spoilage, such as in orange juice (26), apricot and carrot juice (22). However, the efficacy of HPH on reduction of pathogenic and spoilage microorganisms, parameters of HPH treatment to produce a safe Concord grape juice, as well as quality changes during storage, have not been reported.

The aim of this work was to evaluate the effectiveness of high pressure homogenization in producing a safe, refrigerated Concord grape juice with premium quality. Reduction of non-pathogenic *E. coli* ATCC 25922 and a common wine yeast (*Saccharomyces cerevisiae*) inoculated in hot-pressed Concord grape juice by single pass HPH treatment from 150 to 300 MPa (increase at 50 MPa intervals) at an inlet temperature of 10 °C were investigated. Additionally, polyphenoloxidase activity on day 1 after processing, physicochemical properties, antioxidant contents (total phenolics and total anthocyanins) and antioxidant activity after HPH were compared with heat treated juice (71.1 °C, 3 sec) during a 15-week shelf-life study under refrigeration (4 °C).

2. MATERIALS AND METHODS

2.1 Raw materials and chemicals

Concord grapes (*Vitis Labruscana*) were hand-picked from a vineyard located in the finger lakes region (Branchport, NY, USA) in 2021. Grapes were stored at 4 ± 1 °C for 1 day before processed into juice. All chemicals used in this study were of analytical grade.

2.2 Concord grape juice production and experiment design

Fresh organic Concord grapes were processed into juice in a pilot plant (Cornell Agritech, Geneva, NY) according to the procedures depicted in Figure 2.1. Firstly, a grape crusher (Berarducci Bro's, McKeesport, PA) was used to break up skins and separate stalks. Then pectic enzyme (Rapidase®, DSM Food Specialties USA, Inc., South bend, IN, USA) was added into grape mash at a ratio of 0.1 mL/lb and heated in a 40-gallon steam jacketed kettle (Legion industries, Inc., Waynesboro, GA) at 50 °C for 1 hour. After hot press, grape mash was packed into two layers of cheese cloth and pressed into juice using a hydraulic rack and frame presser (Orchard Equipment Co., Conway, MA). Press pressure was hold at 1200-1400 psi for a few minutes to extract most of the juice. Juice samples were kept in glass carboys and stored at 2 °C ± 1 °C for 1 week to allow precipitation of potassium bitartrate. After cold storage, detartration was done by siphoning juice into new containers using an electric pump (313 S, Watson-Marlow fluid technology group, MA).

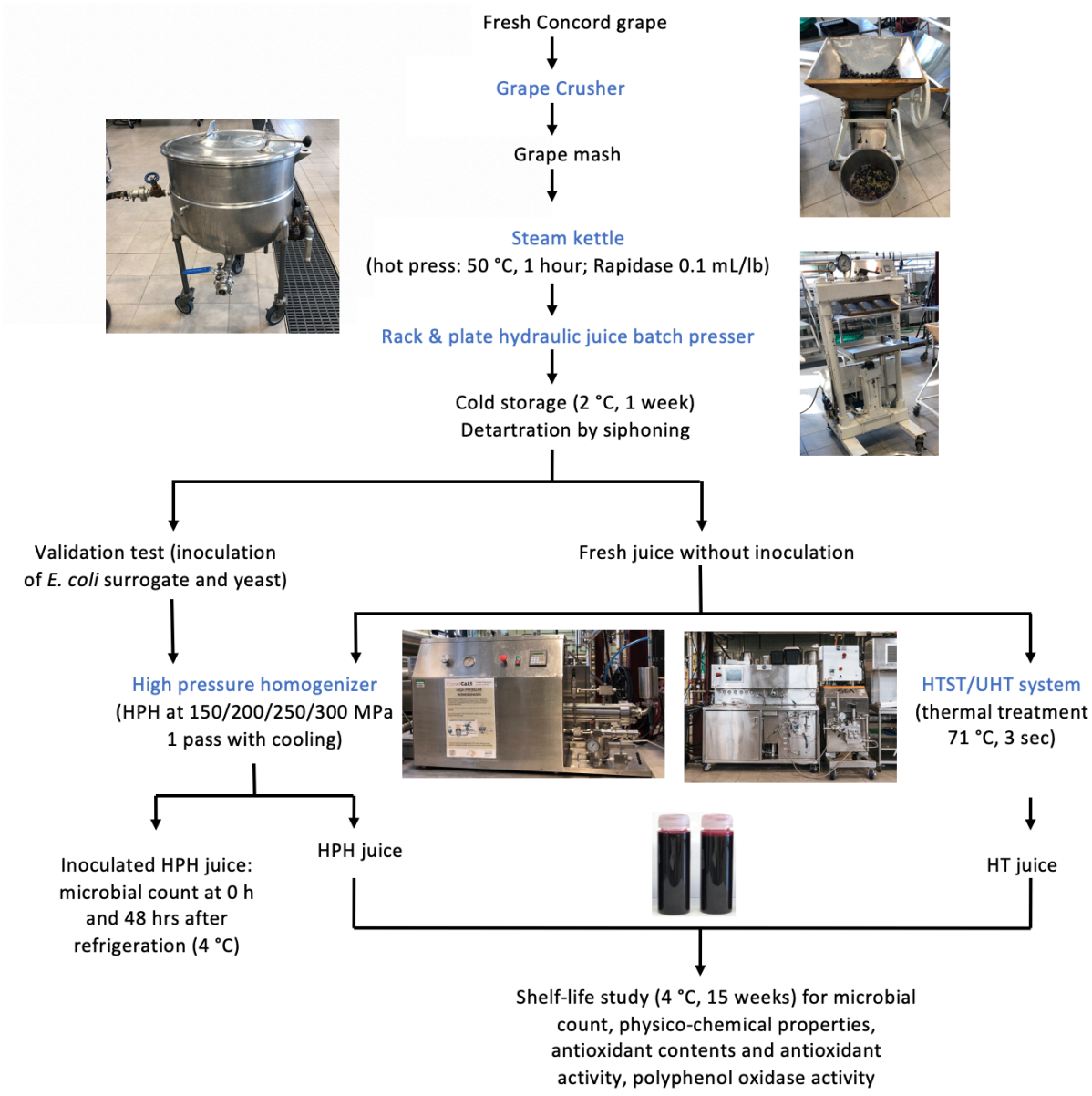

Figure 2. 1 Flow diagram of processing Concord grape juice and experimental design.

For the validation test, juice samples were inoculated with *Escherichia coli* O157:H7 surrogate (*E. coli* ATCC 25922) or *Saccharomyces cerevisiae* (EC 1118, Lalvin, Lallemand Inc., Memphis, TN, US) to an approximately concentration of 7-8 log CFU/mL. Then juices were treated using a high-pressure homogenizer (Mini DeBEE 45, BEE International Co., South Easton, MA) equipped with a diamond nozzle (D5, d=0.15 mm) and a water-cooling heat exchanger. Samples were kept in cold water to ensure that the inlet temperature was around 10 ± 3 °C. Juice samples with or without inoculation were treated at 150, 200, 250 and 300 MPa for one pass with cooling. The temperature in the emulsifying cell (EC) was estimated depending on the inlet temperature and processing pressure according to the equation ($T_o=T_i+1.5*P$, T_o and T_i is the temeperature in °C units at the EC outlet and inlet, respectively, while P is the operating pressure in kpsi units) provided by the manufacturer. To be specific, the maximum temperature in EC was about 78 °C at 300 MPa, 65 °C at 250 MPa, 55 °C at 200 MPa and 42 °C at 150 MPa. Because of the cooling system and the short exposure time in EC, the outlet temperature of all samples did not exceed 17 °C. Before running juice samples, the high-pressure homogenizer was sanitized using 200 ppm chlorinated water and then rinsed once using juice. Samples were processed from the highest pressure (300 MPa) and then decreased to lower pressure at an interval of 50 MPa. All samples were put in ice-water bath immediately after collection.

For heat treatment (HT), juice samples were flash pasteurized in a UHT/HTST Lab-25 HV tubular heat exchanger (MicroThermics Inc., Raleigh, NC) at 71.1 °C for 3 seconds to achieve >5-log reduction of pertinent pathogens as per FDA recommendations (15).

All grape juice samples were collected in 8 oz plastic sanitized bottles and sealed with sanitized caps. Bottles and caps were sanitized in 200 ppm chlorinated water and subsequently rinsed with 4 ppm free chlorine water. All samples were kept at 4 °C under dark for a shelf-life study up to 15 weeks.

2.3 Validation test and microbial counts reduction

2.3.1 Validation test

To validate the effect of HPH on pathogenic and spoilage microorganisms, non-pathogenic *Escherichia coli* (*E. coli* ATCC 25922) and wine yeast (*Saccharomyces cerevisiae*) were obtained from the Food Microbiology Laboratory at Cornell AgriTech (Geneva, NY). Subculture was propagated by inoculating the stock culture of *E. coli* in 5 mL of Tryptic Soy Broth (TSB, Alpha Biosciences Inc., Baltimore, MD), and incubated at 37 °C for 24 h. The subculture was added into a flask containing 2 liters of Concord grape juice produced in Section 2.2 and mixed by manually stirring. Inoculated juice samples were randomly collected to determine the initial population of microorganisms, which was about 7 to 8 log CFU/mL. A common wine yeast (*Saccharomyces cerevisiae*) was propagated in MRS broth and incubated at 32 °C for 40 h. The initial population was about 7 log CFU/mL. Juice samples before and immediately after HPH treatments were collected to study the microbial counts. Additionally, HPH treated juices were also kept at 4 °C for 48 hours and pour plated to determine the microbial count reduction.

2.3.2 Total plate count

Total plate count (TPC) was analyzed to investigate the microbial counts reduction in different groups by sampling juice samples during the 15-week period. Approximately 1 mL juice sample was pour-plated on plate count agar (PCA, CM0325, Oxoid), following by incubation at 30 °C for 48 to 72 h. Results were expressed as log CFU/mL.

2.4 Physicochemical properties analyses

Color was determined by a colorimeter (HunterLab, VI, Hunter Associate Laboratory Inc., VA, USA) and expressed as L^*, a^* and b^* values. The values of the absolute color difference of samples were calculated according to equation (1):

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (1)$$

where L_0, a_0, b_0 are the color measurements of untreated juice samples, L, a, b are the color measurements of treated juice samples.

Additionally, juice color was also examined by spectrophotometry methods commonly adopted by the grape juice industry. Juice samples were mixed with McIlvaine buffer (pH 3.2) at a ratio of 1:40 and set at room temperature for 15 minutes. The absorbance of the diluted juice sample was measured at 430 nm and 520 nm respectively.

Total soluble solids content (TSSC) was determined using a refractometer (model 300055, Sper scientific, Scottsdale, AZ, USA). About 2-3 drops of juice were added to obtain °Brix readings at 20 °C.

The pH value was measured at ambient temperature using a Thermo Scientific benchtop pH meter (Orion 3 Star Series, Fisher Scientific, MA, USA). The instrument was calibrated using pH 4 and pH 7 phosphate buffer before measurements.

2.5 Phenolic compounds content and antioxidant activity

2.5.1 Sample extraction

Juice samples were mixed with acidified methanol (1% HCl) at a ratio of 1:1 (v/v). After brief vortexing, tubes were kept in the water bath (40 °C) for 30 min. Then samples were centrifuged at 10,000 rpm for 10 min. Supernatant was used as extract for chemical analysis.

2.5.2 Total phenolic content (PC)

Total phenolic content was determined according to Waterhouse with minor modifications (27). Briefly, in 1580 µL DI water, 20 µL extract was mixed with 100 µL Folin-Ciocalteau reagent (Sigma-Aldrich St. Louis, MO), then set at room temperature for 6 minutes. After incubation, 300 µL of 20% sodium carbonate solution was added and gently vortexed before incubating at room temperature for 2 hours under dark. Additionally, Gallic Acid (Gallic acid anhydrous, Chem-Impex international Inc.) (0-500 mg/L) was used to create the standard curve. Absorbance was measured at 765 nm using the UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). All results were expressed as mg gallic acid equivalents (GAE) per L extract (mg/L).

2.5.3 Total anthocyanin content (AC)

Total anthocyanin content was determined according to the pH differential method (28). Briefly, juice extracts were diluted (50-fold) in pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) buffers separately. After vortex, the mixture was incubated at room temperature for 20 min. Blank was prepared using DI water. Absorbance was measured at 520 nm and 700 nm using the UV-visible Spectrophotometer. Results were calculated according to equation (2) and expressed as cyanidin-3-glucoside (cyd-3-glu) equivalents (CGE) per L extract (mg/L):

$$\text{AC}\ (CGE, mg/L) = \frac{A \times M_W \times DF \times 10^3}{\varepsilon \times L} \quad (2)$$

where $A = (A_{520nm} - A_{700nm})pH_{1.0} - (A_{520nm} - A_{700nm})pH_{4.5}$; M_W, molecular weight of cyd-3-glu = 449.2 g/mol; DF (dilution factor) = 50; ε is the molar extinction coefficient = 26,900 $L^{-1} \times cm^{-1} \times mol^{-1}$ for cyd-3-glu; L (pathlength) = 1 cm; 10^3 is the conversion of g to mg.

2.5.4 In vitro antioxidant activity (free radical scavenging activity ABTS)

The in vitro antioxidant activity was determined as ABTS$^{\bullet}$ free radical scavenging activity based on the description of Re et al (29). Briefly, 7 mM ABTS [2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt] (Sigma-Aldrich, Poole, Dorset, UK) solution was mixed with potassium persulfate (Honeywell Fluka, North Carolina, USA), to a final concentration of 2.45 mM. Then the mixture was set at dark and room temperature for 12-16 h to generate stable radicals. ABTS$^{\bullet}$ solution was adjusted to 0.700 ± 0.050 at 734 nm before using. Extract (10 uL) was mixed with 2 mL ABTS$^{\bullet}$ solution and set at room temperature under dark for 6 min. Blank was prepared using DI water. Absorbance was measured at 734 nm using the UV-visible Spectrophotometer. Radical scavenging capacity was calculated as the percentage inhibition (%) according to equation (6):

$$\boldsymbol{ABTS\ \%\ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} * 100\% \quad (6)$$

where $A_{control}$ is the absorbance of blank, A_{sample} is the absorbance of sample. Higher values of % inhibition indicates greater antioxidant activity.

2.6 Polyphenoloxidase activity analysis

Polyphenol oxidase (PPO) enzyme extraction and assay were carried out according to Garcia-Palazon et al. (30) with some modifications. All chemicals were purchased from VWR International, LLC., PA, except for Triton-100 (Electron microscopy sciences, PA) and catechol (Tokyo Chemical Industry Co., Ltd, TCI America, OR). Enzyme extraction buffer was prepared by mixing 4% (w/v) poly(vinylpolypyrrolidone) (PVPP), 1% (v/v) Triton X-100 and 1 M NaCl with 0.2 mol/L sodium phosphate buffer (pH 6.5). Equal aliquot (4.5 mL) of extraction buffer and juice samples were mixed vigorously and homogenized for 3 min on ice. Then the mixture was centrifuged at 9,500 rpm for 30 min at 4 °C (Centrifuge 5810R, Eppendorf, CT). The supernatant was collected and used as the crude enzyme extract for the PPO assay.

To start the PPO assay, 200 µL of crude enzyme extract was mixed with 1 mL 0.1 M catechol prepared in 0.05 M sodium phosphate buffer (pH 6.5). The absorbance was monitored at 420 nm every 30 seconds for 10 min. Blank was prepared the same way except that DI water was used instead of enzyme extract.

2.7 Statistical analysis

All treatments were performed in triplicate. Data were presented as mean ± standard deviation (SD) from technical replicates. Data were analyzed using one-way ANOVA at a significance level of $p < 0.05$. Significant differences among mean values were determined by the Tukey's post hoc test following the one-way ANOVA test using SPSS (SPSS statistics, version 22.0, IBM, Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Inactivation of E. coli ATCC 25922 and wine yeast (Saccharomyces cerevisiae)

The initial counts in inoculated grape juice were 7.59 ± 0.14 log CFU/mL for *E. coli* ATCC 25922 and 6.52 ± 0.02 log CFU/mL for *Saccharomyces cerevisiae*. The reduction of both strains was increased with the increment of pressure in HPH treatment (Figure 2.2). Immediately after HPH, a greater than 5 log reduction of *E. coli* was achieved by pressure level above 200 MPa, while HPH-150 MPa and HPH-200 MPa were able to obtain a 3 and 4 log CFU/mL reduction respectively. After refrigerated storage for 48 hours, the viable *E. coli* cell counts in control, HPH-150 MPa and HPH-200 MPa decreased about 2 log CFU/mL. The decrease of *E. coli* in grape juice samples indicated that the low pH of Concord grape juice imposed stress on the survival of *E. coli*. Studies have also reported that *E. coli* ATCC 25922 has less resistance towards ultrasound or ozone treatment in lower pH environments (31,32). However, no cumulative effect of pH and HPH was found in this study in the reduction of *E. coli* ATCC 25922.

This result was in alignment with the results in the studies of apple (pH~3.8) and carrot (pH~5.2) juices, in which a >5 log reduction of *E .coli* K12 was obtained at > 250 MPa homogenization pressure (19). However, no significant difference of reduction rate was found between the two types of juices, even though they have different juice characteristics, especially pH. In another study of the lethality effects of HPH processing on *E. coli* O157:H7 (ATCC 26) cells, results differed in different juice mediums (33). Application of HPH at 200 MPa in apple juice (Brix, % = 10.7; pH 3.45) was able to cause approximately 4-log reduction in *E. coli* cells, which was similar to our findings (Figure 2A). However, similar pressure level could cause a > 5-log reduction in the buffer solution ($NaPO_4$, 0.1 M, pH 7). It was suggested that the differences in food medium/matrix, such as higher soluble solids content, could increase the resistance of *E. coli* cell against the HPH treatment. As Concord grape juice is rich in carbohydrates and acids (17.5 °Brix, pH 3.0), a pressure level no less than 250 MPa was needed to meet the safety requirement. The main mechanism of the reduction of *E. coli* cells was attributed to the damages caused by HPH: injuries due to spatial pressure, cavitation, turbulent flow, shear stress, impingent, etc (19).

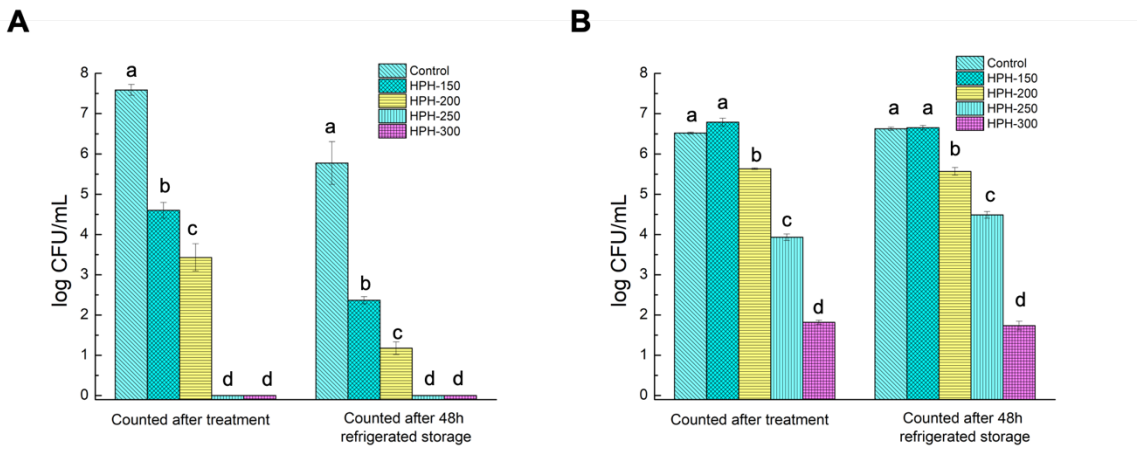

Figure 2. 2 Effect of high pressure homogenization (HPH) on inactivating E. coli 25922 (A) and Saccharomyces cerevisiae (B).

The capacity to tolerate high-sugar and low-pH conditions, as well as being able to grow in refrigerated conditions made yeast a threat to fruit juice spoilage (34). The efficacy of HPH on killing a common wine yeast, *Saccharomyces cerevisiae*, was presented in Figure 2.2B. Similarly, the viable cell counts decreased significantly with the increase of HPH processing pressure. Immediately after HPH-300 MPa treatment, a 4.7 log CFU/mL reduction of *Saccharomyces cerevisiae* was achieved, while HPH-250 MPa and HPH-200 MPa were able to obtain a 2.5 and 0.9 log CFU/mL reduction respectively. The lower reduction rate of *Saccharomyces cerevisiae* suggested that it was less sensitive to high pressure homogenization than the gram-negative *E. coli*, probably due to its much thicker overall structure. Similar reduction of yeast was also seen in a water model system, suggesting that pH level or soluble solids content did not affect the resistance of yeast cells to the HPH process (35). After refrigerated storage for 48 hours, changes of yeast counts were less than 1 log CFU/mL, indicating a higher resistance of yeast to the low-pH condition compared to *E. coli* (Figure 2B).

3.2 Changes of microbial counts and physicochemical properties of Concord grape juice during a 15-week storage under refrigeration at 4 °C

It is important to investigate the evolution of microbiological changes in HPH pasteurized juice to understand the endogenous microflora stability. The total plate count was presented in Figure 2.3 for a shelf-life study of 15-week at 4 °C.

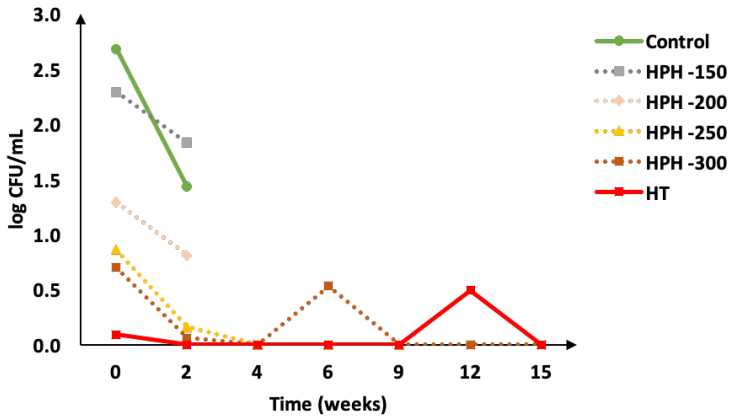

Figure 2. 3 Total plate count of Concord grape juice stored at 4 °C for a shelf-life study up to 15 weeks.

[a]Data are represented as mean ± standard deviation (n=3). HPH-150/200/250/300 MPa: juice samples treated at a pressure level of 150/200/250/300 MPa in the high-pressure homogenizer; HT: juice samples treated by thermal treatment of 71.1 °C for 3 seconds.

Few microbial strains could survive and grow in acidic juices, therefore, yeasts and molds, such as *Saccharomyces cerevisiae*, and *Lactobacilli* could be the dominant spoilage microorganisms that degrade the juice quality. The initial counts in the untreated and HPH-150 MPa treated grape juice samples were above 2 log CFU/mL. After storing for 4 weeks, visible mold was seen in untreated and HPH treated samples at the pressure level lower than 250 MPa. In the study of clear apple juice, HPH treatment lower than 200 MPa could only cause a less than 1 log reduction in total microbial count (36), wich was similar to our results. HPH-250 MPa treated samples were able to extend the microbial stablity for another 2 months, with mold visually present at 12 weeks. After storing for 15 weeks at 4 °C, thermally treated and HPH-300 MPa treated samples were still microbiologically safe. Similarly, the application of HPH at 250 and 300 MPa was able to maintain a minimum shelf-life of 8 weeks in clear apple juice and apple juice with pulp, respectively (36). Suárez-Jacobo also reported that HPH processed apple juice over 200 MPa was microbiologically safe for at least 2 months (37).

The physicochemical properties of treated Concord grape juice during storage are shown in Table 2.1.

Table 2. 1 Changes of physicochemical properties of treated Concord grape juice during a 15- week storage at 4 °C.

		Time (weeks)						
		0	**2**	**4**	**6**	**9**	**12**	**15**
Brix	**Control**	17.5 ± 0.1	17.6 ± 0.1	-	-	-	-	-
	HPH -150	17.5 ± 0.1	17.5 ± 0.1	-	-	-	-	-
	HPH -200	17.4 ± 0.1	17.5 ± 0.1	-	-	-	-	-
	HPH -250	17.4 ± 0.1	17.5 ± 0.2	17.5 ± 0.2	17.4 ± 0.2	17.5 ± 0.2	-	-
	HPH -300	17.3 ± 0.2	17.6 ± 0.1	17.5 ± 0.2	17.5 ± 0.1	17.6 ± 0.1	17.3 ± 0.2	17.0 ± 0.5 a
	HT	17.4 ± 0.2	17.7 ± 0.2	17.6 ± 0.1	17.4 ± 0.3	17.5 ± 0.1	17.2 ± 0.2	16.2 ± 0.4 b
pH	**Control**	3.05 ± 0.01 bA 3.06 ± 0.02	3.08 ± 0.01 abcA	-	-	-	-	-
	HPH -150	abA	3.11 ± 0.01 abA	-	-	-	-	-

		3.07 ± 0.01						
	HPH -200	abB	3.15 ± 0.01 aA	-	-	-	-	-
	HPH -250	3.05 ± 0.03 bA	3.11 ± 0.01 abA	3.05 ± 0.01 bA	3.05 ± 0.01 bA	3.04 ± 0.01 bA	-	-
	HPH -300	3.06 ± 0.02 abB	3.01 ± 0.01 bcAC	3.06 ± 0.01 bB	3.06 ± 0.01 bB	3.04 ± 0.01 aAB	2.97 ± 0.02 bD	2.99 ± 0.01 bCD
	HT	3.12 ± 0.01 aAC	3.05 ± 0.05 cC	3.16 ± 0.01 aAB	3.18 ± 0.02 aB	3.17 ± 0.02 aB	3.09 ± 0.01 aC	3.11 ± 0.01 aC
△E	**HPH -150**	0.20 ± 0.02	1.13 ± 0.4 a	-	-	-	-	-
	HPH -200	0.50 ± 0.25	0.63 ± 0.03 a	-	-	-	-	-
	HPH -250	0.56 ± 0.08 B	0.83 ± 0.09 aA	0.87 ± 0.10 A	0.76 ± 0.05 aAB	0.79 ± 0.05 bAB	-	-
	HPH -300	0.55 ± 0.26 A	0.65 ± 0.07 aA	0.70 ± 0.08 A	1.06 ± 0.19 aB	0.61 ± 0.07 bA	1.00 ± 0.10 A	0.60 ± 0.02 A
	HT	0.54 ± 0.18 B	0.43 ± 0.09 bB	0.70 ± 0.25 B	0.47 ± 0.13 bB	1.36 ± 0.12 aA	0.79 ± 0.04 B	0.61 ± 0.04 B

[a]Different lowercase letters indicate significant difference among treatments; different uppercase letters indicate significant difference during storage time. HPH-150/200/250/300 MPa: juice samples treated at a pressure level of 150/200/250/300 MPa in the high-pressure homogenizer; HT: juice samples treated by thermal treatment of 71.1 °C for 3 seconds.

Generally, no significant differences in soluble solid content, pH and color were found among juice samples with or without HPH and heat treatments on day 1, except the pH value in HT sample was significantly higher than the control and HPH-250 MPa samples. During storage, no significant changes were found in soluble solids content, except that the °Brix value in HT samples were significantly lower than HPP-300 MPa treated samples on 15-week, probably due to microbial growth or precipitation of tartrates. On the other hand, significant decrease in pH and increase in color change were observed at a few sampling points during storage. After storing for 15 weeks, both HPH-treated and heat-treated Concord grape juice samples did not show significant color changes compared to those values on day 1. In addition, the absolute color difference (ΔE) in all groups were below 2 (0.55 for HPP-300 MPa samples and 0.54 for HT samples), indicating that the color changes were not visible.

Similar changes were also found in other juices processed by HPH. In apple juice, stable pH/soluble solid content, negligible color changes were also found in HPH-150 MPa (3 passes, 20 °C) treated samples during 28 days refrigerated storage (33). In mango juice, no significant changes of pH, soluble solid content and titratable acidity were found immediately after HPH and HT treatment, while pH and soluble solid content decreased during storage at 4 °C, probably due to microbial growth (20). In mixed juice (apple, peach and carrot), no significant color changes were found in HPH-140 MPa (1 pass, 25 °C) treated samples (38). Considering the low pH, high soluble solids content and deep color, the changes in physicochemical properties were minor, suggesting HPH could be used as a non-thermal pasteurization method that preserves the juice characteristics.

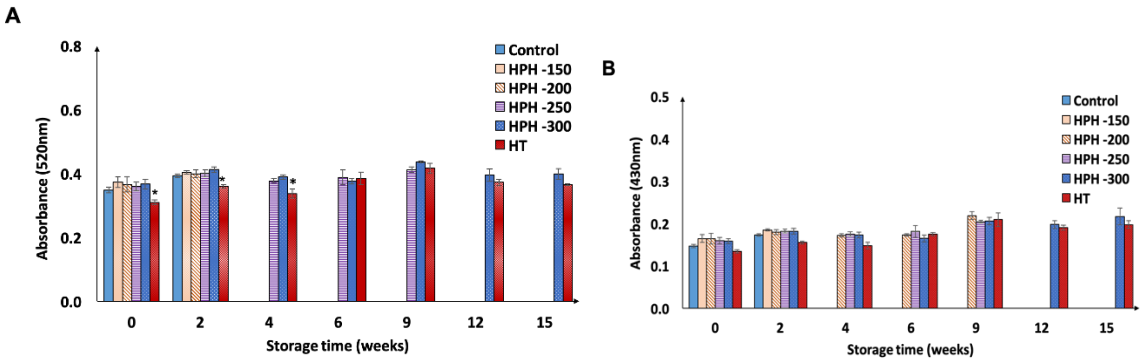

Figure 2. 4 Color changes of Concord grape juice during a 15- week storage at 4 °C: redness at 520 nm (A) and yellowness at 430 nm (B).

[a]Denotation of "*" indicates significant difference among treatments at each sampling point. Standard errors were calculated from technical replicates. HPH-150/200/250/300 MPa: juice samples treated at a pressure level of 150/200/250/300 MPa in the high-pressure homogenizer; HT: juice samples treated by thermal treatment of 71.1 °C for 3 seconds.

No significant changes in yellow color were observed within groups while significant lower redness color was observed in HT treated juice in the first 4 weeks (Figure 2.4). The decrease in redness color in HT samples was caused by the changes of anthocyanins, probably due to co-precipitation of anthocyanins with potassium bitartrate. During storage, for all groups, generally no significant changes of the diluted juice color measured at 520 nm were found, which agreed with the chromatic color changes (ΔE) presented in Table 2.1; while the values at 430 nm significantly increased during storage, probably due to the formation of brown pigment over time.

3.3 Effects of high pressure homogenization and heat on the inactivation of polyphenoloxidase (PPO)

High oxidative enzyme activities in fruit juices can cause quality deterioration. For example, polyphenoloxidase (PPO) can cause oxidative reactions, forming brown pigment in grape juice (39). The residual PPO activities after different treatments are shown in Figure 2.5.

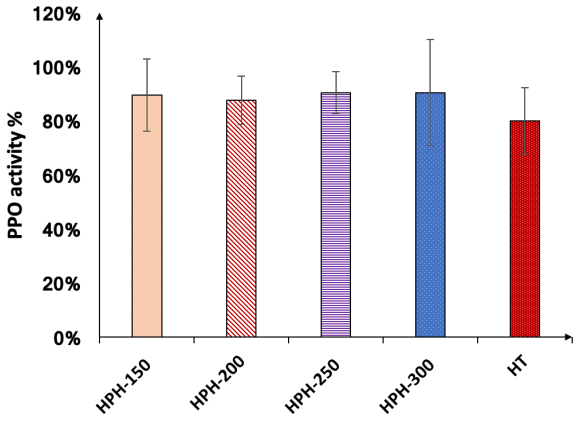

Figure 2. 5 Polyphenol oxidase activity of Concord grape juices after high pressure homogenization and heat treatment.

[a]HPH-150/200/250/300 MPa: juice samples treated at a pressure level of 150/200/250/300 MPa in the high-pressure homogenizer; HT: juice samples treated by thermal treatment of 71.1 °C for 3 seconds.

No significant changes were found among different groups, even though HT (71.1 °C, 3 sec) had the lowest value of PPO activity (80 ± 12%). High pressure homogenization caused about 10 % loss of PPO activity, with higher variances in 150 MPa and 300 MPa, showing no pressure dependence. It is reported that 40% residual PPO activity was founded in heat (90 °C, 1 min) treated grape juice. The heat treatment (71.1 °C, 3 sec) used in this study was intended to minimize the damage caused by heat while producing a safe, refrigerated juice for consumption, which was not effective in the reduction of PPO activity. It is reported that oxidase enzymes were completely inactivated in the crushed muscadine grape (red variety) after heating at 75 °C for 2 min (40). Moreover, hot press (50 °C, 1 hr) can also cause a partial reduction of PPO enzyme activity, leaving the more resistant type of oxidative enzymes, which might not be affected by the HPH and HT treatments. It is reported that heating grape (muscadine) PPO extract at 60 °C for 30 minutes could cause a loss of 70% PPO activity (41). In apple juice, the application of HPH (50-150 MPa, 1 pass, 8 °C) caused negligible reduction of PPO activity, while 50 % reduction was achieved by 10 passes of HPH treatment at 150 MPa (42). In another study of apple juice, residual PPO enzyme activity was not detected after HPH (300 MPa, 1 pass, 4 °C) (43). Contrarily, PPO activity in pear juice was elevated by 183 % after HPH (180 MPa, 1 pass, room temperature), and this increment was greater with the increase of pressure and passes (44). Similar increase of polyphenoloxidase and peroxidase activities after HPH was also reported in carrot juice (45). PPO from different plant sources has different resistance towards processing. Increase or decrease of enzyme activity after HPH was mainly attributed to the high pressure and forces (turbulence, shear stress, etc.) that caused changes in enzyme configuration (46).

3.4 Changes of total phenolics, total anthocyanins and antioxidant activity of Concord grape juice during 15-week storage under refrigeration at 4 °C

The retention of bioactive compounds (total phenolics and total anthocyanins) and antioxidant activities were investigated and compared to the heated juice samples during a 15-week shelf-life study (Figure 2.6).

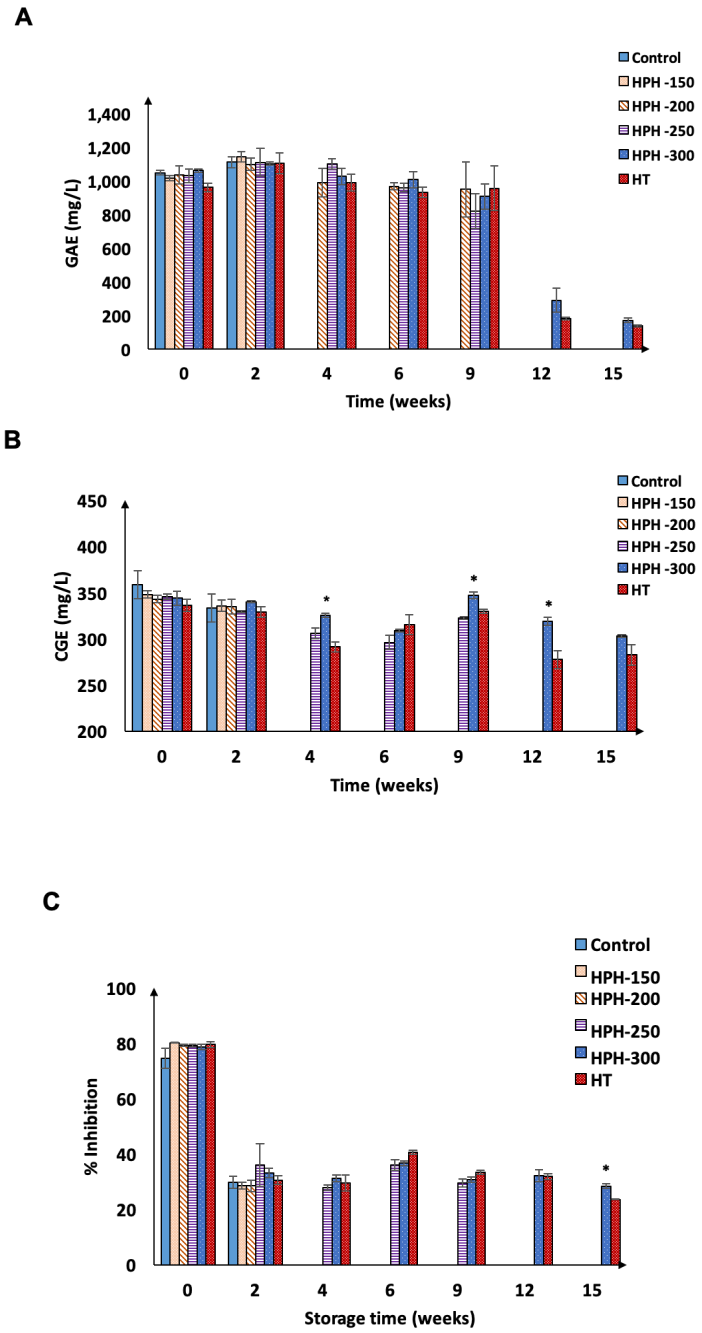

Figure 2. 6 Changes of total phenolics (A), total anthocyanins (B) and antioxidant activity (C) of Concord grape juices immediately after different treatments and during a storage of 15-week at 4 °C.

[a]Denotation of "*" indicates significant difference among treatments at each sampling point. HPH-150/200/250/300 MPa: juice samples treated at a pressure level of 150/200/250/300 MPa in the high-pressure homogenizer; HT: juice samples treated by thermal treatment of 71.1 °C for 3 seconds.

As shown in Figure 2.6A, no significant differences of total phenolics content were found among different treatments at each sampling point. In compliance with the total phenolics, no significant difference in antioxidant activity was found among different groups during storage, except for 15-week, at which HPP-300 MPa treated samples showed significantly higher antioxidant activity than HT samples (Figure 2.6C). Similar to our findings, HPH (100/200/300 MPa, 1 pass, 10/20 °C) treated orange juice did not differ significantly in total phenolics content and antioxidant activity compared to the fresh juice (47). He et al. also reported that HPP (250 MPa, 10 min) treatment did not alter the total phenolic content and phenolic bio accessibility in grape jucie (48). Guan et al. (20) reported that equal or higher total phenolics content, and higher antioxidant activity were found in HPH (190 MPa, 60 °C, 1 pass) compared to HT (90 °C, 5 min) treated mango juice samples immediately after process and during a 60-day storage at 4 °C. For total anthocyanins, the content was found significantly higher in HPP-300 MPa treated samples than in the HT samples at 4, 9 and 12 weeks storage (Figure 2.6B). During storage, generally, significant decreases of total phenolics, total anthocyanins and antioxidant activity were found in all groups despite some fluctuations due to sample variance. This decrease was probably attributed to the residual PPO enzyme activity (Figure 2.5). After storing for 15 weeks, HPP-300 MPa treated samples had similar total phenolics content and total anthocyanins content compared to HT samples, while antioxidant activity was significantly higher in HPP-300 MPa treated samples. The mild thermal treatment (71.1 °C, 3 sec) used in this study cause less detrimental impacts on quality parameters as reported in Mango juice (90 °C, 5 min) (20) and orange juice (90 °C, 1 min) (47), in which the authors reported that significantly higher retention of bioactive compounds and antioxidant activity were found in HPH treated samples than HT treated samples. Moreover, it is reported that wine produced from HPH treated must is more fruity and had better aroma (49), indicating the advantage of using HPH to preserve the flavor agents and aroma compounds in grape. Thus, it is promising to use high pressure homogenization to process a safe, refrigerated Concord grape juice with better retention of nutrients and fresh flavor compared to conventional high temperature pasteurization.

4. CONCLUSIONS

The results in this study indicated that the application of high pressure homogenization (300 MPa, single pass, 10 °C) was able to achieve a greater than 5-log reduction of *E. coli* ATCC 25922, the surrogate for pathogenic *E. coli* O157:H7, and a greater than 4-log reduction of a spoilage microorganism (*Saccharomyces cerevisiae*) in Concord grape juice. No significant changes of physicochemical properties, bioactive compounds, antioxidant activity and polyphenoloxidase enzyme activity were founded among untreated, HPH- and heat- (71.1 °C, 3 sec) treated juice samples. Pressure levels above 200 MPa were able to extend the shelf-life under refrigeration to 12 weeks. During storage, total phenolics, total anthocyanins and antioxidant activity significantly decreased in all HPH- and heat-treated samples, probably due to the high residual PPO enzyme activity. HPH-300 MPa treated samples were still microbiologically safe with acceptable quality parameters after 15-week storage, indicating its feasibility for processing Concord grape juice. Further studies on the changes of individual bioactive compounds and multi-pass HPH treatment on the juice safety, quality and oxidative enzyme activities, as well as a sensory study to compare the HPH- and HT- treated juices are needed to better understand the feasibility of using HPH to produce a fresher Concord grape juice.

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CHAPTER 3

EVALUATION OF PULSED ELECTRIC FIELD AND HIGH-PRESSURE PROCESSING ON THE OVERALL QUALITY OF REFRIGERATED CONCORD GRAPE JUICE PREPARED ON PILOT PLANT SCALE

ABSTRACT

To develop a nutritious, cold-pressed, refrigerated Concord grape juice, high pressure processing (HPP, 600 MPa, 3 min, 5 °C) and pulsed electric field (PEF, 1 kV/cm, 300 pulses) were utilized and the resultant juice was compared with the heat treated (HT, 63 °C, 3 min) juice. HPP was able to preserve the physicochemical properties, reduce microbial load and extend the shelf-life of juice under refrigeration. PEF pretreatment significantly increased the juice yield ($p<0.01$), antioxidants contents and antioxidant activity ($p<0.05$) in fresh-made juice sample. Macroscopic observation indicated that the skin cell structure was altered after PEF. Sensory evaluation showed that HPP treated juice with PEF pretreatment owned highest overall liking ratings and a significantly higher purchase intent ($p<0.05$) compared to HT treated juice. Combination of PEF and HPP can preserve the organoleptic and nutritional properties of refrigerated Concord grape juice while delivering better consumer acceptance.

1. INTRODUCTION

Concord grape is a commercially important grape variety. Among the various grape products, grape juice is a predominant product as it is easily accessible and can be consumed by people of all ages. In the United States, the majority of commercial red grape juices are produced from Concord grape (1). Concord grape juice is rich in polyphenol compounds, mainly flavonoids, which have equal or better antioxidant capacity than α-tocopherol in both in vivo and in vitro studies (2). In a study of 13 commercial fruit juices and fruit drinks, Concord grape juice possessed the highest total phenolic content, the most diverse identified individual phenolic compounds, with half of which had a concentration greater than 10 μmol/L (3). In addition, the health-benefits of Concord grape juice have been reported extensively, such as reducing the risk of cardiovascular disease (4,5), lowering blood pressure (6), improving neurocognitive function in human studies (7,8), and inhibiting mammary tumorigenesis in mouse study (9). The benefits of Concord grape juice are mainly attributed to the antioxidant activity of its phenolic compounds that can reduce the oxidative stress (10).

Processing techniques can affect the quality of fruit juices (11). Conventionally, juice industries utilize high temperature pasteurization (185-190 °F, 1 min) to inactivate pathogens and spoilage microorganisms. Additionally, hot press, enzyme addition, filtration, clarification and concentration are also common practices used in grape juice production, most of which involved the application of heat (1). However, loss of nutrients, aroma and other quality attributes induced by thermal processing leads to quality deterioration in fruit products (12–14). Anthocyanins can become brown pigments after heating which negatively impact the consumer acceptability (15). When heating red grape juice, the rate of anthocyanin degradation and polymeric color formation increased with temperature increase (70-90 °C) (16). Mounting demand for minimally processed,

fresh-like and nutritious fruit products has driven food manufacturers to opt for minimal processing methods (17,18). To this end, non-thermal technologies are promising alternatives to meet consumers' demand for minimally processed fruit juices possessing dense nutrients, fresh-like appearance and flavor (19).

Pulsed electric field (PEF) is a non-thermal technology that has been utilized on many food substances due to its short treatment time and great energy efficiency. Compared to traditional thermal processing, the advantages of PEF processing include: inactivation of microorganisms to ensure food safety at low temperature and thus having minor impacts on organoleptic and sensory qualities; induction of electroporation on fruit cells which leads to higher juice yield, higher juice color intensity and better leaching efficiency of bioactive compounds; inactivation of enzyme activity that cause juice quality deterioration (20, 21). PEF applications in grape studies have been mainly focused on promoting juice yield, enhancing the extraction efficiency of bioactive compounds and color pigments, and facilitating vinification by shortening the maceration time (21–28). Compared to the high electric field strength required for microbial inactivation (29–31), a field strength of 0.5-4 kV/cm is sufficient to induce electroporation on plant cells, enhancement of mass transfer, and extraction of functional compounds from grapes (24, 26, 32–34). It has been reported that PEF pretreated grape products showed greater phenolic extraction efficiency than the traditional enzyme assisted extraction methods, as well as other non-thermal technologies, such as high hydrostatic pressure processing, ultrasound and mild heat pretreatment (34, 35). PEF technology, in a word, is a theoretical and technologically feasible approach to extract more nutritive and pigmentary compounds from fruits (36). The implementation of PEF on Concord grape products has not been reported.

High pressure processing (HPP), on the other hand, is a widely studied non-thermal processing technology in food preservation. HPP can destruct the cells of pathogens and spoilage microorganisms at low temperature. High pressure (300 - 600 MPa) can deform cell structure and cause vegetative cell death due to membrane permeabilization (37). In a study of white grape juice, HPP (600 MPa, 3 min) treatment had equal microbial inactivation efficiency as thermal (90 °C, 1 min) treatment, and HPP treated juice was superior in showing less impacts on antioxidants, higher consumer acceptance while providing similar physicochemical properties as thermally treated sample (38). As for Concord grape juice, HPP treatment at 400 MPa for 2 min was sufficient to achieve a greater than 5-log pathogen (*Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes*) reduction (39). To the best of our knowledge, the effects of combining PEF pretreatment and HPP treatment on cold-pressed Concord grape juice have not been reported.

This study was aimed to evaluate the effects of PEF pretreatment and HPP treatment on the physicochemical properties, antioxidant contents, antioxidant activities, oxidative enzyme activities and sensorial attributes of cold-pressed Concord grape juice in comparison to thermally treated juice. Results from this study will provide knowledge on the processing methods and parameters for producing a minimally processed, fresh-like and nutritious Concord grape juice with extended shelf-life under refrigeration.

2. MATERIALS AND METHODS

2.1 Raw materials and chemicals

Fresh Concord grapes (*Vitis Labruscana*) were provided by Welch's Foods Inc. (Concord, MA, USA), which were harvested at finger lakes region, NY. Grapes were stored in a

refrigerated room at 4 ± 1 °C and processed into juice product within one week after harvest. All chemicals used in this study were of analytical grade.

2.2 Concord grape juice production, preservation and storage

Concord grape juice samples were prepared in the Cornell Food Venture Center pilot plant (Geneva, NY) according to the flow diagram depicted in Figure 3.1.

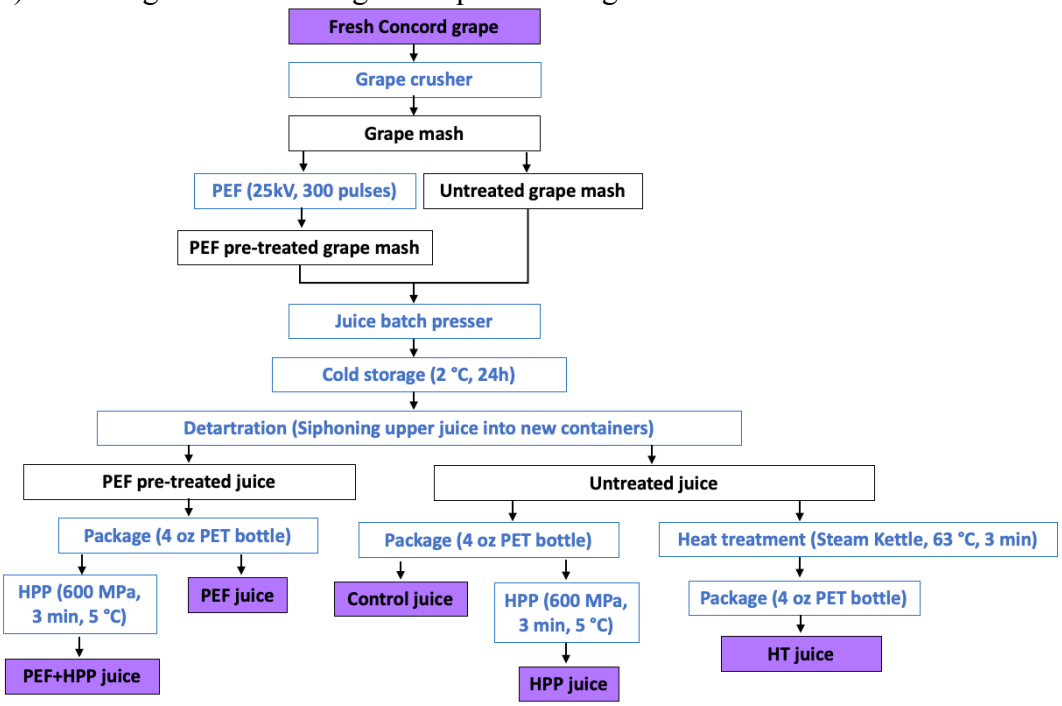

Figure 3. 1 Flow diagram for refrigerated, cold-pressed Concord grape juice.

Three batches of Concord grapes (11kg per batch) were crushed using a grape crusher (Berarducci Bro's, McKeesport, PA) to separate the stems. The grape mash was then packed into two layers of cheese cloth and pressed into juice using a hydraulic rack and frame presser (Orchard Equipment Co., Conway, MA). The press pressure was hold at 1400 psi for a few minutes until most juice was drained. For PEF pretreatment, the grape mash was treated using a lab-scale PEF Pilot^{TM} unit (Elea GambH, Quackenbruck, Germany) before pressing. The applied field strength of PEF treatment was 1 kV/cm. The pulse duration and frequency were 40 µs and 1 kHz respectively. PEF treatment was conducted on grape mash in triplicate at the voltage of 25 kV for 300 pulses, using a rectangular chamber {internal chamber size: 24 cm (L)×30.5 cm (W)× 22.5 cm (H)}. Sample temperature was below 27 °C after PEF treatment.

Juice samples were decanted to 32 oz glass jars (Ball, Broomfield, CO) and stored at 2 °C for 24 hours to accelerate the precipitation of potassium bitartrate. After cold storage, detartrated supernatant juice was siphoned into new containers using an electric pump (313 S, Watson-Marlow fluid technology group, MA). All samples were kept cold in ice-water during the preparation to minimize degradation.

For high pressure processing (HPP), HPP compatible PET bottles (Merrimack Valley Plastics, Methuen, MA) filled with juice samples were packed into PET bags and vacuum sealed. Sample packages were loaded into a 55 L commercial Hiperbaric 55 high pressure processing unit (Hiperbaric, Burgos, Spain) and cold water was used to transmit pressure. Juice samples were pressurized under 600 MPa for 3 min at 5 °C. HPP treated samples for sensory study were

prepared the same way except that an industrial Hiperbaric 525 HPP unit (Hiperbaric, Miami, FL) was used at LiDestri Food and Beverage Inc. (Rochester, NY) to meet the research requirements for sensory study.

For heat treatment (HT), juice samples were heated to 63 °C for 3 min in a 10-gallon stainless steel steam kettle (Orchard Equipment, Conway, MA) and then immediately cooled in an ice-water bucket. When temperature dropped below 38 °C, samples were filled into PET bottles and immediately closed tightly. The mild heat treat used in this study was intended to retain the fresh attributes while ensure the safety (40).

All samples (control, PEF, HPP, PEF+HPP and HT juice) were placed in a refrigerated room at 4 ± 1 °C for a shelf-life study up to 5 months and sampled at a 1-month interval for analyses.

2.3 Microbial count analyses

For total aerobic plate count (TPC) analysis, 1 mL Concord grape juice was directly pour-plated or serially diluted in 9 mL of peptone water and then pour-plated using plate count agar (TPA, CM0325, Oxoid) following by incubation at 30 °C for 48 to 72 h.

Yeast and molds (Y&M) counts were conducted using the same sample interval and preparation procedures expect that potato dextrose agar (PDA, Alpha Biosciences) was used for incubation. Tartaric acid was added in the PDA medium to adjust the pH to 3.5.

TPC and Y&M results were expressed as log Colony Forming Unit per mL (log CFU/mL).

2.4 Physicochemical properties analyses

Total soluble solids content (TSSC) was determined using a portable digital refractometer (model 300055, Sper scientific, Scottsdale, AZ) with automatic temperature compensation. Approximately 2 to 3 drops of grape juice were added on the prisma at ambient temperature to obtain readings and expressed as °Brix.

The value of pH was measured at ambient temperature using a pH meter (Orion 3 Star Series, Fisher Scientific, MA) after calibrating with pH 4 and 7 buffer.

Titratable acidity (TA) was determined according to Iland (41). Briefly, a diluted solution containing 5 mL of grape juice and 45 mL of distilled water was titrated with 0.1 mol/L sodium hydroxide to pH 8.2 using an autotitrator (Mettler compact G20, Mettler-Toledo, LLC, OH). Results were calculated based on equation (1) and expressed as g/L tartaric acid (gram tartaric acid equivalence per liter grape juice):

$$\text{Tartaric acid (g/L)} = \frac{\boldsymbol{V_{NaOH}}\,\mathbf{(L)}\times\mathbf{0.1\ (mol/L)}\times\mathbf{75(g/mol)}}{\boldsymbol{V_{juice}}\,\mathbf{(mL)}\times\mathbf{10^{-3}}} \qquad (1)$$

Where $\boldsymbol{V_{NaOH}}$ is the volume of titrant used until the endpoint was reached; 0.1 mol/L was the concentration of sodium hydroxide; 75 g/mol is the molar mass of titrating one carboxy group of tartaric acid; $\boldsymbol{V_{juice}}$ (mL) is the total juice volume used for titration.

Color attributes of grape juice were determined with the Hunter colorimeter (labscan XE, Hunter associates laboratory, Inc, VA) in reflection mode. Juice samples were poured into a 25 mL optical glass cuvette with 10 mm pathlength for measurement. The values of the absolute color difference of samples were calculated according to equation (2):

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \qquad (2)$$

where L_0, a_0, b_0 are the color measurements of fresh-made control juice samples, L, a, b are the color measurements of PEF, PEF+HPP, HPP, and HT treated juice samples.

The Browning index (BI) were analyzed according to Palou et al. (42) and calculated by equations (3) and (4):

$$BI = \frac{100\times(x-0.31)}{0.172} \quad (3)$$

$$x = \frac{a+1.75\times L}{5.645\times L+a-3.012\times b} \quad (4)$$

Color of Concord grape juice prepared immediately after different treatments were also determined using short pathlength (1 mm) quartz cuvettes in a Genesys UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). The visible red color was determined at the wavelength of 520 nm; color intensity was determined by the sum of absorbance at 420 nm, 520 nm and 620 nm; the browning index was determined by dividing the absorbance at 420 nm by the absorbance at 520 nm.

Grape juice yield for control and PEF treated samples was calculated as the ratio of resulting juice mass to grape mash mass (w/w, %). Each treatment was carried out in triplicate.

Microstructure observation was performed by horizontally cutting the grape from the 1/3 of top grape berries using a scalpel (Miltex kai, Integra LifeSciences, NJ) into a thin slice with a diameter of 8 mm and a thickness of 1 mm. Whole berries were also freeze dried and cut in half from the center afterwards to observe the visible structure changes. All sample preparations were done immediately after the PEF treatment. At least 10-30 grape berries were selected and compared for each treatment and observation.

2.5 Antioxidants content and in vitro antioxidant activity

2.5.1 Extraction

Extraction procedure was developed based on the studies of Jensen et al. (43) and Leong et al. (28). Grape juice sample (1 mL) was mixed with acidified methanol (1% HCl, v/v) at a 1:1 ratio. After vortexing for 30 seconds, sample tubes were incubated in a 40 °C water bath for 30 min. After centrifugation (10,000 rpm, 5 min), the supernatant was used as extract for further analyses.

2.5.2 Total phenolic content (TP)

TP was determined by Folin-Ciocalteau colorimetric assay according to Waterhouse (44) with minor modifications. Generally, 20 µL of juice extracts were mixed with 1,580 µL DI water and 100 µL Folin-Ciocalteau reagent (Sigma-Aldrich St. Louis, MO), then the mixture was incubated at room temperature for 6 min. Then 300 µL of 20% sodium carbonate solution was added and mixture was incubated at room temperature for 2 hours under dark. Absorbance was measured at 765 nm using the UV-visible Spectrophotometer. Gallic Acid (Chem-Impex international Inc., IL) was used to prepare the standard curve from 0 to 500 mg/L. Results were expressed as mg gallic acid equivalents (GAE) per liter grape juice extract (mg/L).

2.5.3 Total monomeric anthocyanin content (TMA)

TMA was determined by the method described by Lee et al. (45). Briefly, 200 µL extract were diluted 5 to 10-fold by pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium

acetate) buffers respectively. Results were calculated according to equation (5) and expressed as mg cyanidin-3-glucoside (cyd-3-glu) equivalents (CGE) per liter of grape juice extract(mg/L):

$$\text{TMA } (CGE, mg/L) = \frac{A \times M_W \times DF \times 10^3}{\varepsilon \times L} \quad (5)$$

where $A = (A_{520nm} - A_{700nm})pH_{1.0} - (A_{520nm} - A_{700nm})pH_{4.5}$; M_W, molecular weight of cyd-3-glu = 449.2 g/mol; DF (dilution factor) =5 to 10; ε is the molar extinction coefficient = 26,900 $L^{-1} \times cm^{-1} \times mol^{-1}$ for cyd-3-glu; L (pathlength) = 1 cm; 10^3 is the conversion of g to mg.

2.5.4 In vitro antioxidant activity (free radical scavenging activity by DPPH and ABTS)

DPPH$^{\bullet}$ and ABTS$^{\bullet}$ free radical scavenging activities were carried out respectively according to the description of Brand-Williams et al. (46) and Re et al. (47) with some modifications. Briefly, DPPH (1,1-diphenyl-2-picrylhydrazyl radical, Tokyo Chemical Industry Co., Ltd, TCI America, OR) was dissolved at a concentration of 0.2 mM in methanol, then the absorbance was adjusted to 0.900 ± 0.050 at 517 nm before test. A stock solution of 7 mM ABTS [2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt] (Sigma-Aldrich Co., Ltd, Dorset, UK) was prepared in methanol. Then potassium persulfate powder (Honeywell Fluka, NC) was added to the ABTS stock solution to reach a final concentration of 2.45 mM. The mixture was stored under dark and at room temperature for 12-16 hours to generate stable radicals. The absorbance of ABTS$^{\bullet}$ solution was adjusted to 0.700 ± 0.050 at 734 nm before using. The juice samples were mixed with methanol at a 1:1 ratio and centrifuged at 10,000 rpm for 5 min using a benchtop centrifuge (Heraeus Biofuge pico, NC). A portion of supernatant (100 µL) was mixed with 900 µL DPPH$^{\bullet}$ solution and equilibrated at room temperature under dark environment for 30 min. For ABTS assay, 100 µL supernatant was mixed with 2mL ABTS$^{\bullet}$ solution and set at room temperature under dark for 6 min. Blank was prepared using methanol. Absorbance was measured at 517 nm for DPPH assay and 734 nm for ABTS assay using the spectrophotometer. Radical scavenging capacity was calculated as the percentage inhibition (%) according to equation (6):

$$\boldsymbol{DPPH\ or\ ABTS\ \%\ inhibition} = \frac{(\boldsymbol{A_{control} - A_{sample}})}{\boldsymbol{A_{control}}} * 100 \quad (6)$$

where $\boldsymbol{A_{control}}$ is the absorbance of blank, $\boldsymbol{A_{sample}}$ is the absorbance of sample. Higher values of % inhibition indicates greater antioxidant activity.

2.5.5 Analysis of polyphenoloxidase (PPO) and peroxidase (POD) activity

PPO and POD enzyme extraction and assays were carried out according to Garcia-Palazon et al. (48) with some modifications. All chemicals were purchased from VWR International, LLC., PA, except for Triton-100 (Electron microscopy sciences, PA) and catecheol (Tokyo Chemical Industry Co., Ltd, TCI America, OR). Enzyme extraction buffer was prepared by mixing 4% (w/v) poly(vinylpolypyrrolidone) (PVPP), 1% (v/v) Triton X-100 and 1M NaCl with 0.2 mol/L sodium phosphate buffer (pH 6.5). Equal aliquot (4.5 mL) of extraction buffer and juice samples were mixed vigorously and homogenized for 3 min on ice. Then the mixture was centrifuged at 10,000 rpm for 30 min at 4 °C (Centrifuge 5810R, Eppendorf, CT). The supernatant was collected and used as the crude enzyme extract for the PPO and POD assays.

To start the PPO assay, 500 µL of crude enzyme extract was mixed with 3 mL 0.07 M catechol prepared in 0.05 M sodium phosphate buffer (pH 6.5). The absorbance was monitored

at 420 nm every 30 seconds for 3 min. Blank was prepared the same way except that DI water was used instead of enzyme extract.

For the POD assay, 200 μL of crude enzyme extract was mixed with 1.5 mL 0.05 M sodium phosphate buffer (pH 6.5) and 200 μL 10 g/L p-phenylenediamine in 0.05 M sodium phosphate buffer (pH 6.5). Hydrogen peroxide solution (200 μL; 1.5%, w/v) was added into the mixture to start the reaction. Readings at 485 nm was recorded at 30 seconds interval for 3 min.

Both PPO and POD results were shown as the residual activity (RA) in % according to equation (7):

$$\boldsymbol{RA}(\%) = \frac{\boldsymbol{A}}{\boldsymbol{A_0}} * 100 \quad (6)$$

where A and A_0 were the enzyme activity of treated and control samples, respectively.

2.6 Sensory study of HPP, PEF+HPP and HT treated juice

A total of 101 untrained panelists were recruited to participate in the sensory study of 3 different samples (HPP, PEF+HPP and HT treated Concord grape juice). The fresh-prepared juice samples were stored at 4 °C before testing at the sensory evaluation center (Department of Food Science, Cornell University, NY). Samples were randomly coded with 3 digits. Panelists were asked to assess the consumer liking of appearance, aroma, flavor and overall liking using a 9-point hedonic scale rating (1 = dislike it extremely, 9 = like it extremely); intensity of sweetness, sourness, bitterness, flavor, authenticity to Concord grape (1 = not at all, 5 = very) and purchase intent (1 = definitely would not, 2 = probably would not, 3= may or may not, 4 = probably would, 5 = definitely would) on a 5-point scale; color preference on a "just about right" (JAR) question (1 = too dark, 2 = moderate dark, 3 = just about right, 4 = moderate light, 5 = too light) and finalizing with product ranking test.

The sensory evaluation was conducted under approval and requirements from the Cornell's Sensory Evaluation Center and Cornell University Institutional Review Board (IRB) protocols in respect to juice study for human consumption. All samples were served based on a randomized order and under the procedure guidance provided by the Sensory Evaluation Center. Data were collected by the REDJADE® Sensory Software (RedJade Software Solutions, LLC, Redwood Shores, CA).

2.7 Statistical analysis

All trials were performed in triplicate. Data were presented as mean ± standard deviation (SD). Data were analyzed using student t- test or one-way ANOVA as appropriate at a significance level of $p < 0.05$. Significant differences among mean values were determined by the Tukey's post hoc test following the one-way ANOVA test using SPSS (SPSS statistics, version 22.0, IBM, NY). Correlation was determined by Person's test in SPSS.

3. **RESULTS AND DISCUSSION**

3.1. Microbial stability during 5-month refrigerated storage

Initial total aerobic plate count (TPC) and yeast & molds count (Y&M) in freshly made control grape juice were 6.2 log CFU/mL and 6.1 log CFU/mL respectively (Figure 3.2). As shown in Figure 3.2, TPC and Y&M counts declined by less than 1 log CFU/mL after PEF treatment. The slight

microbial reduction in PEF juice was as expected. Stronger imposed intensity of the PEF treatment, such as higher electric-field strength and longer treatment time, or in combination with antimicrobial factors, could contribute to a better efficacy of microbial inactivation (49–51). According to a previous study, high PEF field strength (20 to 35 kV/cm) with different treatment time and pulse frequency were able to achieve a 0.5 to 4 log count reduction of yeast and bacteria present in grape juice (31). As the field strength applied in this study was 1 kV/cm, the impact of PEF treatment on grape samples was mainly reflected on facilitating juice extraction instead of microbial reduction.

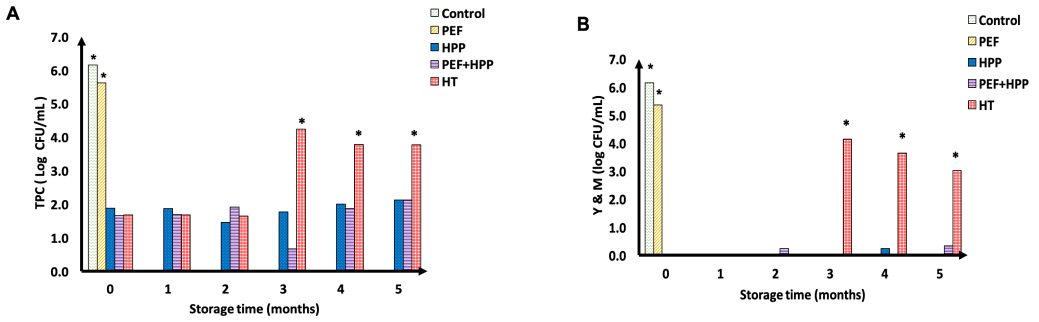

Figure 3. 2 Total aerobic plate count (TPC) (A) and yeast & molds count (Y&M) (B) changes during 5-month refrigerated storage.

[a]Denotation of "*" indicate significant differences among treatments at each sampling point ($p<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

HPP (600 MPa, 3 min, 5 °C) and HT (63 °C, 3 min) treatments were able to reduce the TPC count respectively to 1.9 and 1.7 log CFU/mL immediately after processing. There was no significant difference in microbial counts between HPP and PEF+HPP treated samples, indicating that HPP and PEF treatments did not work synergistically to inactivate microorganisms. Petrus et al. (39) reported that HPP treatment at 400 MPa for 2 min was able to achieve a larger than 5-log reduction of pathogens (cocktail of *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes*) in Concord grape juice. Therefore, the HPP (600 MPa, 3 min, 5 °C) treatment used in this study was able to meet the FDA's juice HACCP regulation of 5-log reduction of pertinent pathogen. It was also reported that 600 MPa was able to extend shelf-life and maintain quality of white grape juice compared to thermal treatment (90 °C, 1 min) (38).

During 5-month refrigerated storage, TPC and Y&M counts remained at the same level in both HPP and PEF+HPP treated samples, while these values in the HT treated samples started to increase in the third month and was significantly higher than the values in HPP treated samples from the third to the fifth month. To be specific, after 5-month storage, the Y&M counts in HPP and PEF+HPP treated samples were still below the detection limit (1 log CFU/mL), however the counts in HT treated samples was about 3.0 log CFU/mL. These results indicate that HPP (600 MPa, 3 min, 5 °C) is an effective preservation method in producing Concord grape juice with a shelf-life of at least 5 months at 4 ° C. The equivalent mild heat treatment (63 °C, 3 min) used in this study was not as effective as the HPP treatment to achieve the same shelf-life length, probably due to post process contamination during bottling, which is eliminated in the HPP treated juice.

3.2. Juice physicochemical properties

3.2.1 pH, total soluble solids content (TSSC) and titratable acidity (TA) of Concord grape juice during refrigerated storage.

Untreated grape juice had a TSSC value of 18.1 ± 0.1 °Brix, TA of 6.6 ± 0.1 g/L tartaric acid, pH of 3.19 ±0.01. The TSSC and TA values in PEF (18.6 ± 0.1 °Brix, 7.6 ± 0.1 g/L tartaric acid) and PEF+HPP (18.7 ± 0.1 °Brix, 7.4 ± 0.1 g/L tartaric acid) treated samples were significantly increased compared to that of control juice on day 1 (Table 3.1). Electric fields affected the grape cell membrane integrity and thus promoted cellular components leaching into juice during pressing (52). There was no significant difference between HPP and HT treated samples in terms of TSSC and TA values on day 1. During refrigerated storage, significant changes (lower TSSC and TA values, higher pH values) in HT treated samples were observed compared to other groups starting from the third month. After 5-month storage, pH values in all samples increased significantly. The pH increase was probably caused by the growth of spoilage microorganisms.

Table 3. 1 Physico-chemical changes of Concord grape juice during 5-month refrigerated storage.

		Storage time (months)					
		0	**1**	**2**	**3**	**4**	**5**
Total Soluble solids (°Brix)	**Control**	18.1 ± 0.1 b	-	-	-	-	-
	PEF	18.6 ± 0.1 a	-	-	-	-	-
	HPP	18.3 ± 0.1 abX	18.3 ± 0.1 a	18.2 ± 0.2 b	18.6 ± 0.1 a	18.6 ± 0.1 ab	18.6 ± 0.1 b
	HPP+PEF	18.7 ± 0.1 aX	18.2 ± 0.3 aX	18.7 ± 0.1 aXY	18.8 ± 0.1 aY	18.7 ± 0.2 aXY	18.9 ± 0.1 aY
	HT	18.4 ± 0.1 abXZ	18.0 ± 0.1 aXY	18.3 ± 0.0 abXYZ	18.0 ± 0.2 bY	18.4 ± 0.1 bXYZ	18.5 ± 0.1 bZ
Titratable acidity (g/L)	**Control**	6.6 ± 0.1 b	-	-	-	-	-
	PEF	7.6 ± 0.1 a	-	-	-	-	-
	HPP	6.7 ± 0.1 b	6.9 ± 0.2 a	7.1 ± 0.2 ab	7.0 ± 0.1 a	7.0 ± 0.1 a	6.7 ± 0.2 a
	HPP+PEF	7.4 ± 0.1 a	7.7 ± 0.1 b	7.6 ± 0.3 a	7.9 ± 0.2 b	7.6 ± 0.1 b	7.4 ± 0.2 b
	HT	6.6 ± 0.1 bXY	6.8 ± 0.1 aX	6.9 ± 0.1 bX	7.2 ± 0.1 aW	6.5 ± 0.1 cYZ	6.4 ± 0.1 aZ
pH	**Control**	3.19 ± 0.01 b	-	-	-	-	-
	PEF	3.17 ± 0.01 b	-	-	-	-	-
	HPP	3.19 ± 0.01 bW	3.16 ± 0.02 bW	3.25 ± 0.01 bX	3.41 ± 0.01 aY	3.54 ± 0.01 aZ	3.52 ± 0.01bZ
	HPP+PEF	3.17 ± 0.01 bW	3.23 ± 0.01 aX	3.23 ± 0.01 bX	3.38 ± 0.01 bY	3.49 ± 0.01 bZ	3.53 ± 0.01bZ
	HT	3.23 ± 0.01 aW	3.21 ± 0.02 abW	3.30 ± 0.01 aX	3.43 ± 0.01 aY	3.53 ± 0.01 aZ	3.59 ± 0.01aZ
Brix/ TA	**Control**	2.7 ± 0.1 b	-	-	-	-	-

PEF	2.4 ± 0.1 a	-	-	-	-	-
HPP	2.8 ± 0.1 bXY	2.6 ± 0.1 aX	2.6 ± 0.1 aX	2.7 ± 0.1 aXY	2.7 ± 0.1 aXY	2.8 ± 0.1 aY
HPP+PEF	2.5 ± 0.1 a	2.4 ± 0.1 b	2.4 ± 0.1 b	2.4 ± 0.1 b	2.4 ± 0.1 b	2.5 ± 0.1 b
HT	2.8 ± 0.1 bY	2.7 ± 0.1 aX	2.7 ± 0.1 aX	2.5 ± 0.1 cW	2.8 ± 0.1 cYZ	2.9 ± 0.0 aZ

[a]Different lowercase letters indicate significant differences among treatments at each sampling point; different uppercase letters indicate significant differences during storage for each treatment ($p<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

The °Brix/TA ratio is an important indicator for the balanced taste of sweetness and sourness in fruit products. This value could also be used to predict the volatile compositions which represented the "foxy" or "grapey" character of Concord grape, and more importantly, to predict the consumer acceptability and the viability of product on the market (53,54). As shown in Table 3.1, the values of °Brix/TA in HPP (2.8) and HT (2.8) were similar to the control (2.7), indicating that HPP did not negatively affected the flavor of Concord grape juice compared to fresh juice or thermally treated juice. PEF pretreated samples had the highest titratable acidity and lowest ° Brix / TA ratio (2.4 for PEF and 2.5 for PEF+HPP), which could indicate the presence of more trans-2-hexenal (herbaceous aroma) in PEF treated samples as TA and °Brix/TA ratio are good indicators of volatile compounds in Concord grape juice (54).

3.2.2. Effect of PEF pretreatment on juice yield, visible color and macro-structure.

Immediately after pressing, grape juice yields in control and PEF treated groups were 61±2 % and 76±2 %, respectively. The yield increments of 15 % was significant at $p<0.01$. PEF treatment can cause electroporation and induce mass transfer which result in increased juice yield. This phenomenon is also reported in other grape varieties, such as Pinot Noir (5%) (28) Chardonnay (8%) (25), Muscadelle, Sauvignon and Semillon (24%-27%) (26).

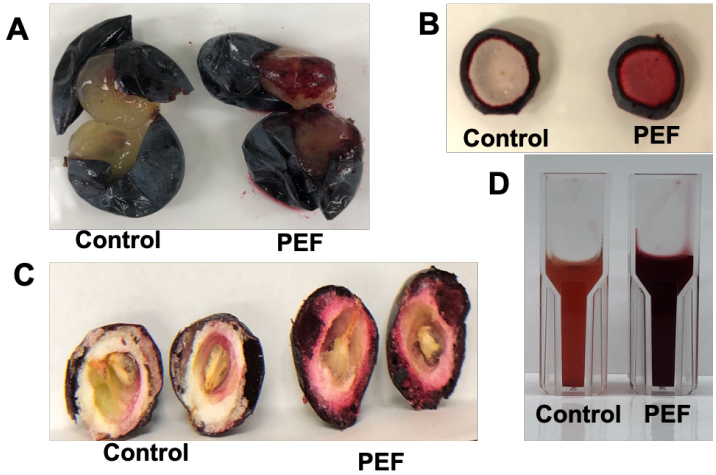

Figure 3. 3 Visual observation of control and PEF (1 kV/cm, 300 pulses) treated Concord grape berries after crushing (A), fresh sample cross section (B), freeze dried sample center cross section (C) and grape juice (D).

PEF treatment at 1 kV/cm for 300 pulses promoted the anthocyanin release from the skin cells compared to the untreated ones (Figure 3.3). After crushing the grapes, part of the hypodermis cells that are adjacent to the pulp can be easily separated from skin in PEF treated berry (Figure 3.3A), indicating a PEF induced electroporation impact on the inner layer of skin. PEF treatment prompted anthocyanin release from the skin and thus the color of the pulp was stained red while the control pulp remained green and transparent (Figure 3.3B). Freeze drying is utilized to fix the structure changes as the berries are very soft and it is difficult to observe intact and uniform cross sections from the center where the seeds were located. After freeze drying, similar red color diffusion and transmission in the inner hypodermis tissues were also observed (Figure 3.3C). As the inner hypodermis cells are the largest cells of the hypodermis, higher transmembrane potentials are posed on this layer in the electrical field (55). The histological changes, especially for the inner hypodermis, induced by PEF treatment (0.7 kV, 200 ms) in Cabernet Sauvignon grapes have been studied using light microscopy (LM) and transmission electron microscopy (TEM) (33,56). Our observations in the Concord grape variety supported previous findings. Additionally, we provided a direct and macroscopic proof of changes induced by PEF treatment, which is an easy, fast and environmentally friendly process.

3.2.3 Color changes of Concord grape juice treated by different methods.

PEF pretreated samples had the highest ΔE value on day 1 (Table 3.2). This color change was caused by the significantly lower a*, b* and L* values compared to freshly made control sample. With more anthocyanins leaking to the final juice of PEF pretreated samples, a darker (lower L*) grape juice with more red (higher a*) and blue (lower b*) color were expected. The colorimeter results were as expected except for the a* value changes. The reason for this disagreement could be the lower sensitivity of Hunter LAB analysis when detecting the grape juice sample with intense dark blue purplish color, compare to the spectrophotometry method using the short pathlength cuvette (1 mm) (Figure 3.4). HPP treated samples had similar a* value and browning index (BI) in comparison to that in the untreated sample. During the refrigerated storage of 5 months, the values of L*, a*, b*, ΔE, and BI in all treatments did not show significant changes, except that the a* and ΔE values had some fluctuations at a few sampling points. These a* and ΔE value changes were insignificant when comparing to the day 1 and 5-month samples.

Table 3. 2 Color changes of grape juice treated by different methods during refrigerated storage at 4 °C.

		Storage time (months)					
		0	1	2	3	4	5
L*	Control	15.4 ± 0.1 b	-	-	-	-	-
	PEF	5.9 ± 0.1 c	-	-	-	-	-
	HPP	18.1 ± 0.5 a	21.0 ± 1.4 a	19.9 ± 0.5 a	18.7 ± 1.1 a	19.9 ± 1.2 a	18.5 ± 3.4 a
	PEF+HPP	7.4 ± 0.4 c	7.6 ± 0.8 b	7.8 ± 0.2 c	7.1 ± 0.3 b	7.4 ± 0.2 c	7.0 ± 0.1 b

	HT	15.0 ± 0.6 b	17.9 ± 2.1 a	15.9 ± 0.2 b	17.0 ± 0.5 a	16.3 ± 0.1 b	16.3 ± 2.4 a
a*	Control	24.9 ± 0.1 a	-	-	-	-	-
	PEF	16.2 ± 0.1 c	-	-	-	-	-
	HPP	24.7 ± 0.3 a	26.5 ± 0.4 a	26.3 ± 0.2 a	24.8 ± 0.4 a	24.7 ± 1.3 a	22.9 ± 4.8 a
	PEF+HPP	15.2 ± 0.7 c	13.9 ± 1.1 b	15.3 ± 2.1 c	11.4 ± 0.8 c	13.3 ± 0.9 c	13.1 ± 1.5 b
	HT	20.6 ± 0.9 b	25.0 ± 2.6 a	20.5 ± 0.1 b	19.5 ± 1.3 b	21.0 ± 0.4 b	20.2 ± 3.2 a
b*	Control	20.0 ± 0.1 b	-	-	-	-	-
	PEF	3.5 ± 0.1 e	-	-	-	-	-
	HPP	23.2 ± 0.7 a	27.4 ± 2.1 a	26.0 ± 0.8 a	23.6 ± 1.7 a	23.2 ± 2.2 a	21.8 ± 7.4 a
	PEF+HPP	5.4 ± 0.8 d	4.3 ± 0.6 b	4.4 ± 1.2 c	3.6 ± 0.1 c	4.3 ± 0.3 c	4.5 ± 0.4 b
	HT	15.8 ± 0.7 c	22.6 ± 5.0 a	16.6 ± 0.1 b	16.0 ± 2.4 b	17.1 ± 0.5 b	18.5 ± 5.5 a
△E	Control	-	-	-	-	-	-
	PEF	20.9 ± 0.1 a	-	-	-	-	-
	HPP	4.3 ± 0.4 b	9.4 ± 2.5 b	7.7 ± 1.0 b	4.9 ± 2.0 b	5.9 ± 1.2 b	8.6 ± 1.6 b
	PEF+HPP	19.2 ± 1.1 a	20.7 ± 1.1 a	19.8 ± 1.9 a	22.8 ± 0.4 a	21.1 ± 0.7 a	21.2 ± 1.2 a
	HT	6.0 ± 1.1 b	6.0 ± 1.7 b	5.5 ± 0.1 b	6.9 ± 2.4 b	4.9 ± 0.5 b	7.5 ± 1.1 b
BI	Control	400 ± 0 a	-	-	-	-	-
	PEF	220 ± 0 c	-	-	-	-	-
	HPP	390 ± 40 a	410 ± 10 a	410 ± 0 a	380 ± 10 a	340 ± 70 a	340 ± 90 a
	PEF+HPP	220 ± 20 c	180 ± 10 b	190 ± 40 c	160 ± 10 c	180 ± 10 b	200 ± 20 b
	HT	290 ± 10 b	390 ± 70 a	290 ± 10 b	250 ± 50 b	290 ± 10 a	320 ± 80 a

[a]Different letters indicate significant differences among groups at each sampling point ($p<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

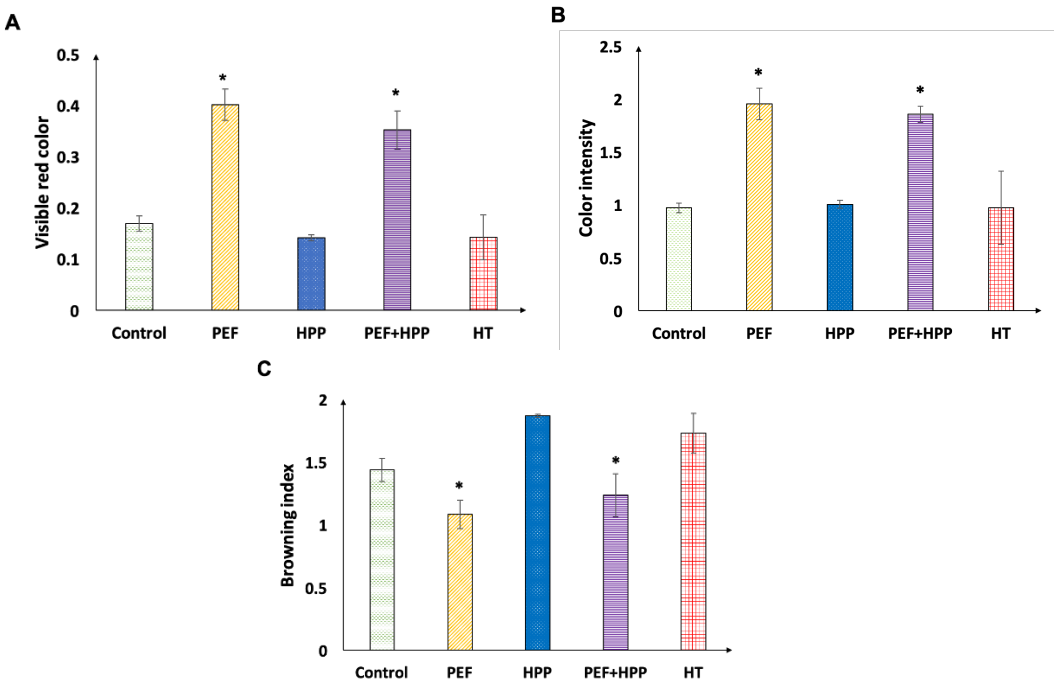

Figure 3. 4 Visible red color (A), color intensity (B) and browning index (C) of Concord grape juice treated by different methods.

[a]Denotation of "*" indicates significant difference among treatments ($p<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

As the color of Concord grape juice is very dark and intense, spectrophotometry method was also utilized to better illustrate the color differences of the Concord grape juice immediately after treatment. The visible red color (Figure 3.4A) and color intensity (Figure 3.4B) in PEF pretreated samples were significantly increased by 100% compared to samples without PEF pretreatment. Darra et al. (57) also reported that PEF treatment (5 kV/cm, 1 ms) could enhance the color intensity by 75% in Cabernet Franc and 68% in Cabernet Sauvignon. Leong et al. (28) observed that the color intensity in the PEF (1.5 kV/cm, 234 and 1033 pulses) treated juice was significantly higher than the untreated grape juice sample during the first 8 days of maceration. The differences in BI were generally in accordance with the results showed in Table 3.2. In the current study, medium PEF (1 kV/cm, 300 pulses) pretreatment was sufficient to induce electroporation and increase membrane permeability on grape skin cells, which prompted the release of anthocyanin pigments from grape skin into juice, with less time and operation cost than the traditional juice and wine production.

3.3. Changes in total phenolic content (TP) and total monomeric anthocyanin (TMA) content

On day 1, PEF treatment significantly increased the TP values in PEF treated and PEF+HPP treated samples to 660 ± 80 mg/L and 750 ± 50 mg/L as gallic acid equivalents (GAE) respectively, which were almost doubled compared to 290 ± 10 mg/ L as GAE in control juice sample (Figure 3.5A). Therefore, PEF pretreatment was able to produce a nutritive cold-pressed grape juice with high phenolic content. The anthocyanin content in PEF+HPP treated sample was the highest (73 ± 22 mg/L as cyanidin-3-glucoside equivalents, CGE) on day 1 and during storage (Figure 3.5B). TP and TMA

values in HPP and HT samples had similar values on day 1 and during storage. Similar to our findings, PEF treatment (1.5 kV/cm, 243 to 1033 pulses) could increase the amounts of major anthocyanins and total phenolics in Pinot Noir grape juice, which consequently enhanced the DPPH scavenging activity and bioprotective capacity on the cellular level (28). The increment of TP and TMA values in PEF treated samples was due to the increased cell membrane permeability that allowed more polyphenolic compounds, which approximate 28-35% of them were located in the skin and 10% or less in the pulp (58), leaching into the juice (59,60).

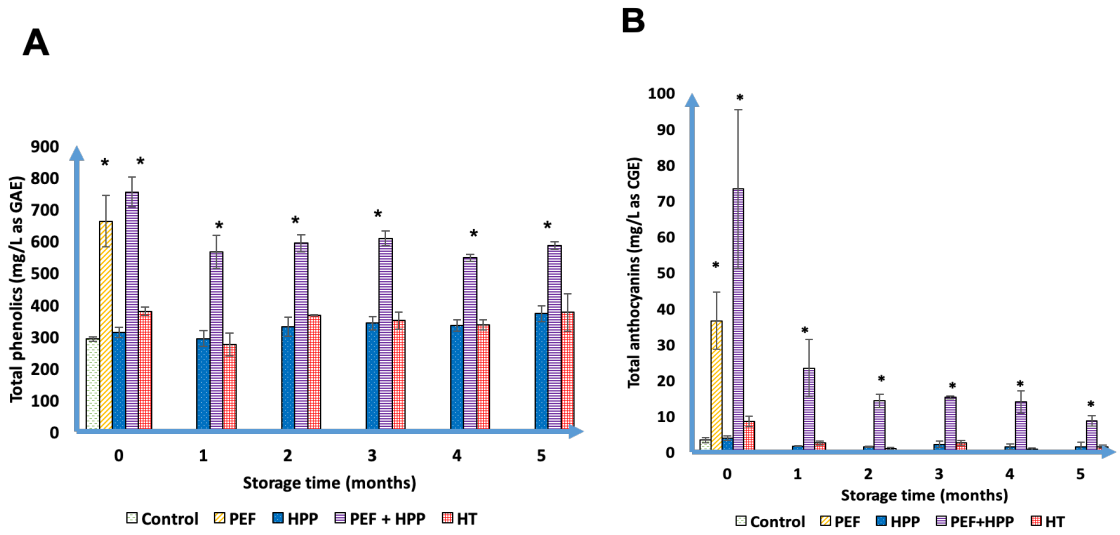

Figure 3. 5 Changes in total phenolic content (A), total monomeric anthocyanins content (B) of Concord juice during 5-month storage at 4 ° C.

[a]Denotation of "*" indicate significant difference among treatments at each sampling point ($P<0.05$). GAE: gallic acid equivalents; CGE: cyanidin-3-glucoside equivalents; PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

During refrigerated storage, the TP and TMA values of all juice samples were decreasing, except for the TP values in HT treated samples, which could be attributed to the inactivation of PPO and POD enzyme activity during thermal treatment (Section 3.5). The TP and TMA values in PEF+HPP treated samples were significantly higher than that in HPP and HT treated samples at each sampling point. Although anthocyanin content of all treated juice samples degraded significantly during storage, PEF+HPP treated juice still showed higher TMA values (8.6 ± 1.5 mg/L as CGE) after 5-month refrigerated storage compared to the freshly made control juice (3.3 ± 0.7 mg/L as CGE). Moreover, after storing for 5 months, TP in PEF+HPP juice was 54% higher than that in the freshly made HT juice, while the TMA content was similar. These results suggested that the combination of PEF and HPP treatment provide a more nutritive, cold pressed, grape juice product compared to heat treatment even after 5-month storage at 4°C.

3.4. In vitro total antioxidant activity of Concord grape juice

Antioxidant activity is one of the most valuable attributes of grape juice products because of the high level of phenolic compounds (61–64). It has been reported that the total antioxidant activity values differ depending on the methods utilized, thus different analyses are recommended to evaluate the best proximity of antioxidant activity (65). DPPH and ABTS assays were applied in this study to evaluate the in vitro total antioxidant activities and shown as the percentage inhibition of free radicals in Figure 3.6. The percentage inhibition of free radicals on day 1 ranged from 31% to 73%, which is similar to the values reported in the PEF treated Pinot Noir juice (from 49% to 74%) (28) and three other common grape varieties (ranging from 20% to 80%) (66). Generally, PEF treatments significantly affected the antiradical ability of grape juice samples determined both by the DPPH and ABTS assays ($p<0.05$). On day 1, PEF+HPP treated juice had 32% higher antiradical activity than HT juice determined by DPPH assay. For ABTS assay, the increment in antiradical activity for PEF+HPP treated juice was 125% compared to the control juice, and 68% compared to HT juice. Pressure and thermal treatment utilized in this study did not significantly affect the antioxidant activity of the juice samples in comparison to the control juice. The significant increment in antioxidant activity of the PEF treated samples is mainly caused by the improved mass transfer, which prompted the infusion of bioactive compounds into the juice (34). During storage, there were some fluctuations of the percentage inhibition values due to sample variation, test sensitivity and microbial contamination. However, there were no significant changes in the antioxidant activity, especially when comparing the day 1 and 5-month juice samples.

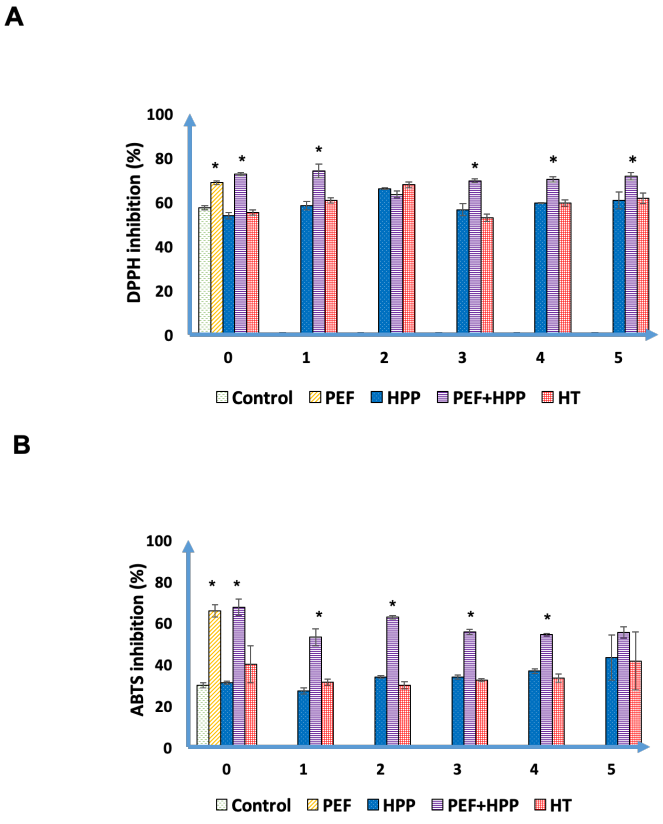

Figure 3. 6 Changes in total antioxidant activity of Concord juice determined by DPPH (A) and ABTS (B) analysis during 5-month storage at 4 ° C.

[a]Denotation of "*" indicate significant difference among treatments at each sampling point ($P<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

As mentioned above, phenolic compounds were considered to greatly contribute to the antioxidant activity in the juice sample. In Pearson's correlation analysis, both DPPH and ABTS assays results had significant correlation with TP and TMA contents ($p<0.01$). Total phenolic contents had a strong positive correlation (0.89) with ABTS results and a lower correlation coeffecient (0.74) with DPPH results ($p<0.01$). On the other hand, TMA contents showed significant correlations with DPPH ($r=0.53$) and ABTS ($r=0.71$) assay result ($p<0.01$), which was lower than the numbers found between the TP and antioxidant activity assays. Orak (67) also reported that antioxidant activity had stronger correlation with phenolics content than with anthocyanin content. Additionally, our results support the viewpoint that among different categories of phenolic compounds, anthocyanins are the main significant contributors to the antioxidant activity (68). These results indicated that the total phenolic contents in the juice samples had a strong correlation with the juice antiradical capacity, and particularly, ABTS assay was more appropriate to estimate the total antioxidant activity in Concord grape juice. Hence, utilizing PEF and HPP can produce a cold-pressed Concord grape juice with high bioactivity, as the antioxidant activity of PEF+HPP juice was almost doubled compared to the HPP and HT juice.

3.5 Analysis of PPO and POD enzyme activities

Oxidative enzyme activities in fruit juices have impacts on the juice quality and shelf-life. The changes of PPO and POD enzyme activities during 5- month refrigerated storage at 4 °C were shown in Figure 3.7A and 3.7B, respectively. PEF pretreatment had very slight impact on inactivation of PPO and POD enzyme activity in Concord grape juice, in which the residual activities were 89±9 % and 78±7 % respectively. Additionally, the residual activity of PPO and POD in HPP and PEF+HPP treated samples had very similar values, ranging from 13 % to 50 % for PPO and 36 % to 100 % for POD. On the other hand, HT treatment was effective in inactivating PPO and POD enzyme activities, and the residual activities of the HT treated juice were 6 ± 1 % and 29 ± 20 % respectively. These results agreed with previous findings which support the conclusion that PPO and POD enzyme activities are more bar-tolerant than heat treatment (69). In white grape juice, HPP (600 MPa, 20-38 °C, 3 min) treated samples had higher PPO and POD residual enzyme activities (43-51 % and 45-52 %, respectively) than heat treated (90 °C, 60 seconds) samples (33-42 %, and 26-31 %, respectively) (38). During storage, PPO and POD enzyme activities showed signs of reactivation as the values increased at some sampling points. The sample variances also caused some fluctuations during storage. Overall, the PEF+HPP treated samples had the highest PPO and POD residual enzyme activities, while HT had the lowest. In alignment with this, antioxidant contents in PEF+HPP treated samples had larger extent of decrease than those observed in HPP and HT samples (Figure 3.5). Though the high residual oxidative enzyme activities can affect the quality and shelf-life of PEF+HPP treated juice, our results, as shown in Section 3.3 and 3.4, indicated that PEF+HPP juice still had the highest total phenols, total anthocyanins and antioxidant activities after 5-month storage. This was attributed to the significant increment of antioxidant compounds content in the freshly made PEF+HPP juice, as well as the low enzyme reaction rate during refrigerated storage.

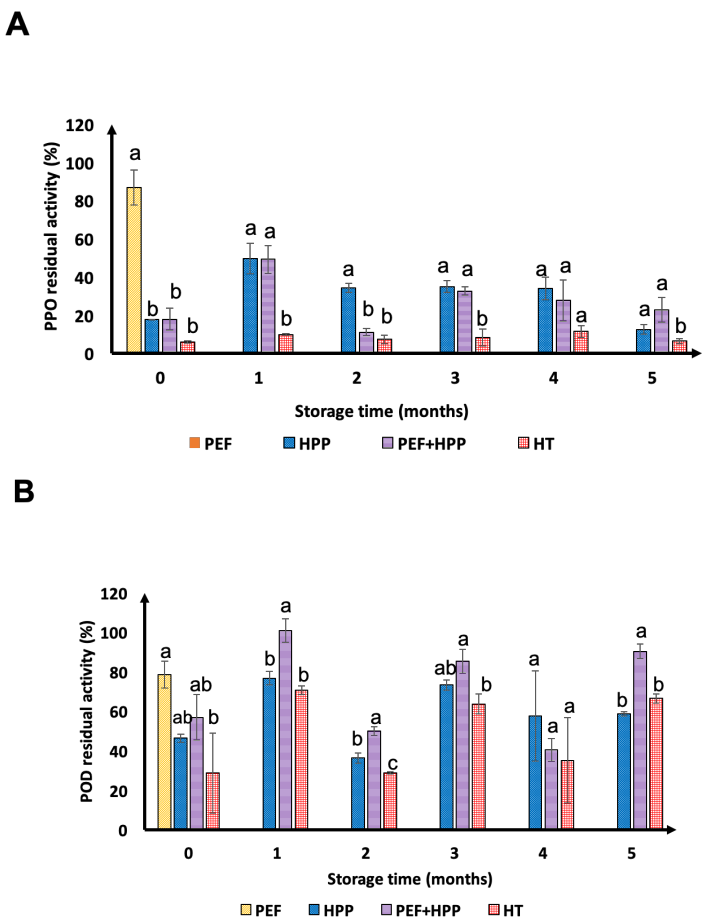

Figure 3. 7 PPO (A) and POD (B) residual enzyme activities of Concord grape juice during 5- month refrigerated storage at 4 °C.

[a]Different letters indicate significant difference among treatments at each time point ($p<0.05$). PEF: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash; HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

3.6 Sensory study

It is very important to know the consumer acceptability of food products that are processed by innovative technologies, and the best way to assess it is to evaluate the consumers' experience during the product tasting (70).

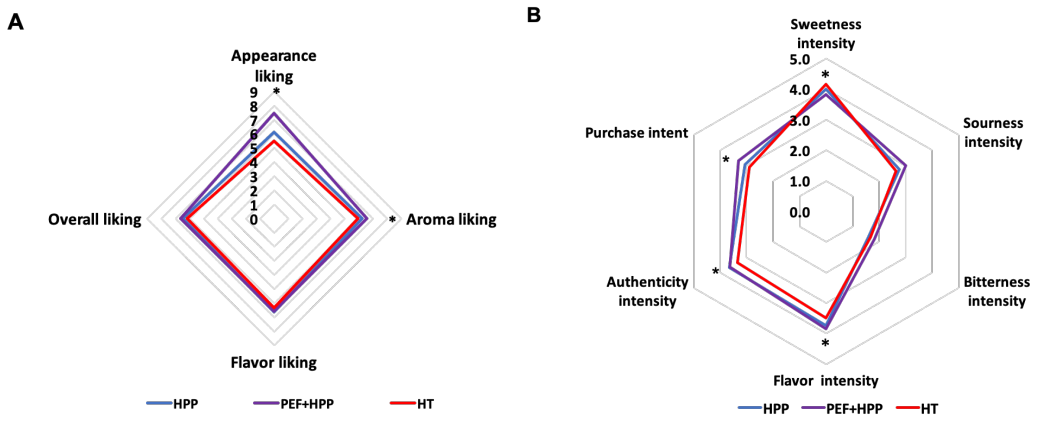

Figure 3. 8 Sensory analysis of consumer liking (A) on a 9-point scale (1 =dislike it extremely, 9 = like it extremely); intensity of different attributes (1 = not at all, 5 = very) and purchase intensity (1 = definitely would not, 5 = definitely would) on a 5-point scale.

[a]Denotation of "*" indicate significant difference among treatments at each time point ($p<0.05$). HPP: juice was pressurized by high pressure processing at 600 MPa, 5 °C for 3 min; PEF+HPP: juice pressed from PEF (1 kV/cm, 300 pulses) pretreated grape mash and sequentially pasteurized by high pressure processing at 600 MPa, 5 °C for 3 min; HT: juice was pasteurized by heating to 63 °C for 3 min.

From the consumer liking results (Figure 3.8A), PEF+HPP juice had significantly higher ratings in aroma liking and appearance liking than HT juice ($p<0.05$). As discussed earlier, the higher °Brix/TA ratio in PEF pretreated samples indicated that PEF pretreated samples had higher level of herbaceous aromatic components (Section 3.2.1). For color preference, about 83% of panelists rated the PEF+HPP juice color as "just about right", while this preference in HPP and HT samples were about 53% and 33%, respectively. This was attributed to the lighter color in HPP and HT samples perceived by the panelists, which is in alignment to the significantly higher color intensity of PEF+HPP juice determined by the spectrophotometer (Figure 3.4B). There was no significant difference among three treatments regarding to flavor liking, overall liking (Figure 3.8A), sourness and bitterness intensity (Figure 3.8B) in the sensory study. PEF+HPP juice had significant higher TA value and lower Brix/TA ratio than HT juice (Section 3.2.1), which may contribute to the higher sweetness perception by panelists in HT juice. When compared with HT juice, PEF+HPP juice had significantly higher ratings in Concord grape flavor intensity and purchase intent, while HPP sample had significantly higher ratings in authenticity.

The reason for higher ratings in the aroma, flavor and authenticity intensities of non-thermally treated juice is probably due to the retention of flavor and aroma compounds, which represented the "fresh and natural" note in fruit products (71); while the thermal treatment can degrade a variety of volatile compounds, with some of the degradation rate as high as 90 to 100% (72). Finally, juice preference ranking for PEF+HPP juice is the highest (44 % panelists ranked it first), following by HPP juice (32%) and HT juice (25%). Hence, PEF+HPP treated sample had the highest consumer acceptability in the context of without knowing the increased phenolic contents and their beneficial health effects. According to Olsen et al. (73), the health-conscious consumers preferred HPP or PEF prepared juice over thermally pasteurized ones in the sensory evaluation if the consumer knew the benefits of the processing method.

4. CONCLUSION

In this study, cold-pressed, non-thermally and thermally treated concord grape juice samples were prepared and studied during refrigerated storage. HPP was able to extend the refrigerated shelf-life to a minimum of 5-month. PEF pretreatment could extract significantly higher amounts of color pigment and phenolic components into the juice, which resulting significantly higher values in antioxidant activieties and consumers' acceptance (appearance, aroma, flavor and purchase intent). Compared to heat treatment, non-thermal treatments were able to produce a fresher juice that meet consumers' demand. Though the PPO and POD enzyme activities in the non-thermally treated samples were high, the quality and nutritional values in these samples were comparable or even higher than the thermally treated juice during refrigerated storage. In conclusion, combination of PEF and HPP treatments can produce a cold-pressed Concord grape juice that has intense color, enhanced nutrients, fresh flavor and extended shelf-life, all of which meeting consumers' demands.

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CONFLICT OF INTERESTS:

None.

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CHAPTER 4

HIGH PRESSURE PROCESSING VS. THERMAL PASTEURIZATION OF WHOLE CONCORD GRAPE PUREE : EFFECT ON NUTRITIONAL VALUE, QUALITY PARAMETERS AND REFRIGERATED SHELF LIFE[*]

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ABSTRACT

High pressure processing (HPP) has been utilized for food preservation as it can ensure product safety at low temperature, meeting consumers' demand for fresh-like and minimally processed products. The purpose of this study was to determine the effects of HPP (600 MPa, 3 min, 5 °C) and pasteurization by heat treatment (HT, 63 °C, 3 min) on producing a novel whole Concord grape puree product (with skin and seeds, no waste), and the shelf-life of the puree under refrigerated storage (4 °C). Microbial load, physicochemical properties, phenolics content and antioxidant activity, composition and sensorial attributes of puree samples were evaluated. HPP and HT treated purees were microbiologically stable for at least 4 months under refrigeration, with less microbial growth and longer shelf-life for HPP samples. HPP and HT samples had similar level of phenolics contents and antioxidant activities throughout the 4-month refrigerated storage, even though HPP retained >75% PPO and POD enzyme activities while HT were less than 25%. Inclusion of seeds in the puree product significantly increased the fiber, protein, total fatty acids and linoleic acid contents. Sensory results showed that HPP treated puree retained more fresh-like grape attributes, had better consistency, and showed significantly higher ratings in consumer overall liking, product ranking and purchase intent than the HT puree ($p<0.05$).

KEYWORDS

High pressure processing; Concord grape; Microbial inactivation; Physicochemical properties; Antioxidant activity; Nutritional value; Sensorial attributes.

1. INTRODUCTION

Grape is one of the most favored fruits worldwide with the highest total value of production. Consumer demand for both table grapes and processed grape products has driven the total global grape production to 79 million tons in 2018, increasing by 4 million tons compared to 2014 [1]. Despite its palatable characteristics, the health-promoting functions of grape are another aspect highly valued by the consumers. Researchers have assessed the health benefits of grape and grape products with both animal and human studies [2]. Flavonoids are the most abundant phytonutrients found in grapes. Anthocyanins are a subclass of flavonoids which are responsible for the attractive skin color in red grape varieties [3].

Concord grape is a dark purple to blue colored variety belonging to Vitis labrusca. The health promoting properties of Concord grape are reported as cardiovascular protection [4–8], neuroprotection [9, 10], antiaging [11, 12] and anti-tumoral effect [13]. Compared to culinary grape products, grape-derived commercial products, such as grape seed extracts, grape seed oil, grape powder (grape skin dietary fiber), are rapidly expanding in the marketplace as they can be classified as high value-added nutraceuticals or cosmetics. This is attributed to the highly valued phenolic compounds that can reduce the oxidative stress which leads to many chronic diseases and deterioration of normal body function. The phenolic compounds are mainly located in the skin (55%) and seed (44%) of grapes [14], which are considered as the processing waste or byproducts of the wine and juice producing industries. Grape seed extracts have been studied and reported as antimicrobial [15], anti-tumor [16, 17], anti-cancer [18, 19] and anti-inflammation agents to lessen Alzheimer's disease [20, 21]. As the most widely accepted grape product, grape juice has been reported to face the disadvantages of high sugar content and antimicrobial addition for preservation. Wine has been favored for its cardioprotection effect for many years, even though it can only extract a maximum of 50% of total phenolic compounds from grape berries. However,

there are rising concerns for wine, such as alcohol addiction and the reverse effects against health [22]. Alternatively, grape puree can be directly consumed or applied as an ingredient. One aim of this research is to develop a whole Concord grape puree product (inclusion of seeds and skin, eliminating waste), to meet consumers' demand for healthy fruit products. Conventional thermal processing is often applied to preserve food products, especially for highly perishable fruits with short harvest seasons, such as Concord grape. Despite the effective reduction of microbial loads and inactivation of deleterious enzymatic activity, thermal processing can cause adverse effects on fruit product quality. Quality parameters such as color, nutrient content and sensory attributes are crucial factors that affect consumers' acceptability, and can be deteriorated during thermal processing [23, 24]. Alternatively, nonthermal technologies can meet the consumers' demand for minimally processed products with clean label, high nutrition, fresh-like appearance and taste. High pressure processing (HPP) is one of the most widely investigated nonthermal technologies that has been successfully utilized on many food products. HPP ensures food safety by killing vegetative cells of microorganisms due to cell membrane rupture under extreme pressure, leading to loss of normal function and integrity [25, 26]. Regarding food quality, high pressure affects the morphology and function of macromolecular food components, such as proteins, enzymes, lipids and polysaccharides, while low molecular food components, such as vitamins, flavoring and coloring compounds, which are often the important components determining the nutritional and sensorial attributes, are less affected as covalent bonds are not disrupted [27, 28].

HPP has been recognized as the most successfully commercialized nonthermal technology in the food industry, and its application on fruit products is rising [29]. Previous research has laid the groundwork for the application of HPP in industry-scale food processing. For instance, raspberry puree showed the smallest anthocyanin degradation rate when pressurized at 200 to 800 MPa during storage at 4 °C [30], and the red color loss was closely associated with the residual enzyme activity after HPP [31]. HPP treatment at 400 to 600 MPa for 5 min could provide a microbial-safe aronia berry puree product that retained physicochemical properties and nutritional values [32, 33]. Similar studies have also been conducted on HPP treated acidified apple [34], strawberry [35 – 37], blackberry [38], plum [39], pineapple [40] and banana purees [41, 42]. Application of HPP on grape products has been mainly reported in studies of grape juice, pomace and wine [43–46], whilst no report on grape puree products has been published.

The objective of this study was to assess the effects of HPP and mild heat pasteurization on the quality of whole Concord grape puree during its refrigerated shelf-life. The relevance of this study to the food industry is that it supports sustainable approaches to eliminate waste while providing evidence of using HPP to develop a fresh-like and nutritious whole Concord grape puree with extended refrigerated shelf-life.

2. MATERIALS AND METHODS

2.1 Material and chemicals

Fresh Concord grapes (*Vitis Labrusca L.*) provided by Welch's Foods Inc. (Concord, MA), were harvested in October 2019 from the finger lakes region, NY. Grapes were stored in a refrigerated room at 4 ± 1°C and processed into puree within one week after harvest. The grapes used in this study were from the same batch.

All chemicals used were of analytical grade. Gallic acid anhydrous was obtained from Chem-Impex International, Inc., IL. Folin-Ciocalteu's phenol reagent, ABTS [(2,2′-Azinobis-(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt)] and methanol were purchased from Sigma-Aldrich, Inc., MO. DPPH (1,1-diphenyl-2-picrylhydrazyl radical), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and catechol were purchased from Tokyo Chemical Industry, TCI America, OR. Potassium persulfate was purchased from Honeywell Fluka, North Carolina. Triton-100 was purchased from Electron Microscopy Sciences, Inc., PA. Poly(vinylpolypyrrolidone) (PVPP), p-phenylenediamine, hydrogen peroxide, sodium chloride and monobasic and dibasic sodium phosphate were purchased from VWR International, LLC., PA.

2.2. Concord grape puree preparation

Figure 4.1 shows the processing procedure followed. Concord grapes were destemmed by hand and then ground using a food processor (R302V, Robot Coupe, Inc., MS) for 30 min. Grape seeds in the puree were broken into small pieces after grinding. Then the slurry was sheared in the Ross high shear homogenizer equipped with the fine screen stator (HSM-100LSK, Charles Ross & Son Company, NY) at 9,500 rpm for 2 min to attain a smooth puree. Ice water bath was used to maintain the temperature of the grape slurry below 40 °C during processing.

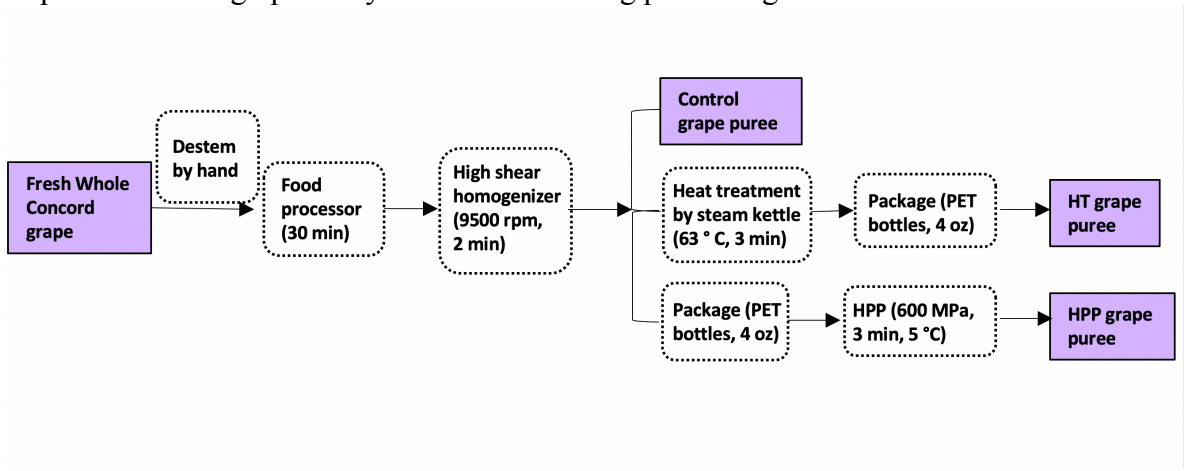

Figure 4. 1 Flow diagram for the production of whole Concord grape puree.

2.3. Puree Preservation

2.3.1. High pressure processing (HPP)

For quality and shelf-life studies, samples were processed at the Cornell HPP Validation Center (Geneva, NY), following biosafety level 2 guidelines, which prohibits testing for sensory analysis. HPP compatible PET bottles (4 oz, Merrimack Valley Plastics, MA) filled with sheared puree samples were packed into PET bags and vacuum sealed. Then each bag was bagged and sealed again to prevent any leakage in the HPP system. Packages were loaded into a 55 L commercial high pressure processing unit (Hiperbaric 55, Hiperbaric, Burgos, Spain) and cold water was used to transmit pressure. Puree samples were pressurized using current industry standards of 600 MPa for 3 min at 5 °C. The HPP parameters (600 MPa, 3 min) are commonly used in the food industry, which have been tested to achieve a greater than 5-log reduction of relevant pathogens in acid /acidified juices /beverages (pH < 4.5) [47]. After HPP, all bags were discarded, and bottles were wiped dry. HPP treated samples for the sensory study were prepared the same way except that the HPP treatment was completed at a commercial food plant, LiDestri Food and Beverage (Rochester, NY) with an industrial HPP unit (Hiperbaric 525, Hiperbaric, FL).

2.3.2. Heat treatment (HT)

A mild heat treatment (63 °C, 3 min) was applied for thermal processing to retain fresher attributes. This time and temperature combination was calculated based on a D_{52} value of 23 min, a z-value of 4.8 °C, with an additional 5-fold safety factor to achieve a >5-log reduction process for *E. coli O157:H7*, *Salmonella*, and *Listeria monocytogenes* [48]. Puree samples were pasteurized for safety in a steam kettle (TDA-10 QT, IL) to achieve >5-log reduction of pertinent pathogens (FDA, 2004) and then immediately cooled in an ice water bucket. When temperature dropped below 38 °C, samples were poured into clean 4 oz PET bottles and immediately closed tightly using screw caps.

2.4. Refrigerated storage

Samples used for the sensory study were kept under refrigeration at 4 °C for 1 week, to simulate commercial distribution time required to reach stores, before sensory analysis. Control (untreated puree), HPP and HT treated samples for physicochemical and other quality analyses were stored at 4 ± 1 °C and sampled at 1-month intervals for the shelf-life study for up to 5 months. All samples were prepared according to the flow diagram (Figure 4.1), except for samples for the proximate composition analysis.

2.5. Microbial analyses

2.5.1. Total aerobic plate count

Total aerobic plate counts (APC) were determined by taking puree samples monthly during refrigerated storage. Twenty-five grams of Concord grape puree sample was diluted (1:10 w/w) in 0.1% sterile peptone water and homogenized using a stomacher (Stomacher 400 Circulator, Seward Medical, London, UK) at 200 rpm for 1 min at ambient temperature. The homogenized solution was then serially diluted in 9 mL of peptone water and pour-plated for APC using Plate Count Agar (PCA, CM0325, Oxoid Limited, Thermo Fisher Scientific Inc., Hampshire, UK), followed by incubation at 30 °C for 48 to 72 h. APC was expressed as log of colony forming units per gram of puree by fresh weight (log CFU/g FW).

2.5.2. Yeast and mold count

Yeast and mold (Y&M) counts were assessed at the same sample interval and using the same preparation procedures as APC analyses expect that Potato Dextrose Agar (PDA, Alpha Biosciences Inc., MD) was used as growth medium. Tartaric acid was added in the PDA medium to adjust the pH to 3.5. Y&M counts were expressed as log CFU/g FW.

2.6. Physicochemical properties analyses

2.6.1. Total soluble solids content (TSSC)

Total soluble solids content was determined using a portable digital refractometer (model 300055, Sper Scientific, Scottsdale, AZ). Approximately 5 g of grape puree was filtered through Whatman No.4 filter paper, and 2 to 3 drops of filtrate were added onto the prism at room temperature to obtain readings, expressed as °Brix.

2.6.2. pH

pH was measured at room temperature using a pH meter (Orion™ 3-star, benchtop pH meter, Thermo Scientific™, Fisher Scientific, MA) which was calibrated prior to each measurement with the standard phosphate buffers at pH 4 and 7.

2.6.3. Titratable acidity (TA)

TA was determined according to Iland [49] (pp. 39 - 43) with some modifications. After calibrating the pH meter using the pH 4 and pH 7 buffers, a diluted solution containing 5 g of grape puree sample and 45 mL of distilled water was titrated with 0.1 mol/L sodium hydroxide to pH 8.2 using an autotitrator (Mettler compact G20, Mettler-Toledo, LLC, OH). Results were calculated based on equation (1) and expressed as % tartaric acid (% gram tartaric acid equivalence per gram of grape puree):

$$\text{Tartaric acid (\%, w/w)} = \frac{V_{NaOH}(L) \times 0.1(\frac{mol}{L}) \times 75(\frac{g}{mol})}{m_{puree}(g)} \times 100 \quad (1)$$

2.6.4. Color measurement

Color components {L* (lightness), a*(greenness [−] to redness [+], and b* (blueness [−] to yellowness [+])} of puree samples were determined by a Hunter colorimeter (Labscan XE, Hunter Associates Laboratory, Inc., VA) in the reflection mode. The colorimeter was first standardized with a white tile and samples were measured in a 10 mm pathlength quartz cuvette. Two measurements for each sample were conducted, and an average value was reported as the color result of the sample. The values of the absolute color difference of a sample were calculated according to equation (2) as shown below:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (2)$$

where L_0, a_0, b_0 are the color measurements of fresh-made control puree samples, L, a, b are the color measurements of HPP or HT treated puree samples.

The browning index (BI) was analyzed according to Palou et al. [41] and calculated by equations (3):

$$BI = \frac{100 \times (x - 0.31)}{0.172} \quad (3)$$

where $x = \frac{a + 1.75 \times L}{5.645 \times L + a - 3.012 \times b}$ in equation (3).

2.6.5. Particle size distribution (PSD), serum separation and viscosity

The particle size distribution was measured by a laser diffraction particle size analyzer (Malvern Mastersizer 2000, Malvern instruments Ltd., MA). Distilled water was used as dispersant and the samples were measured when the concentration of added sample reached 5% obscuration. The particle size distribution profiles were measured in triplicate.

Serum separation rate (SSR) was measured as an indicator of syneresis by the centrifugation method described by Eliasson and Kim [50]. Ten grams of puree samples were loaded into a 50 mL centrifuge tube and centrifuged at 6,000 rpm for 10 min at room temperature (Centrifuge 5810R Eppendorf, CT). Supernatant was decanted and the weight of remaining solids was recorded. SSR was calculated based on equation (4):

$$\text{SSR (\%, w/w)} = \frac{(m_t - m_r)}{m_t} \times 100 \quad (4)$$

Where m_t is the total puree weight before centrifugation, m_r is the remaining solids weight after centrifugation.

Viscosity was measured by a Brookfield DV-III ultra programmable rheometer (Brookfield Engineering Laboratories, Ltd., MA) with the V-73 spindle. Samples were equilibrated to room temperature before measuring at 250 rpm for 3 min.

2.7. Phenolics content and in vitro antioxidant activity

2.7.1. Extraction of total phenols and anthocyanins from whole Concord grape puree

Extraction procedure was based on the methods reported by Iland [49] (pp. 45 - 48) and Jensen et al. [51] with some modifications. Generally, fresh whole Concord grape puree was mixed with acidified methanol (1% HCl, v/v) on a 1:1 ratio (w/v). After vortexing the mixture for 1 min, tubes were put into a 40 °C water bath for 30 min. Then the supernatant was transferred into new vials after centrifugation ($12{,}000 \times g$, 5 min). The supernatant was diluted 5 to 10 folds using distilled water and then used as total phenols and anthocyanins solution for future determination. Puree weight (m_p) and supernatant volume (V_s) were recorded for calculation.

2.7.2. Total phenolic content (TP)

Total phenolic content was determined by Folin-Ciocalteau colorimetric assay according to the method reported by Waterhouse [52] with minor modifications. Generally, 20 µL of diluted extract was mixed with 1,580 µL DI water and 100 µL Folin-Ciocalteau reagent. The mixture was vortexed and incubated at room temperature for 6 min. After incubation, 300 µL of 20% (w/v) sodium carbonate solution was added and gently vortexed before incubating at room temperature for 2 h in the dark. Absorbance was measured at 765 nm by a Genesys UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). Gallic acid solutions (0 to 500 mg/L) were used to determine the standard curve. Results were expressed as gallic acid equivalents (GAE) mg/g of fresh weight of whole Concord grape puree. Calculation was carried out according to equation (5):

$$GAE\left(\frac{mg}{g}\right) = \frac{C\left(\frac{mg}{L}\right) \times V_s(L)}{m_p(g)} \quad (5)$$

2.7.3. Total monomeric anthocyanin content (TMA)

Total monomeric anthocyanin content was determined by the method described by Lee et al. [53]. Briefly, 200 µL extracts were diluted 10-fold with pH=1.0 (0.025 M, potassium chloride) and pH=4.5 (0.4 M, sodium acetate) buffers separately. The mixture was gently vortexed and equilibrated at room temperature for 20 min. Blank was prepared using DI water. Absorbance readings were taking both at 520 nm and 700 nm using the Genesys UV-visible Spectrophotometer. Results were calculated by equation (6) and expressed as cyanidin-3-glucoside equivalents (CGE):

$$\text{CGE (mg /kg)} = \frac{A \times M_W \times DF \times 10^{-3} \times V_S}{\varepsilon \times L \times m_p} \quad (6)$$

where $A = (A_{520nm} - A_{700nm})pH_{1.0} - (A_{520nm} - A_{700nm})pH_{4.5}$; M_W (molecular weight) of cyd-3-glu = 449.2 g/mol; DF (dilution factor) =10; ε is the molar extinction coefficient = 26,900 $L^{-1} \times cm^{-1} \times mol^{-1}$ for cyd-3-glu; L (pathlength) =1 cm; V_s is the puree extraction volume (mL) and 10^{-3} is the conversion of mL to L; and m_p is the fresh puree weight (g) used for extraction.

Results were calculated and expressed as mg CGE / kg of fresh weight of whole Concord grape puree (mg /kg as CGE).

2.7.4 In vitro total antioxidant activity

The in vitro total antioxidant activity was determined by DPPH and ABTS assays according to the description of Brand-Williams et al. [54] and Re et al. [55] with some modifications. Briefly, DPPH radical solution was prepared at a concentration of 0.2 mM in methanol, and the absorbance of DPPH solution was adjusted to 0.900 ± 0.050 at 517 nm before testing. ABTS solution was prepared in methanol at the concentration of 7 mM. Then potassium persulfate powder was added to the ABTS solution at a final concentration of 2.45 mM in the mixture. The mixture was left at room temperature for 12-16 h to generate stable radicals. The absorbance of $ABTS^{\bullet}$ solution was adjusted to 0.700 ± 0.050 at 734 nm before using. The puree extracts as described in Section 2.7.1 were used for the DPPH and ABTS assays. The supernatant of puree extracts (100 μL) was mixed with 900 μL $DPPH^{\bullet}$ solution and measured at 517 nm after 30 min equilibration under dark and room temperature conditions. For ABTS assay, 50 μL diluted supernatant (dilution factor=10) was mixed with 2 mL $ABTS^{\bullet}$ solutions and determined spectrophotometrically after 6 min sitting in dark environment at room temperature. Blank was prepared the same way except that DI water was used instead of puree supernatant. Trolox standards (ranging from 0 to 800 μM) were prepared by diluting the stock solution (2.5 mM). Radical scavenging capacity was expressed as TEAC (Trolox equivalent antioxidant capacity) in μmol/g of FW (fresh weight) puree sample.

2.7.5 Enzymatic activities: polyphenoloxidases (PPO) and peroxidases (POD)

Enzyme extraction and assays were carried out to determine the activities of PPO and POD according to Garcia-Palazon et al. [31] with some modifications. Enzyme extraction solution was prepared by mixing 4% (w/v) PVPP, 1% (v/v) Triton X-100 and 1 M NaCl with 0.2 mol/L sodium phosphate buffer (pH 6.5). The extraction solution (4.5 mL) and 4.5 g of grape puree sample were mixed vigorously and homogenized for 3 min. Then the mixture was centrifuged at 10,000 rpm for 30 min at 4 °C. The supernatant was collected and used as crude enzyme extract in the PPO and POD assays.

For the PPO assay, catechol solution (3 mL, 0.07 M) was prepared by adding catechol powder in 0.05 M sodium phosphate buffer (pH 6.5), then 500 μL of enzyme extract was added to start the reaction. The absorbance at 420 nm was monitored for 3 min and readings were recorded every 30 s. Blank was prepared in the same way except that DI water was used instead of enzyme extract.

For the POD assay, 200 μL of enzyme extract was mixed with 1.5 mL 0.05 M sodium phosphate buffer (pH 6.5) and 200 μL of 10 g/L p-phenylenediamine in 0.05 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 μL 1.5% (w/v) hydrogen peroxide was added. Readings were recorded at 485 nm using a spectrophotometer. The initial liner region of the absorbance-time curve was used for the enzyme activity analyses.

Both PPO and POD results were shown as the residual activity (RA) in % according to equation (7):

$$\boldsymbol{RA} = \frac{\boldsymbol{A_t}}{\boldsymbol{A_0}} \times 100\% \qquad (7)$$

where A_t is the enzyme activity of treated samples, A_0 is the enzyme activity of control samples.

2.8 Proximate composition analysis

Whole Concord grape puree made with seeds (Control, HPP and HT samples as described in Section 2.3) were used for proximate composition analysis. Additionally, a control without seeds sample was prepared by using 1.5 kg Concord grapes that were manually deseeded and then processed into puree. Four groups of samples, namely, untreated control with seeds (C/W), untreated without seeds (C/O), HPP treated with seeds (HPP/W) and HT treated with seeds (HT/W), were prepared in triplicate for composition analysis. Moisture and dry matter of the fresh puree samples were determined by the oven drying method (930.15, AOAC). Total ash was determined by 942.05, AOAC method. Water soluble carbohydrates (WSC) were determined spectrophotometrically after acid hydrolysis and colorimetric reaction with potassium ferricyanide [56]. Minerals contents were determined by Thermo iCAP 6300 inductively coupled plasma radial spectrometer after sample digestion using a CEM microwave accelerated reaction system (MARS6). Crude fiber and crude protein were analyzed according to AOAC 954.02 and AOAC 992.23, respectively [57]. Total fatty acids profile were analyzed according to O'Fallon et al. [58]. Fatty acid methyl esters (FAME) synthesis was conducted in the presence of up to 33% water. Samples were permeabilized and hydrolyzed for 1.5 h at 55°C in 1 N KOH in MeOH containing C13:0 as an internal standard. After neutralization of KOH, samples are methylated by H_2SO_4 catalysis for 1.5 h at 55°C. Hexane was then added to the reaction tube, vortex-mixed and centrifuged. The hexane layer pipetted into gas chromatography vials and then analyzed using a thermo trace 1310 gas chromatograph fitted with a Supelco SP-2560, 100 m x 0.25 mm x 0.20 µm capillary column and a flame ionization detector. All composition analyses were performed at Dairy One Co-Op, Inc., Ithaca, NY.

2.9 Sensory study of HPP and HT treated puree

A total of 101 untrained panelists were recruited to participate in the sensory evaluation of HPP and HT treated whole Concord grape purees. After processing, the puree samples were kept at 4 °C for 1 week before transporting to the Cornell Sensory Evaluation Center (Ithaca, NY). Samples were stored refrigerated before pouring into 2 oz plastic cups coded blindly with 3 random digits and covered with lids. Panelists were asked to assess the consumer liking of appearance, aroma, texture, flavor and overall liking using a 9-point hedonic scale rating. Intensity of sweetness, sourness, bitterness, smoothness, flavor, authenticity of Concord grape and purchase intent were assessed based on a 5-point scale. Intensity of color was evaluated using a 9-point red color board with different shades (1 = darkest). A Just about right (JAR) question was added to assess color preference (1 = too dark, 2 = moderate dark, 3 = just about right, 4 = moderate light, 5 = too light). The evaluation concluded with a preference ranking test between the HPP and HT treated samples. This study was conducted under approval and requirements from the Institutional Review Board of Cornell University.

All samples were served based on a randomized design and following all hygienic and procedural guidelines provided by the Sensory Evaluation Center. Water and crackers were provided for all panelists to consume between samples to reduce the influence from previous samples. Data were collected and analyzed using the REDJADE® Sensory Software (RedJade Software Solutions, LLC, Redwood Shores, CA).

2.10. Statistical analysis

All results are presented as mean values of the data from experiments performed in triplicate. Data are presented as mean ± standard deviation (SD). Data were analyzed using student's t-test or

one-way ANOVA as appropriate. Significant differences among mean values were determined by the Tukey's post hoc test following the one-way ANOVA test using SPSS (SPSS statistics, version 22.0, IBM, NY). Correlation coefficients were determined by Pearson's correlation test with SPSS.

3. RESULTS AND DISCUSSION

3.1. Microbial count in whole Concord grape puree during 5-month refrigerated storage

According to FDA Juice HACCP regulations (FDA, 2004), fruit juice and its related products should achieve a >5-log reduction of potential pathogens after pasteurization to ensure microbial safety. Petrus et al. [59] reported that moderate HPP treatment (400 MPa, 2 min) was sufficient to achieve the 5-log reduction in the pathogen challenge test (mixed cocktails of *Escherichia coli O157:H7, Salmonella enterica* and *Listeria monocytogenes*) in Concord grape juice, thus the applied 600 MPa for 3 min industrial process met the safety regulatory requirements. The effect of HPP on microbial counts of puree samples during storage at 4 °C is shown in Figure 4.2. The mean values of APC and Y&M counts in the fresh-made untreated puree were 6.33 and 6.31 log CFU/g FW respectively. Both HPP (600 MPa, 3 min at 5 °C) and HT (63° C, 3 min) treatments were able to cause a 5-log reduction in the fresh-made samples. HPP treatment was able to reduce the Y&M count to a level below the minimum detection limit, which was less than 1.0 log CFU/g FW, while HT treatment decreased the Y&M count to 1.22 log CFU/g FW. The results were consistent with Chang's findings [44], which reported that HPP (600 MPa, 3 min) and heat (90 ° C, 1 min) treated white grape juice had similar values in APC (1.2 log CFU/mL), coliforms and Y&M counts (<1.0 log CFU/mL) on day 1 and after 20-day storage at 4 °C, indicating that HPP is very effective in eliminating fungi and vegetative cells of bacteria in grape puree, with limited effect on bacterial spores.

During the 5-month refrigerated storage, the microbial counts in both treatment groups increased gradually, with values in HT samples showing a larger increment than that in HPP samples. After 4-month storage, the APC count in HPP samples was 2.0 log CFU/ g FW, and Y&M count was still under detectable limit, while the APC and Y&M counts in HT samples were 3.6 and 3.1 log CFU/g FW, respectively. After 5-month refrigerated storage, the APC and Y&M counts in the HPP samples were 2.7 and 2.4 log CFU/g FW respectively, while the HT samples showed indications of spoilage (>6 log CFU/g FW) with visible fungi colonies on the surface. The thermal treatment applied in this study was not as effective as HPP in killing spoilage microorganisms which led to higher increment of microbial count during storage in HT samples. These findings suggest that HPP is a feasible preservation alternative for fruit puree products, being as effective as mild heat pasteurization in reducing microbial populations and extending refrigerated product shelf-life. Because HPP is applied to the packaged product, post process contamination is eliminated, contributing to the extended shelf-life of refrigerated foods.

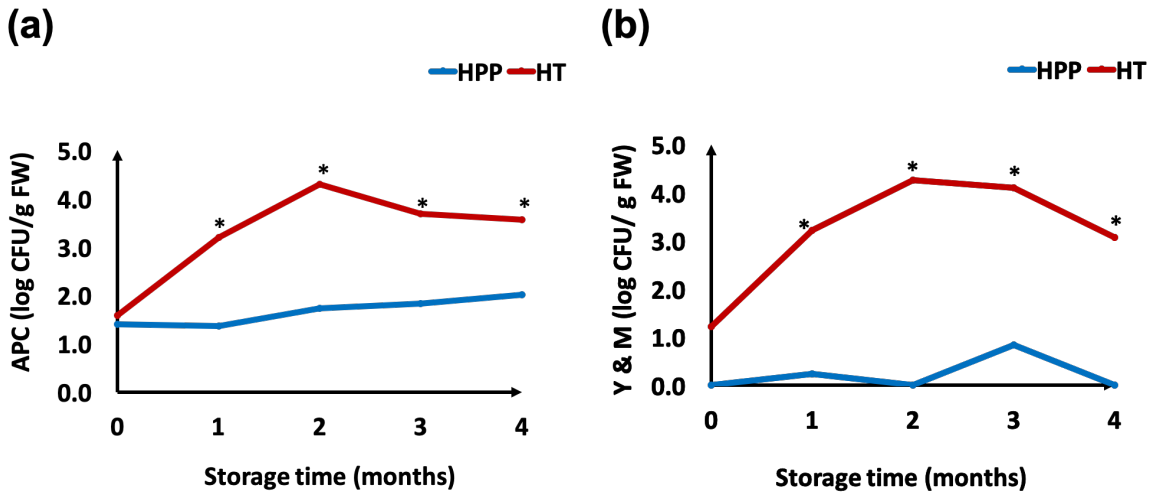

Figure 4. 2 Microbial counts of HPP (600 MPa, 3 min) and HT (63 °C, 3 min) treated puree samples during 4-month refrigerated storage: (a) total aerobic plate counts (APC); (b) yeast and mold counts (Y&M).

[a] Denotation of "*" indicates significant differences between treatments at each sampling point.

3.2. Physicochemical properties of whole Concord grape puree

3.2.1. Total soluble solids content (TSSC), pH, and titratable acidity (TA) of whole Concord grape puree during 4-month refrigerated storage.

The control puree had a water activity of 0.98, pH of 3.35 ± 0.01, TSSC of 18.0 ± 0.1 °Brix and TA of 1.2 ± 0.1% tartaric acid on day 1. Heat treatment did not cause significant changes to these parameters immediately after processing (pH=3.33 ± 0.05, TSSC=18.1 ± 0.2 °Brix and TA=1.3 ± 0.1% tartaric acid) and these values kept constant during storage, except for pH. The pH value in HT samples increased to 3.42 ± 0.05 after 4-month refrigerated storage, due to the observed microorganism growth within the puree. HPP treated samples had similar values to those in HT samples on day 1 (pH=3.35 ±0.01, TSSC=18.3 ± 0.3 °Brix and TA=1.1 ± 0.1% tartaric acid) and during storage (data not shown).

3.2.2 Effect of HPP and HT treatments on color changes of whole Concord grape puree

Color and visual appearance, flavor, texture, and nutritional value are four important attributes that indicate the quality of fruit products. Among them, color and visual appearance are the first quality attributes that impact the perception and acceptance by consumers [60]. For red grape varieties, anthocyanins in the skin cells are responsible for the unique red, purple, or dark blue colors observed in Concord grapes. As anthocyanins are water soluble and sensitive to both heat and pH, the color degradation in Concord grape products during storage could be impacted by mechanical harvest, postharvest conditions, heating, enzyme addition, enzymatic browning, tartration and chemical changes [61, 62].

Visual appearance of freshly produced samples can be seen in supplementary Figure S2. Instrumental color changes of Concord grape puree with or without treatments during refrigerated storage of 4 months are shown in supplementary Table S1. There were no significant changes in L*, a* and ΔE values in either the HPP or HT treated samples on day 1 and the color parameters remained unchanged after refrigerated storage of 4 months. The b* (yellow/blue) value in HT treated samples, however, was significantly higher compared to the HPP treated samples,

indicating that more yellow to brown colors were generated in the HT treated puree samples. Mild heat treatment increased the enzyme (mainly PPO) mediated browning reaction rate during processing, while the oxidation reaction rate was very slow during HPP processing (5 °C) and refrigerated storage (4°C). Brown pigments can also be generated through non-enzymatic activity, such as Maillard reaction during juice heating [63, 64]. As a consequence of the increased b* value, the Browning Index (BI) in HT treated samples was significantly higher than that in the HPP treated and control samples on day 1 and during storage. On day 1, ΔE changed to a noticeable level (ΔE >2) in HT treated samples while the change in HPP treated samples was not noticeable (ΔE <1.5) according to the color change threshold reported in previous studies [65, 66]. This finding was in accordance with the results reported in strawberry and blackberry purees, which concluded that color changes in pressurized (400-600 MPa, 10-30 °C, 15 min) purees were minor compared to those in thermally treated (70 °C, 2 min) samples [38]. Marszałek et al. [37] reported that thermal processing at 90 °C for 15 min was able to inactivate 97.7% enzyme activity in strawberry puree, while the residual PPO enzyme activity of HPP (500 MPa, 0 °C, 5 min) treated strawberry was still as high as 90%. Based on previous puree studies, HPP treated puree products had higher residual enzyme activity than thermally treated puree, hereby HPP treated puree may only have a transitory shelf-life and more color loss during storage. In our study, the color change (ΔE) of both HPP and HT treated Concord grape puree remained consistent after a refrigerated storage of 4 months, suggesting that the unique grape puree matrix containing grape skin and seeds was able to preserve the fresh-like color despite of the possible deleterious enzyme activity in HPP treated Concord grape puree.

3.2.3 Effect of HPP and HT on particle size distribution (PSD), serum separation rate (SSR) and viscosity of Concord grape puree

Grape puree can be utilized as an intermediate ingredient for product development, such as beverages, jam, jelly, bakery products, yogurt and ice cream. Understanding the rheological properties, which determine the textural appearance and viscoelastic parameters, is essential in developing new products as they affect the processing conditions (pumping, mixing, evaporation and pasteurization) and final consumer acceptance (appearance, consistency and stability) [67]. Parameters, such as TSSC, SSR, PSD and viscosity are good indicators of rheological behaviors in puree products [68–72]. The SSR in HPP treated puree was 44.3 ± 3.5%, which was similar to the value of untreated sample but significantly lower than HT treated samples (supplementary Figure S1). After 4-month refrigerated storage, SSR in HPP treated puree increased significantly to 65.8 ± 0.3%, while the HT treated samples gelled, likely due to enzyme activity, impeding the serum separation from the pulp based on the applied centrifugal force. After 4-month storage, HPP treated puree had good consistency which resembled the freshly made untreated puree with good runniness, while the HT puree had large lumps and small amounts of serum syneresis on the surface (Figure S2b). The HPP puree was smooth, lump free, homogenous and more like a liquidized, thin puree, while the HT puree surface was rough and lumpy, resembling an uneven puree with gelled components, unsuitable for pumping. The visual lumps and syneresis not only affect the appearance, but also can negatively affect the processing operations, such as pumping, mixing and filling. Syneresis in the 4-month HT samples indicated that repelling of water and aggregation of grape components, possibly caused by the entanglement of pectin chains after HT treatment, were happening which greatly deteriorated the product physical stability [73].

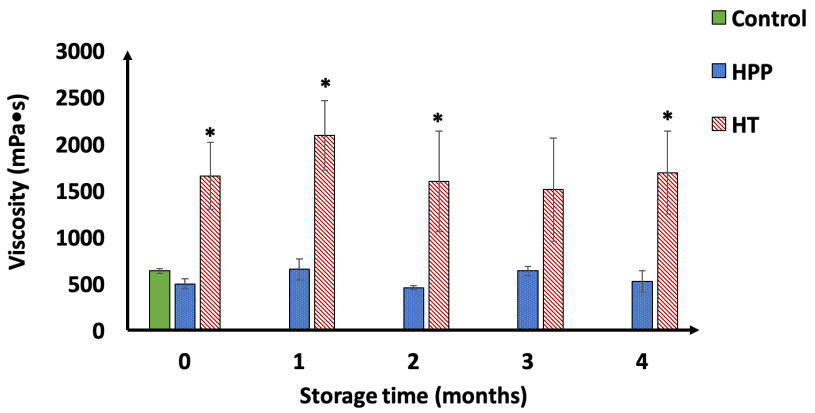

Figure 4. 3 Viscosity changes of control, HPP (600 MPa, 3 min) and HT (63 °C, 3 min) treated puree during 4-month storage at 4 °C.

[a] Denotation of "*" indicates significant difference among treatments at each sampling point ($p<0.05$).

Freshly-made grape puree showed a bimodal particle size distribution (supplementary Figure S2a), regardless of the processing applied, as the puree is comprised of a mixture of particles from skins, pulp and seeds. In HPP treated puree a larger proportion of small particles were present, likely due to the physical disruption of the grape components and breakage of cell clusters by the high presure applied [74]. HT treated puree had more large particles as a result of aggregation of cell components. After 4-month storage, the D90 value in HT samples was 294 ± 36 μm, which was significantly higher than that in the HPP samples at 218 ± 35 μm (data not shown). The heat treatment significantly increased the whole Concord grape puree viscosity compared to the control and HPP, and the same tendency was also found during storage (Figure 4.3). The viscosity increment in HT sample was probably associated with the presence of more large particles and the structural changes of polysaccharides and pectic substances which respectively constitute 30% and 20% of the grape pomace. On one hand, apparent viscosity increased as particle size increased, an effect also seen in tart cherry puree and apple puree [75, 76]. On the other hand, polysaccharides were hydrated and swollen during heat treatment, which could lead to the viscosity increment and polysaccharide aggregation. Low temperature of heat treatment (50 °C to 80 °C) activates the endogenous pectin methylesterase (PME) which results in pectin demethoxylation and cross-linking with calcium ions. Cross-linked pectin could aggregate to form gels, and eventually cause phase separation, which was also supported by the syneresis phenomenon observed in the HT treated puree. After 4 months of refrigerated storage, the viscosity of both HT and HPP treated samples did not change significantly; HPP treated puree still had good liquidity and consistency, while HT treated puree was jam-like paste without flow. The high viscosity of HT treated puree would make it difficult for processing, for instance in mixing and piping operations. These results suggested that HPP treatment was able to produce the whole Concord grape puree with more fresh-like texture and consistency compared to thermally processed grape puree.

3.3 Total phenolic content (TP), total monomeric anthocyanin content (TMA) and antioxidant activity of puree samples

Concord grape and its derivate products are rich in polyphenol compounds, especially in flavonoids, such as anthocyanins which contribute to its distinct purple hue. The benefits of Concord grape polyphenol compounds have been reported by many researchers: consumption of

Concord grape juice can improve memory in senior adults with cognitive impairment, due to the anti-inflammatory effect and influence on neuronal signaling [10, 77]; mice fed with Concord grape juice showed better motor function and cognitive performance due to neuronal and behavioral defects in aging, caused by the accumulated oxidative stress and inflammation [12]; supplementation of Concord grape juice can help to reduce blood pressure in hypertensive patients [6]; consumption of grape can reduce the risk of cardiovascular disease [78]; supplementation of $10\ mL \bullet kg^{-1} \bullet d^{-1}$ Concord grape juice can achieve the same level of antioxidant capacity and protection of low density lipoprotein against oxidation to that obtained with 400 IU α-tocopherol/d supplementation in healthy adults, and significantly lower native plasma protein oxidation rate than that in α-tocopherol treated group [79]. Results of total phenolics, total anthocyanins content and total antioxidant activity of whole Concord puree samples are presented in Table 4.1.

Table 4. 1 Changes in total phenolic (TP) compounds content, total monomeric anthocyanins (TMA) content and antioxidant activity of whole Concord grape puree during 4-month storage at 4 ° C.

		Storage time (months)				
		0	**1**	**2**	**3**	**4**
TP (mg/g as GAE)	**Control**	3.0 ± 0.1 a	-	-	-	-
	HPP	3.8 ± 0.5 bX	3.0 ± 0.6 aY	2.9 ± 0.2 aY	3.2 ± 0.3 aXY	2.6 ± 0.0 aY
	HT	3.6 ± 0.0 abX	3.6 ± 1.0 aX	2.9 ± 0.3 aX	3.2 ± 0.7 aX	2.9 ± 0.3 aX
TMA (mg/kg as CGE)	**Control**	628 ± 35 a	-	-	-	-
	HPP	620 ± 110 aX	590 ± 40 aX	510 ± 50 aX	520 ± 60 aX	560 ± 30 aX
	HT	790 ± 120 aX	840 ± 40 bX	650 ± 170 aX	610 ± 180 aX	610 ± 170 aX
DPPH (TEAC µmol/g)	**Control**	12.6 ± 0.3 a	-	-	-	-
	HPP	12.7 ± 0.1 aY	13.2 ± 0.1 aX	12.2 ± 0.2 aZ	12.7 ± 0.2 aY	12.6 ± 0.1 aY
	HT	13.4 ± 0.1 aX	13.2 ± 0.1 aX	12.8 ± 0.2 aY	12.8 ± 0.1 aY	12.8 ± 0.1 aY

ABTS (TEAC µmol/g)	**Control**	34.7 ± 0.6 a	-	-	-	-
	HPP	38.1 ± 0.2 bW	36.6 ± 0.5 aX	32.3 ± 0.5 aZ	36.6 ± 0.7 aX	33.9 ± 0.5 aY
	HT	37.8 ± 0.5 bY	42.1 ± 0.6 bX	36.1 ± 1.0 bY	36 ± 0.4 aY	33.6 ± 1.4 aZ

[a]Different lowercase letters indicate significant difference among treatments at each predefined sampling point; while different uppercase letters indicate significant difference during storage for HPP (600 MPa, 3 min) or HT (63 °C, 3 min) treated samples ($p<0.05$). GAE: gallic acid equivalents; CGE: cyanidin-3-glucoside equivalents. TEAC: trolox equivalent antioxidant capacity.

The TP and TMA values in untreated Concord grape puree were 3.0 ± 0.1 mg/g as gallic acid equivalents (GAE) and 628 ± 35 mg/kg as cyanidin-3-glucoside equivalents (CGE), respectively (Table 4.1). These results agree with TP values (2.9 mg/g as GAE) reported in Grenache grape, which is a widely planted red wine grape variety [80]. The TMA value is within the detected range of red grape varieties (40.3 mg/kg to 990.8 mg/kg fresh weight) prepared without seeds [3]. After HPP treatment, the TP value of fresh-made puree was significantly ($p<0.05$) higher than control puree, while there were no significant differences in TMA values among different treatments. Significant increment in phenolic content after HPP treatment was also reported in strawberry puree and blackberry puree [38]. Stated reason for the increased phenolic content in HPP treated samples is that the high pressure prompted the mass transfer, cell membrane permeability and the release of bound phenolic compounds [81]. After 4-month refrigerated storage, the TP values in HPP treated samples significantly decreased from 3.8 mg/g to 2.6 mg/g, while that in HT treated samples decrease insignificantly from 3.6 mg/g to 2.9 mg/g. The TMA values in both HPP and HT treated samples did not show significant changes after 4-month refrigerated storage. PPO and POD are deleterious enzymes that catalyze the oxidation of phenols, which leads to quality degradation in fruit products, such as discoloration [82]. Greater decrease in phenolic compounds in HPP samples was attributed to the higher PPO and POD residual enzyme activities (75.2% and 80.7%, respectively), compared to the HT treated samples (22.6% and 10.2%, respectively). Nevertheless, there were no significant differences in both TP and TMA values between HPP and HT treated samples after 4-month refrigerated storage, probably due to the low reaction rate under refrigerated conditions. These results revealed that HPP is a feasible processing approach to preserve as many nutritive components in Concord grape puree as the conventional thermal processing with extended shelf-life, even though HPP was not effective in inactivating deleterious enzymes compared to thermal processing.

Antioxidant activity is determined by the DPPH and ABTS assays and shown as the capacity of scavenging free radicals expressed as Trolox equivalent antioxidant capacity (TEAC). Higher TEAC values indicate higher antioxidant activities. The TEAC results in Table 4.1 range from 12.2 to 42.1 µmol/g FW, which are higher than the Trolox equivalent (TE) values determined in the supernatant of Concord grape slurry (5 to 20 µmol TE/g FW) [83]. As shown in Table 4.1, HPP and HT treated samples had higher TEAC values than fresh-made control samples, probably

due to releasing of bound phenolic substances after treatment. No significant differences were found between HPP and HT treated samples in antioxidant activity on day 1 and after 4-month refrigerated storage. After 4 months, the antioxidant capacity in both HPP and HT treated samples remained at similar level as in freshly made control puree.

Correlations between antioxidant content (TP and TMA) and antioxidant activity (DPPH and ABTS) were checked by the Pearson's correlation test. The correlations between antioxidant contents and DPPH were not significant (R=0.185 for TP and R=0.284 for TMA). The ABTS results showed stronger correlations (R=0.661 and 0.604, respectively) with both TP and TMA at a significance level of 0.01. Previous studies have reported that antioxidant activity had strong correlation with phenolic compounds in fruits, grape juice and red wine [84–89]. In our study, the ABTS assay seemed to be a more precise method in determining the antioxidant capacity of the puree product. Floegel et al.[88] also reported the ABTS assay as a better method compared to DPPH to assess the antioxidant activity of fruit, especially those rich in pigments and hydrophilic antioxidants, after testing 50 antioxidant rich fruits, vegetables and beverages. In the evaluation of 16 red grape cultivars, Orak [3] reported that antioxidant activity had significant positive correlations with total phenolics content (R=0.806) and total anthocyanins (R=0.455) (p=0.01). However, Kallithraka et al.[89] reported that antioxidant activity had statistically insignificant correlation with total anthocyanin content after analyzing the grape skin extracts from 17 red grape varieties. In our study, as the Concord grape puree contains the whole grape with skin, pulp and seeds, it is reasonable that the complex variety and rich amounts of phenolic compounds in the puree matrix led to significant positive correlations not only between antioxidant activity and total phenolics, but also between antioxidant activity and total anthocyanins.

3.4 PPO and POD enzyme activity

The effects of high pressure and thermal treatment on the PPO and POD enzyme activity of puree are presented in Figures 4.4a and 4.4b respectively. Compared to the PPO and POD activity in freshly-made control sample, the PPO residual activity in HPP and HT samples were 75.2 ± 7.4% and 22.6 ± 2.4%, while the POD values in HPP and HT samples were 80.7 ± 6.8% and 10.2 ± 1.1% respectively. During refrigerated storage, both PPO and POD enzyme activity in puree samples did not change significantly. The significantly lower enzyme activity in HT samples indicates that the oxidative enzymes are more sensitive to heat than high pressure in the grape puree. Similar inactivation effects of HPP treatment on oxidative enzymes had been reported in previous studies. Castellari et al. [43] reported that the Trebbiano grape still had 90.7 ± 5.6% PPO activity after HPP (600 MPa, 6 min, 4-22 °C) treatment. Yen and Lin [90] conducted HPP (600 MPa, 25 °C, 15 min) on guava puree and achieved a residual activity of 63% for PPO and 74% for POD; during a 60-day storage at 4 °C, the PPO and POD activity increased gradually and reached 81% and 83%, respectively. Chakraborty et al. [91] reported that 25% inactivation of PPO and POD activity by HPP (500 MPa, 15 min, 30 °C, pH 3.5) can be achieved in treated pineapple puree. Consequently, the total phenolics content and antioxidant activity (by ABTS assay) decrease significantly after a refrigerated storage of 4 month in HPP samples as shown in Section 3.3. However, the overall quality and antioxidant activity of HPP treated puree remained comparable to the freshly made samples even after 4-month storage, suggesting that HPP is able to preserve the grape puree quality despite its inefficiency in enzyme inactivation.

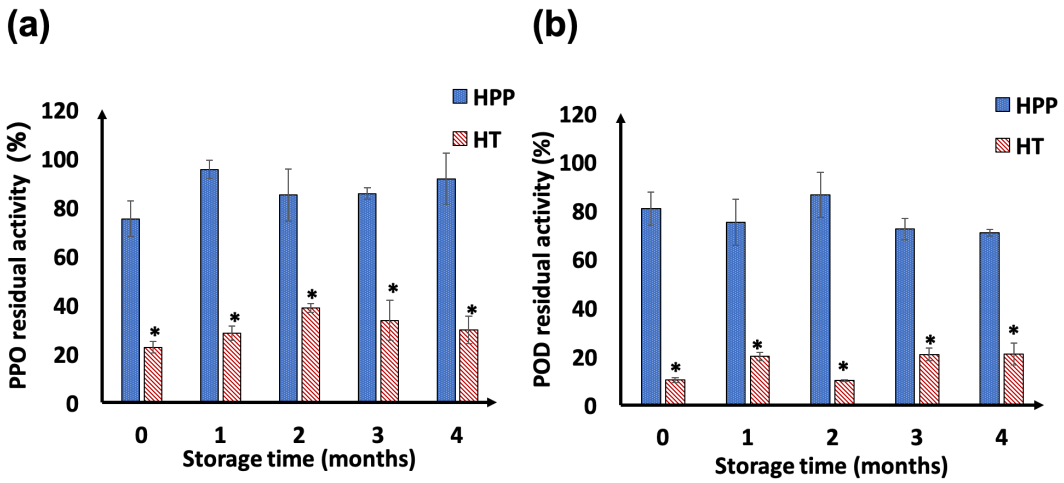

Figure 4. 4 Changes of PPO (a) and POD (b) residual enzyme activities in HPP (600 MPa, 3 min) and HT (63 °C, 3 min) treated puree during 4-month storage at 4 °C.

[a]Denotation of "*" indicates significant difference between different treatments at each sampling point ($p<0.05$).

3.5 Proximate composition analysis

The aim of this study is to produce a nutritious grape product that utilize all the potential bioactive components of the Concord grape with minimal processing and no waste. Therefore, an analysis of the composition and nutritional values of the puree product is imperative. Proximate composition and the fatty acids profile of the untreated control samples with seeds (C/W), untreated samples without seeds(C/O), HPP treated samples with seeds (HPP/W) and HT treated samples with seeds (HT/W) are presented in Table 4.2 and Figure 4.5, respectively. The moisture and dry matter content in puree made with seeds was around 75% and 24%, respectively. When analyzed on fresh basis, the moisture content in C/O was significantly higher than samples made with seeds, and accordingly, the dry matter in C/O was significantly lower than samples made with seeds ($p<0.001$). Crude protein, crude fiber, total fatty acids and rumen unsaturated fatty acids (RUFAL) values in puree samples made with seeds were significantly higher than C/O samples($p<0.001$). Crude fiber content in puree samples made with seeds was about 5 times higher than the value in samples made without seeds. Despite crude fiber, grape pomace also contains antioxidant dietary fiber, such as condensed tannins, which are the main constituent (16% in white grape seeds) of nonextractable polyphenols in grape pomace [92]. These polymetric tannins can lower the cholesterol absorbed by the rats fed with high-cholesterol diets [93], showing potential use in preventing cardiovascular disease. HPP/W and HT/W samples had significantly higher phosphorus content than C/O sample ($p<0.05$); and HPP/W sample had higher copper and manganese contents than both C/W and C/O samples ($P<0.05$). No significant differences among water soluble carbohydrates (WSC), ash, magnesium, potassium, sodium, iron, zinc and molybdenum were found among different groups. Grape seeds account for 38-52% dry weight of pomace [94]. The grape seeds are mainly (w/w) composed of 40% fiber, 16% fatty acids, 11% protein, 7% phenolic compounds, and others minor components including sugars and minerals [95]. Grape pomace consists of over 60% (dry matter) of indigestible components, including not only dietary fiber, but also condensed tannin and resistant protein, which is unusual compared to other dietary fiber rich vegetables; moreover, these unique tannins and protein show distinct physiological and nutritional properties [92]. Therefore, incorporating seeds and skin in the puree

product significantly improved the contents of functional components which resulted in a healthier product.

When analyzed as dry matter (supplementary Table S2), the crude protein, potassium and manganese content in C/O samples were significantly lower than that in the puree samples made with seeds, regardless of the treatment ($p<0.05$). Calcium, phosphorus, crude fiber, total fatty acids and RUFAL in puree samples made with seeds were significantly higher than C/O samples ($p<0.001$). Insignificant differences of WSC, ash, sodium, iron, zinc and molybdenum among different groups were found.

Table 4. 2 Proximate composition analysis of Concord grape puree made by different processes.

	Fresh basis			
	Control / W	**Control / O**	**HPP / W**	**HT / W**
Moisture (%)	75.90 ± 0.33 a	78.40 ± 0.16 b	75.33 ± 0.12 a	75.07 ± 0.38 a
Dry matter (%)	24.10 ± 0.33 a	21.60 ± 0.16 b	24.67 ± 0.12 a	24.93 ± 0.38 a
Crude protein (%)	0.93 ± 0.05 a	0.70 ± 0.00 b	0.90 ± 0.00 a	0.97 ± 0.05 a
Crude Fiber (%)	2.53 ± 0.05 a	0.53 ± 0.05 b	2.50 ± 0.00 a	2.53 ± 0.09 a
WSC (%)	14.43 ± 0.52 A	13.97 ± 0.73 A	14.67 ± 1.05 A	15.80 ± 1.44 A
Total Fatty acids (%)	0.29 ± 0.02 a	0.06 ± 0.01 b	0.28 ± 0.02 a	0.32 ± 0.01 a
RUFAL (%)	0.24 ± 0.02 a	0.05 ± 0.01 b	0.23 ± 0.02 a	0.27 ± 0.01 a
Ash (%)	0.93 ± 0.09 A	1.14 ± 0.22 A	0.90 ± 0.12 A	1.13 ± 0.09 A
Calcium (%)	0.02 ± 0.00 A	0.01 ± 0.00 B	0.02 ± 0.00 A	0.02 ± 0.00 A
Phosphorus (%)	0.02 ± 0.00 AB	0.02 ± 0.00 A	0.03 ± 0.00 B	0.03 ± 0.00 B
Magnesium (%)	0.01 ± 0.00 A	0.01 ± 0.00 A	0.01 ± 0.00 A	0.01 ± 0.00 A
Potassium (%)	0.34 ± 0.01 A	0.35 ± 0.00 A	0.32 ± 0.02 A	0.36 ± 0.00 A
Sodium (%)	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A
Iron (ppm)	6.33 ± 3.30 A	2.67 ± 0.94 A	5.00 ± 0.82 A	3.67 ± 0.47 A
Zinc (ppm)	<1.00 A	<1.00 A	<1.00 A	<1.00 A
Copper (ppm)	1.00 ± 0.00 A	1.00 ± 0.00 A	2.00 ± 0.00 B	1.67 ± 0.47 AB
Manganese (ppm)	3.00 ± 0.00 A	2.00 ± 0.00 B	3.00 ± 0.00 A	3.00 ± 0.00 A
Molybdenum (ppm)	<1.00 A	<1.00 A	<1.00 A	<1.00 A

[a]Different uppercase letters indicate significant differences among different treatments ($p<0.05$); different lowercase letters indicate significant differences among different treatments ($p<0.001$). Control/O: untreated samples without seeds; Control/W: untreated samples with seeds; HPP/W: HPP (600 MPa, 3 min) treated samples with seeds; HT/W: HT (63 °C, 3 min) treated samples with seeds; WSC: water soluble carbohydrate; TFA: total fatty acids; RUFAL: rumen unsaturated fatty acids. "-" means not determined.

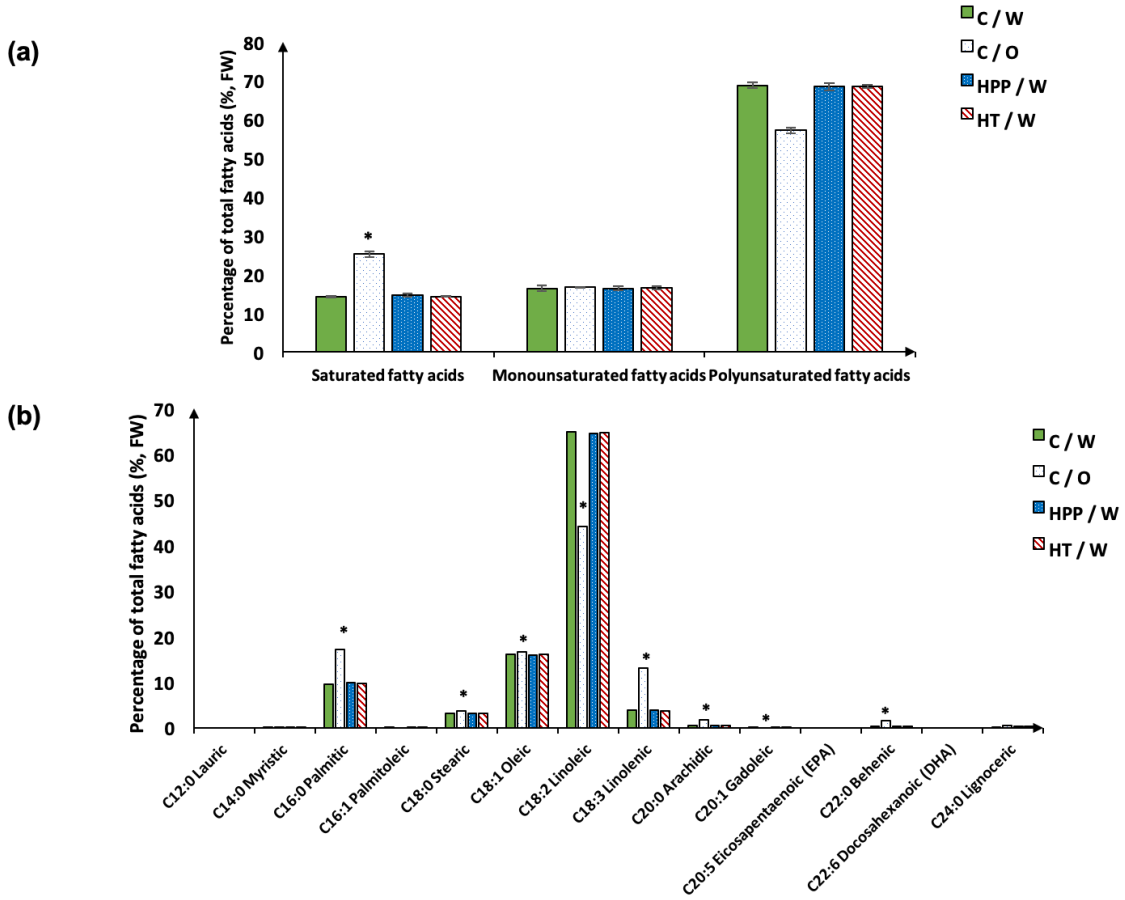

Figure 4. 5 Total fatty acids profile: percentage of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (a) and percentage of individual fatty acid (b) in Concord grape puree made by different processes.

[a]Denotation of "*" indicates significant difference among different treatments ($p<0.001$). FW: fresh basis; C/O: control puree made without seeds; C/W: Control puree made with seeds; HPP/W: HPP(600 MPa, 3 min) puree made with seeds; HT/W: HT (63 °C, 3 min) puree made with seeds.

As shown in Figure 4.5, in grape puree samples made with seeds, polyunsaturated fatty acids (PUFAs) account for 68% (w/w) of the total fatty acids, representing almost 5 times higher than the amount of saturated fatty acids, while this ratio was only about 2 times in seedless puree. The most abundant fatty acid in the Concord grape puree was linoleic acid (LA), which counted for 65% in the puree made with seeds and 44% in the C/O samples. Lutterodt et al.[96] reported that LA was the major fatty acid, accounting for 75.3% (w/w), in the cold pressed Concord grape seed oil. PUFAs are essential nutrients and have important effects on prevention and treatment of coronary heart disease, while LA, also known as omega-6, is the primary and the basis of the n-6 fatty acids of PUFAs [97]. C/O samples had significantly lower level of LA than those made with seeds ($p<0.001$), suggesting that mixing grape seeds in the whole Concord grape puree product provide more dietary and nutritional benefits, especially in providing essential fatty acids, compared to the traditional fruit puree product.

3.6 Sensory study

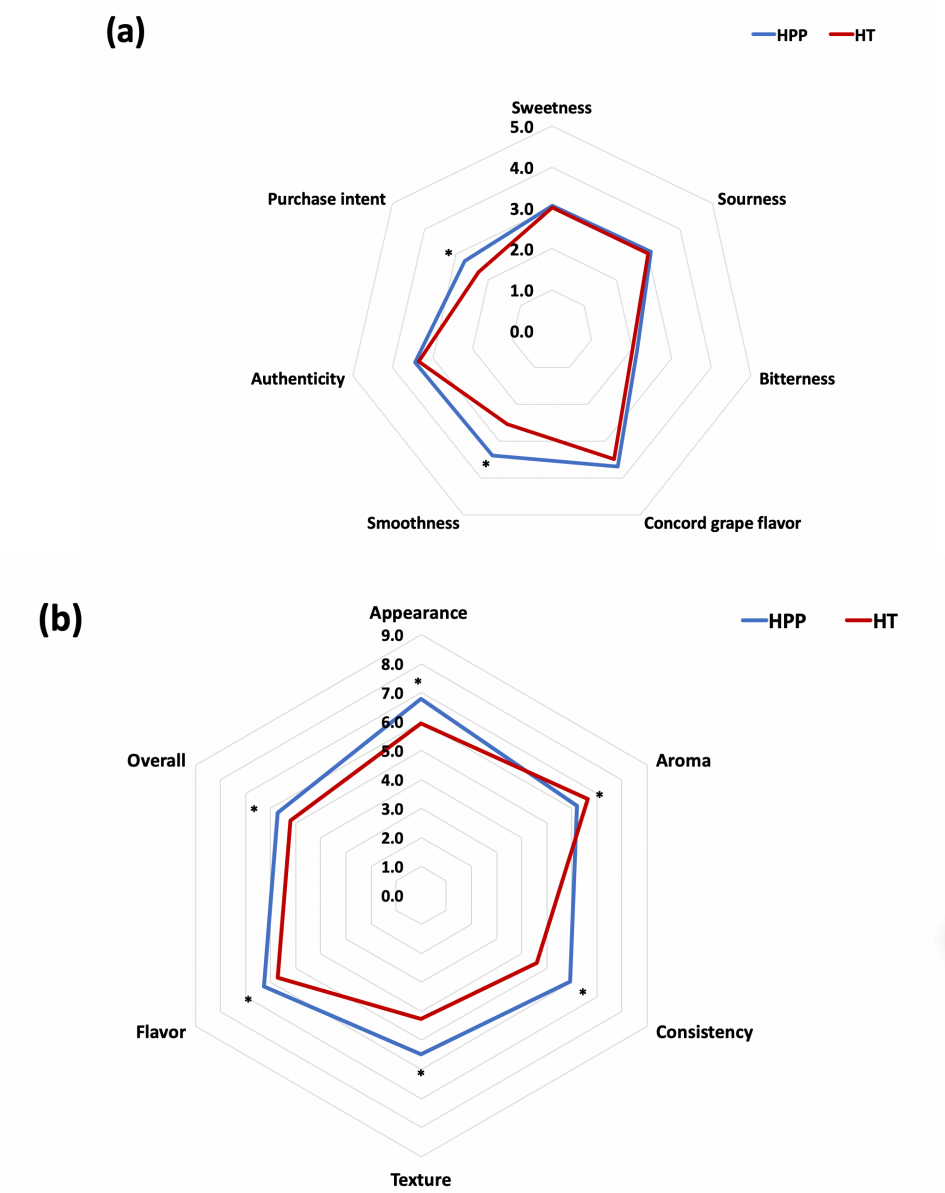

Figure 4. 6 Sensorial attributes of HPP (600 MPa, 3 min) and HT (63 °C, 3 min) treated puree: intensity of different characteristics (a) on a 5- point hedonic scale (1= low, 5= high) and consumer liking (b) on a 9- point hedonic scale (1= dislike it extremely, 9= like it extremely)

[a]Denotation of "*" indicates significant difference between different treatments (n=101, $p<0.05$).

Sensory evaluation of puree products was conducted to assess the consumer acceptability of whole Concord grape puree samples (Figure 4.6). According to Figure 4.6b, HPP treated sample obtained significantly higher scores than HT treated samples in all liking categories except for aroma. The higher level of aroma perceived in the HT samples was probably induced by the higher hydrolysis rate of aroma precursors during heating. It was reported in apple juice and nectar that pasteurization (80 °C, 2 min) led to the development of more aromatic compounds [98]. Moreover, consumers' familiarity with the traditional cooked flavor may have impacted the aroma ratings.

After 4-month storage, however, HPP treated puree still had the attractive "foxy" (floral and fruity) aroma of Concord grape, while little aroma could be detected in heated samples, based on informal evaluation. Sweetness, sourness and flavor are important attributes in evaluating the fruity taste of fruit products. There were no significant differences in all tested intensity categories except for smoothness and purchase intent (Figure 4.6a). HPP (600 MPa, 3 min) is able to provide similar taste profile as that in the heated samples, as there were no significant differences in the sweetness and sourness ratings. Moreover, the low bitterness rating indicated that inclusion of seeds and skin in the product did not compromise the taste profile as it contains skin and seed tannins. The most obvious difference between HPP and HT treated samples was based on texture which affected the consistency, visual color, mouth-feel and appearance. HPP treated samples had significantly higher ratings in "smoothness" than HT treated samples ($p<0.05$). For the color attribute, color ratings using the color board showed that HPP sample was perceived as darker (79.2 % panelists chose the darkest color on the board) compared to the HT sample (74.3 % panelists chose the darkest color on the board). When assessing color preference by the JAR scale, there was no significant difference (for HT sample the value was 2.5 ± 0.6, while HPP was 2.4 ± 0.7). When comparing the appearance (supplementary Figure S2), HPP treated puree had better consistency, more homogenous appearance, while the HT treated sample had uneven surface (lumpy appearance). These results led to a decline in consumer acceptability for the HT treated puree according to the panelists' comments. HPP samples had significantly higher overall product liking, purchase intent and product preference ranking (59% panelists ranked it first) than the HT samples ($p<0.05$). The HPP treatment delivered a whole Concord grape puree product with better consumer acceptability than the conventional thermal processing, although the sensory results indicate that the puree is more suited as an ingredient (the traditional use) than as a stand-alone product, due to the positive but modest ratings obtained.

4. CONCLUSIONS

This study compared the quality of HPP (600 MPa, 3 min, 5 °C) and HT (63 °C, 3 min) treated whole Concord grape puree during refrigerated storage. HPP was more effective in achieving lower microbial counts, thus providing extended shelf-life of at least 5 months. There were no significant changes in color values, total phenolic and total anthocyanins contents between HPP and HT samples after 4-months of refrigerated storage. HPP treated puree had significantly higher overall liking and purchase intent due to its fresh-like appearance and better consistency, compared to the thermally treated puree, while providing similar taste profile. Proximate composition analysis revealed that incorporating seed and skin in the whole Concord grape puree significantly increased the crude fiber, protein, total fatty acids and linoleic acid contents, while eliminating waste. This study provides a sustainable way to create a bioactive compounds rich product containing carbohydrates, protein, fiber and essential fatty acids. However, the textural and flavor changes observed after different processes and during refrigerated storage need to be further investigated. Future studies are needed to better understand the nutritional and sensorial quality of this novel product, such as the effect of soluble and insoluble tannins on digestibility and gut microbiome, textural changes due to pectin methylesterase activity, as well as changes in aroma compounds.

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AUTHOR CONTRIBUTIONS:

O.P. conceptualize the research project and funding acquisition; Y.L. performed the experiments, analyzed the data, drafted the paper; O.P. reviewed and edited the manuscript.

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CONFLICT OF INTERESTS:

The authors declare no conflict of interest.

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CHAPTER 5

EVALUATION OF DIFFERENT DRYING METHODS IN DEVELOPING A DEHYDRATED WHOLE CONCORD GRAPE SNACK

ABSTRACT

To develop a novel dehydrated whole Concord grape snack with intact shape and crunchy structure, three drying methods were evaluated in this study: freeze-drying (FD), hot air drying at 70 °C (HAD) alone or combined with microwave vacuum drying (MVD) at 1 W/g (MVD-1), 1.5 W/g (MVD-1.5), and 2 W/g (MVD-2), respectively. Drying method efficacy, physico-chemical properties of dried samples and rehydration properties were compared. The retention of antioxidant contents and antioxidant capacity were investigated during six months of storage time. Freeze-drying, as the gold standard, produced samples with the best quality, especially in color, shape, rehydration property and antioxidants contents. However, drying time for FD was the longest. Sequentially using MVD after concentrating samples by hot air, greatly reduced the drying time and produced product with better qualities than HAD alone. Compared to HAD samples, MVD caused significant changes: lower hardness, higher antioxidants contents (35% higher total phenolics, 150% higher total anthocyanins), higher crude protein and copper content ($p<0.05$). Moreover, MVD was able to produce puffed products with better powder and rehydration property. Samples produced using whole Concord grape (including seeds and skins) had significantly higher fiber and fatty acids contents compared to samples produced without seeds ($p<0.05$).

1. INTRODUCTION

Concord grape (*Vitis labruscana*) is a commercially important grape variety that has mainly been used to make juice, jams and jellies in US. However, its production and price has been affected, despite the oversupply and weather, by consumers' preferences of less carbohydrate-rich products. Current popular grape products, namely juice and jellies do not contain grape seeds and skins. However, polyphenols, which have antioxidant activity, are mostly stored in grape skins (28-35 %) and seeds (60-70 %) (1). Moreover, grape seeds contain fiber, protein and fatty acids (2); grape skin contain high level of anthocyanins which can be used as a FDA-approved natural colorant. Therefore, using the whole Concord grape, including seeds and skin, could add dietary fiber, protein, essential fatty acids and more polyphenolics in the naturally high sugar Concord grape based products, contributing to a more balanced and healthy food matrix.

Currently, most of the dried grape products commercially available are either raisins produced from seedless varieties, or dried wine grape pomace, which requires further grinding into powder (3,4). Threfore, there is a unique opprotunity to develop a novel dehydrated product using whole grapes (including seed and skin).

Traditionally, grapes are dried by solar energy. Solar drying is the oldest drying method for raisin production with many disadvantages, such as microbial deterioration, debris contamination, and most of all the difficulties posed by inclement weather. Alternatively, controllable convective drying uses hot air to remove the moisture from the surface, which its efficacy largely depends on temperature and air velocity. With a higher air temperature, less

drying time is required, whereas nutrient degradation can happen at a more vigorous rate. Shrinkage and case hardening are also undesirable changes in hot-air dried fruit products (5).

Freeze drying, on the other hand, removes water from frozen fruits by sublimation, which preserves color, texture and nutrients and produces products with premium quality (6). However, this process requires more energy and longer time than other traditional methods because of the refrigeration and vacuum systems. As the capital cost of freeze drying is high, it is often used to produce dehydrated products that are of high quality and high value, such as pharmaceutical herbal products.

In recent studies, microwave-vacuum drying (MVD) has gain interest in preparation of dried products. For example, a microwave vacuum dryer system was first used in California to produce puffy dried grapes (7). Microwave vacuum drying requires less processing time while drying fruits and vegetables at much lower temperature than hot air drying, hence it protects the nutrients and color of food substrates. On one hand, food substrates are heated quickly by the electromagnetic waves in the microwave because of dipole rotation and ionic conduction (8). On the other hand, low pressure in the MVD decreases the water boiling temperature which allows the food to be dehydrated at lower temperature (9). Quality of dehydrated products produced by microwave-vacuum drying is comparable to freeze drying, and is better than that dried by convective drying (10).

Drying fruit puree is difficult as they are sugary, juicy, viscous and can be very sticky during drying. Foam mat drying can be used for drying fruit puree, such as drying blue honeysuckle berry (11). However, foaming agents and stabilizers are required for formulating the puree. Moreover, the end products need to be further grinded into powder. Alternatively, hot air drying can be used in the first stage of drying in which the drying rate is very high. When the drying rate decreased in the second stage, more energy and longer time are required to remove the bound water. It is reported two thirds of the drying time is required to remove the smaller portion of moisture in the second stage of hot air drying (12). On the other hand, it is not feasible to directly dry fruit puree in a microwave vacuum dryer due to the splashing of puree caused by the violent evaporation of water. Thus, using MVD sequentially after HAD is fesasible, and more efficient, to dry fruit puree without adding other agents. Moreover, MVD can be used to create puffed berry products (13).

The aim of this work was to develop a dried whole Concord grape snack with crunchy texture that could also be used as a drink mix or ingredient in other products. Freeze-drying, hot air drying alone or sequentially combined with microwave vacuum drying were used and compared. Drying efficacy, microbial reduction, changes in physico-chemical properties and compositions were compared and evaluated. The retention of bioactive compounds and antioxidant activity were investigated during a six-month shelf-life study at room temperature.

2. MATERIALS AND METHODS

2.1 Raw materials and chemicals

Concord grapes (*Vitis Labruscana)* were harvested in the finger lakes region (NY, USA) and frozen in a cold room at -20 ± 1 °C before processing. All chemicals used in this study were of analytical grade.

2.2 Dehydrated Concord grape snack production and experimental design

Frozen whole Concord grapes (including seeds and skin) were thawed at room temperature for 2 hours and processed in the pilot plant (Cornell Agritech, Geneva, NY) according to Figure 5.1.

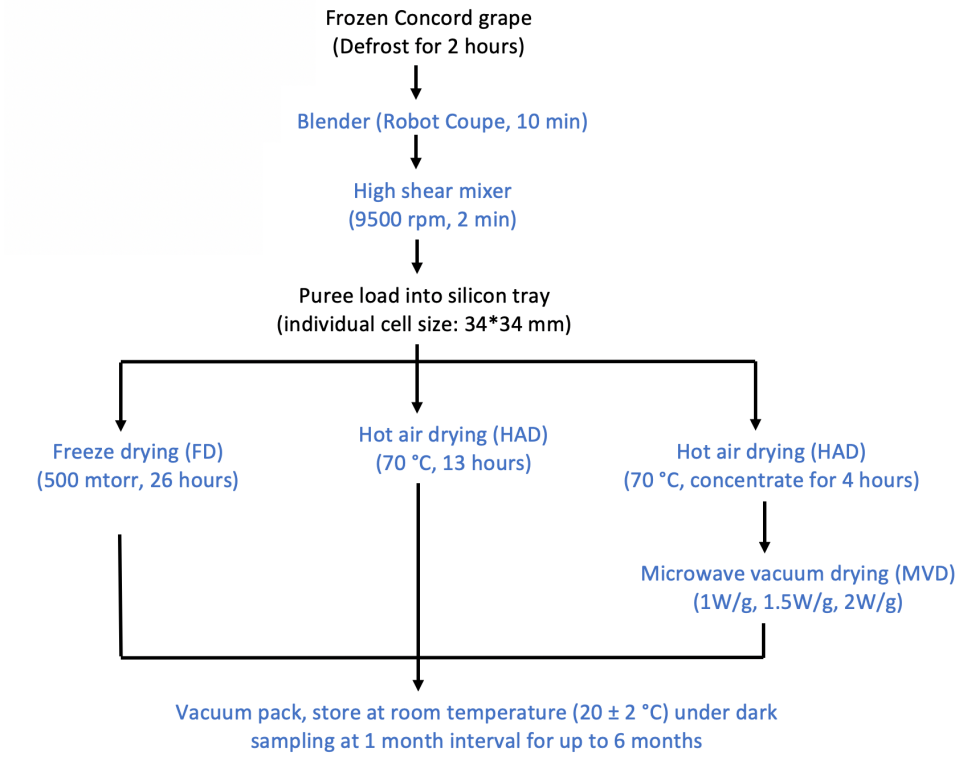


Figure 5. 1 Flow diagram for developing dehydrated whole grape snacks.

Cold grapes were pureed in a food processor (R302V, Robot Coupe USA. Inc., Ridgeland, MS, USA) for 10 minutes at high speed. After grinding, the grape seeds were crushed into small pieces. Then a Ross high shear mixer (HSM-100LSK-I, Charles Ross & Son Company, Hauppauge, NY, USA) was used to further process the puree at 9800 rpm for 2 min, after which the temperature was below 20 °C. After processing, the puree samples were transferred into silicone ice cube trays to be further dehydrated. For each cell, 7.4 g puree was loaded (15 cells per tray). The size of one cell is 3.4 mm*3.4 mm, and the height of sample was about 6 mm.

Hot air drying was carried out using a digital touch screen food dehydrator (Model D-20, The Sausage Maker Inc., Buffalo, NY). Temperature was controlled at 70 °C, relative humidity was around 4 % and fan speed was set at high. Tray locations were altered every hour to decrease the impact of temperature and air velocity differences within the dehydrator. A total drying time of 13 hours was able to achieve the endpoint with weight change less than 0.5 g per tray. At least three samples were randomly selected every hour to determine the moisture content and water activity during drying.

Freeze-drying was carried out by a freeze dryer (Harvest Right, Salt Lake City, UT, USA). Samples were freeze dried at a final shelving temperature of 55 °C and vacuum of 500 mTorr. The total processing time for freeze-drying was about 26 hours.

Microwave vacuum drying was conducted using a 10 kW EnWave microwave vacuum dryer (pilot scale, EnWave, Delta, BC, Canada). A rotating system was included to decrease hot spots, the rotating speed was set at 35%. Whole grape puree was first concentrated by hot air

drying for 4 hours, after which the moisture content is reduced from 76 % to 43 %. Then the concentrated puree samples were subject to microwave vacuum drying at different microwave energy levels (1 W/g, 1.5 W/g and 2 W/g). Samples were taken out and measured at set time intervals until the weight changes of any tray was less than 0.5 g.

All dried grape samples were vacuum packed (Vacmaster, VP215, Overland park, Ks, USA) in vacuum bags to avoid moisture absorption. Samples were kept at ambient temperature (20 ± 2 °C) under dark for a shelf-life study up to 6 months.

2.3 Microbial counts reduction

Total plate count (TPC), yeast and molds (Y&M) count were analyzed to investigate the microbial reduction effect of different drying methods by sampling puree samples before and after drying. A total weight of 25 g of puree or dried samples were diluted (1:10 w/w) in 0.1% sterile peptone water and homogenized at 200 rpm for 1 min in a stomacher (Stomacher 400 Circulator, Seward Medical, London, UK) at ambient temperature. The homogenized solution was pour-plated on Plate Count Agar (PCA, CM0325, Oxoid) for TPC, and Potato Dextrose Agar (PDA, Alpha Biosciences) for Y&M, following by incubation at 30 °C for 48 to 72 h. The pH of PDA medium was adjusted to 3.5 using tartaric acid. Results were expressed as log CFU/g.

2.4 Drying efficacy of different drying methods

Samples before, during and after drying were randomly taken at least in triplicates for moisture content analysis. Moisture content was determined by drying samples at 105 °C in a convection oven (VWR 1310E, Sheldon manufacturing, Inc, Cornelius, OR, USA) till constant weight. Samples were equilibrated in the desiccator when cooling down and then weighted using an analytical balance (A & D weighing, HR-120, San Jose, CA, USA). Moisture content was calculated according to equation (1):

$$\boldsymbol{M_c\,(moisture\ content,\%) = \frac{M_0 - M_t}{M_0} * 100} \qquad (1)$$

Where M_t is the final weight after drying, M_0 is the sample weight before drying.

Water activity was measured using a water activity meter (Aqua Lab Dew Point Water Activity Meter 4TE, Decagon, Pullman, WA, USA). Surface temperature was monitored using an infrared thermometer (lasergrip 1080, Etekcity, Vesync Co., Ltd, CA, USA).

2.5 Physicochemical properties analyses

2.5.1 Appearance and texture

Color was determined by a colorimeter (HunterLab, VI, Hunter Associate Laboratory Inc., VA, USA) and expressed as L^*, a^* and b^* values. The values of the absolute color difference of samples were calculated according to equation (2):

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \qquad (2)$$

where L_0 , a_0 , b_0 are the color measurements of FD samples, L, a, b are the color measurements of HAD and MVD treated samples.

Length and thickness of the final dried samples were measured using a digital caliper (Electron Microscopy Sciences, PA, USA) with 0.01 mm precision. For length, at least 6 measurements were taken. For thickness, 6 samples were stacked and measurements were taken from 3 different spots.

Apparent density ($\boldsymbol{\rho_a}$) was calculated by dividing the weight (g, A & D weighing, HR-120, San Jose, CA, USA) of ten samples by their corresponding volume ($\boldsymbol{cm^3}$); sample volume was determined by volume difference occupied by millet with and without 10 samples in a 100-mL beaker using a graduated cylinder:

$$\boldsymbol{\rho_a = \frac{m}{V}} \qquad (3)$$

True density ($\boldsymbol{\rho}$) and porosity ($\boldsymbol{\varepsilon}$)was calculated according to Silva-Espinoza et al. (14). True density ($\boldsymbol{\rho}$) was calculated according to equation (4):

$$\boldsymbol{\rho = \frac{1}{\frac{X_W}{\rho_W}+\frac{X_{CH}}{\rho_{CH}}}} \qquad (4)$$

Where $\boldsymbol{X_W}$ $\boldsymbol{and}$ $\boldsymbol{X_{CH}}$ are the mass of water and carbohydrates in the dehydrated samples respectively; $\boldsymbol{\rho_W}$ (0.9976 $\boldsymbol{g/cm^3}$) and $\boldsymbol{\rho_{CH}}$ (1.4246 $\boldsymbol{g/cm^3}$) are their corresponding densities.

Porosity ($\boldsymbol{\varepsilon}$) was calculated according to equation (5):

$$\boldsymbol{\varepsilon}\ (\%) = \left(\mathbf{1} - \frac{\rho_a}{\rho}\right) * \mathbf{100} \qquad (5)$$

Hardness was determined by using a texture analyzer (TA-XT2, Texture Technologies Corp, NY, USA). The dehydrated sample was cut in half on the slotted insert supported with BHK blade. The test speed was 1 mm/s and puncture depth was 5 mm. Hardness was expressed as the peak force of compression. At least three measurements from one batch of treatment were made and the mean value was reported as hardness in kilogram.

2.5.2 Rehydration properties: water solubility and water absorption index, total soluble solids content (SSC), pH, titratable acidity (TA) and particle size distribution (PSD) of rehydrated samples.

Dehydrated grape samples were grinded into fine powder using an electric grinder (Mr. Coffee, IDS 77, Sunbeam Products, Inc., Boca Raton, FL, USA). Powder was mixed with DI water on a ratio of 1:10 (w/w) in a 50-mL centrifuge tube. After brief vortexing, all centrifuge tubes were put into a water bath shaker (SW22, Julabo GmbH, Seelbach, Germany) and rehydrated at 30 °C and 200 rpm overnight. Rehydrated samples were used for SSC, pH, TA and PSD.

Water solubility index (WSI) and water absorption index (WAI) were determined according to the method described by Grabowski et al (15) with some modifications. A total

mass (m_o) of 3 g powder obtained as described above was mixed with 30 mL DI water in a 50-mL centrifuge tube. All samples were equilibrated at 30 °C and 200 rpm for 30 minutes in the water bath shaker (SW22, Julabo GmbH, Seelbach, Germany). After homogenization, samples were centrifuged at 10,000 rpm for 15 minutes. Supernatant was decanted into aluminum weighing pan and then dried at 105 °C for 4 hours (m_d). The mass of remaining solids after centrifugation was recorded as m_s. WSI was calculated as percentage of m_d in m_o, while WAI was calculated as percentage of m_s in m_o.

Soluble solids content (SSC) was determined using a refractometer (model 300055, Sper scientific, Scottsdale, AZ, USA). Five milliliters of rehydrated sample were filtrated using No. 4 qualitative filter paper (Whatman Inc., Maidstone, United Kingdom). Then 2 drops of filtrate were added to refractometer to obtain °Brix readings at 20 °C.

The pH value was measured at ambient temperature using a Thermo Scientific benchtop pH meter (Orion 3 Star Series, Fisher Scientific, MA, USA). The instrument was calibrated using pH 4 and pH 7 phosphate buffer before measurements.

Titratable acidity (TA) was determined according to the description of Iland (16). Briefly, a diluted solution containing 5 **mL** of rehydrated sample and 45 **mL** of distilled water was titrated with 0.1 **mol/L** sodium hydroxide to pH 8.2 using an autotitrator (Mettler compact G20, Mettler-Toledo, LLC, OH, USA). Results were calculated based on equation (6) and expressed as gram tartaric acid equivalence per liter rehydrated sample:

$$\text{Tartaric acid } (\mathbf{g/L}) = \frac{\mathbf{V_{NaOH}\ (L) \times 0.1\ (mol/L) \times 75(g/mol)}}{\mathbf{V_{puree}\ (mL) \times 10^{-3}}} \qquad (6)$$

The particle size of rehydrated samples was measured by a particle size analyzer (Malvern Mastersizer 2000, Malvern instruments Ltd., MA). The refractive index was measured by a Leica Auto Abbe Refractometer (Leica Inc., Buffalo, NY). Distilled water was used as dispersant and the samples were measured when the concentration of added sample reached 5% obscuration. Particle size values were measured in triplicate.

2.6 Phenolic compounds content and antioxidant activity

2.6.1 Sample extraction

Extraction was conducted according to the description of Taşeri et al. (17) with some modifications. Dehydrated samples were ground into fine powder and mixed with acidified methanol (1% HCl) on a solid to solvent ratio of 1:10 (w/v). After brief vortexing, tubes were shaken at 70 rpm for 2 h at room temperature in the water bath shaker. Then samples were centrifuged at 9,500 rpm at 4 °C for 10 min. Supernatant was transferred into opaque vials and stored at -20 °C for chemical analysis.

2.6.2 Total phenolic content (PC)

Total phenolic content was determined according to Waterhouse (18) with minor modifications. Briefly, in 1580 **µL** DI water, 20 µL extract was mixed with 100 **µL** Folin-Ciocalteau reagent (Sigma-Aldrich St. Louis, MO), then mixture was equilibrated at room

temperature for 6 minutes. At last, 300 µL of 20% sodium carbonate solution was added and gently vortexed before incubating at room temperature for 2 hours under dark. Additionally, standard Gallic Acid solutions (Gallic acid anhydrous, Chem-Impex international Inc.) (0-500 mg/L) were used to create the standard curve. Absorbance was measured at 765 nm using the UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). All results were expressed as mg gallic acid equivalents (GAE) per L extract (mg/L).

2.6.3 Total anthocyanin content (AC)

Total anthocyanin content was determined by the pH differential method (19). Briefly, 100 µL extracts were diluted (25-fold) in pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) buffers separately. After vortex, the mixture was incubated at room temperature for 20 minutes. Blank was prepared using DI water. Absorbance was measured at 520 nm and 700 nm using the UV-visible Spectrophotometer. Results were calculated according to equation (7) and expressed as cyanidin-3-glucoside (cyd-3-glu) equivalents (CGE) per L extract (mg/L):

$$\text{AC (CGE, mg/L)} = \frac{A \times M_W \times DF \times 10^3}{\varepsilon \times L} \qquad (7)$$

where $A = (A_{520nm} - A_{700nm})pH_{1.0} - (A_{520nm} - A_{700nm})pH_{4.5}$; M_W, molecular weight of cyd-3-glu = 449.2 g/mol; DF (dilution factor) = 25; ε is the molar extinction coefficient = 26,900 $L^{-1} \times cm^{-1} \times mol^{-1}$ for cyd-3-glu; L (pathlength) = 1 cm; 10^3 is the conversion of g to mg.

2.6.4 In vitro antioxidant activity (free radical scavenging activity ABTS)

The in vitro antioxidant activity was determined as $ABTS^{\bullet}$ free radical scavenging activity based on the description of Re et al. (20). Briefly, 7 mM ABTS [2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt] (Sigma-Aldrich, Poole, Dorset, UK) solution was mixed with potassium persulfate (Honeywell Fluka, North Carolina, USA), which the final concentration is 2.45 mM. Then the mixture was set at dark and room temperature for 12-16 hrs to generate stable radicals. $ABTS^{\bullet}$ solution was diluted to the absorbance of 0.700 ± 0.050 at 734 nm before using. Grape extract (10 uL) was mixed with 2 mL $ABTS^{\bullet}$ solution and set at room temperature under dark environment for 6 minutes. Blank was prepared using DI water. Absorbance was measured at 734 nm using the UV-visible Spectrophotometer. Radical scavenging capacity was calculated as the percentage inhibition (%) according to equation (8):

$$\textbf{ABTS \% inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} * 100\% \qquad (8)$$

where $A_{control}$ is the absorbance of blank, A_{sample} is the absorbance of sample. Higher values of % inhibition indicates greater antioxidant activity.

2.7 Proximate composition Analysis

Dehydrated samples made with seeds (FD/W, HAD/W and MVD-1.5/W) and samples made without seeds (FD/O) were used for approximate composition analysis. The following composition analyses were performed at Dairy One Co-Op, Inc. (Ithaca, NY, USA): ash, minerals, crude fiber, crude protein, crude fat, total fatty acids and fatty acids profile.

2.8 Statistical analysis

All experiments were performed in triplicate, and the results were presented as mean values of at least 3 measurements. Data were presented as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA at a significance level of $p < 0.05$. Significant differences among mean values were determined by the Tukey's post hoc test following the one-way ANOVA test using SPSS (SPSS statistics, version 22.0, IBM, Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Microbial counts reduction

The total plate count in original puree samples was 2.85 log CFU/g. Total plate count decreased to 1.0-2.0 log CFU/g (Figure 5.2) after different drying procedures. HAD had the highest microbial counts reduction (1.88 log CFU/g), following by MVD-1.5 (1.46 log CFU/g) and MVD-2 (1.33 log CFU/g), and lastly by FD and MVD-1 (1.0 log CFU/g reduction). No presence of yeast or mold was found in all the samples. HAD samples had significantly lower total plate count compared to that in the original puree due to the long exposure time at higher temperature. No microbial growth during storage is expected as the water activity is below 0.3 in the final product samples (Figure 5.3).

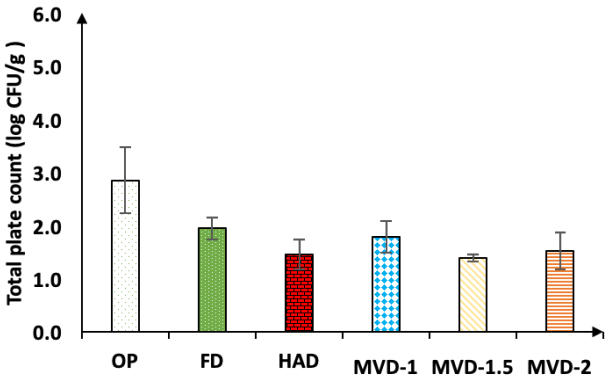


Figure 5. 2 Total plate count of whole grape puree and dehydrated samples.

[a]Data are represented as mean ± standard deviation (n=3). OP: original puree; FD: freeze-dried samples; HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

3.2 Drying efficacy analysis: moisture content (M_c), water activity(a_w) and drying rate

As expected, the total drying time for freeze drying is the longest (26 hours), followed by hot air drying (13 hours). After concentrating the samples using HAD for 4 hours, the moisture content of the puree decreased from 75.9% to 42.5%. MVD sequentially decrease the drying time from 9 hours to less than 80 minutes, depending on different energy levels. The efficacy of HAD and MVD in dehydrating whole Concord grape puree was evaluated by measuring moisture content (M_c), water activity (a_w) (Figure 5.3) and drying rate (Figure 5.4). The final moisture content in FD samples was the lowest (1.84 ± 0.03 %), followed by MVD-1.5 samples (2.98 ± 0.07 %), while the rest of the

samples (4.35-6.05 % for MVD-2, HAD and MVD-1) had significantly higher water contents than FD samples ($p<0.05$). Water activity (a_w) of dried samples ranges from 0.12 to 0.16 in all groups except MVD-2, in which the a_w value was the highest (0.26 ± 0.03). These results are similar to the reported values in dried wine grape pomace (M_c: 4.40 - 7.65 %; a_w: 0.14 - 0.42) obtained by 4 different drying methods (21). During hot-air drying, the surface temperature started to reach 50 °C after 6 hours, leading to an extended exposure (>50 °C) as long as 420 minutes, which was 8 times higher than the time in MVD-1 (50 min). Moreover, the exposure time to high temperature (>50 °C) in MVD-1.5 and MVD-2 was only about 12-18 minutes. Short exposure time during drying preserves the quality of the dehydrated samples, such as anthocyanin, as could be seen in section 3.3. Sequentially drying the sample by MVD after HAD increased the drying efficiency by dramatically decreasing the drying time from 540 minutes to 20-80 minutes. Though freeze-drying can minimize the damage of heat during drying, its efficiency, in terms of time, is the lowest.

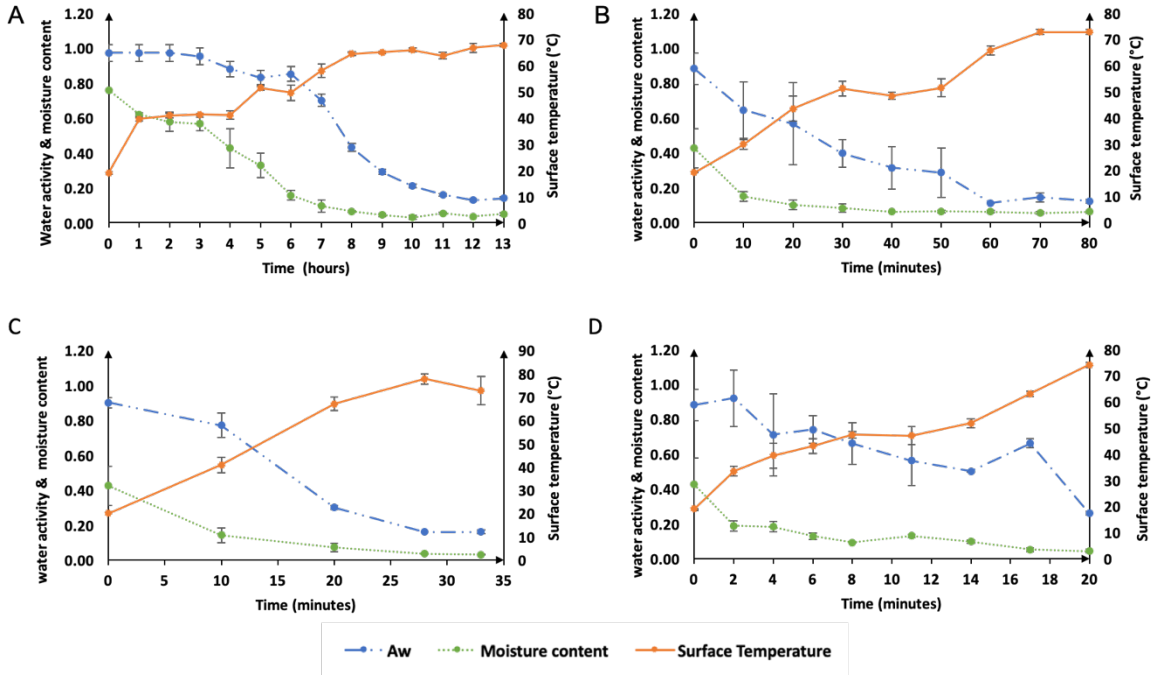


Figure 5. 3 Drying kinetics of Concord grape puree: water activity, moisture ratio and surface temperature changes of HAD (A), MVD-1 (B), MVD-1.5 (C) and MVD-2 (D).

[a]Data are represented as mean ± standard deviation (n=3). HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

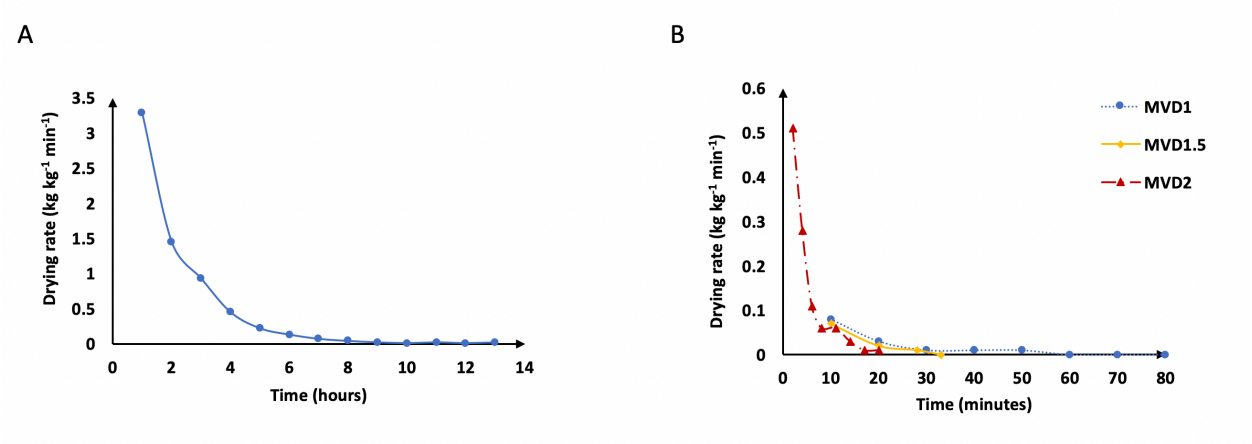


Figure 5. 4 Drying rate of hot air-dried samples (A) and Microwave vacuum dried samples (B).

The drying rate of whole grape puree for HAD and MVD kept falling during the process (Figure 5.4) and could be divided into two drying stages. First was the rapid drying stage, which can be seen from the first 5 hours for HAD and first 10 minutes for MVD (Figure 5.4). In this stage, the drying rate is high and the majority of water is removed during this stage (Figure 5.3). The water removal was much slower during the second drying stage, while the temperature was raising rapidly due to the removal of monolayer water (Figure 5.3). Additionally, the higher energy input could lead to the higher drying rate. As MVD-2 had higher energy input level, its drying rate was about 5 times higher than MVD-1 and MVD-1.5 in the rapid drying stage. Therefore, it is economical to reduce the moisture by HAD in the first stage when the drying rate was high, and sequentially drying by MVD to greatly decrease the drying time. Moreover, directly drying puree in microwave vacuum dryer is very difficult as it is juicy, sugary and viscous, which could cause violent splashing in the machine. In this study, we developed a way to dehydrate viscous semi-liquid samples with high sugar content, producing an intact squared structure instead of a powder, which had been done in former grape studies.

3.3 Physicochemical properties

3.3.1 Appearance and texture

It is evident that freeze-drying preserved the shape and color of the dehydrated sample (Figure 5.5). Distinct visual differences were caused by the different drying methods utilized. FD samples retained the red color, which was also confirmed by the color measurements (Table 5.1). As FD removes water from the grape sample at a much lower temperature than MVD and HAD, the degradation of anthocyanins is largely prevented. FD sample had significantly higher L* and a* values than the other four groups, indicating a lighter and more reddish color perceived in the FD samples. The L* value in FD samples is around 39 which is similar to that reported in the freeze-dried wine grape samples (21). The b* value in FD samples is significantly higher than HAD and MVD dried samples. The high porosity in the FD samples may allow more yellowish color detected. Liang et al. (22) reported that b* values were negatively correlated with anthocyanin content in their study of CIELAB color of berry skins, while a* values were the opposite. Using FD samples as standards, the ΔE color changes in HAD and MVD changes were above 3, which was defined as noticeable color change according to previous researcher's report

(23). In conclusion, FD preserved the red color of grape samples, while HAD and MVD samples had similar color changes, which were darker than FD samples. The unappealing dark color in HAD and MVD samples was caused by the elevated temperature which prompted enzymatic and non-enzymatic browning. Though mild heat (about 50 °C) can reduce the oxidative enzyme activities in fruits and vegetables, it has been reported that a significant reduction of PPO activity in table grapes was achieved at temperature higher than 70 °C (24). Anthocyanins may be oxidized at the beginning of the drying process by oxidative enzymes. It was reported that the oxidation cleavage of covalent bonds enhanced oxidation reactions as temperature rises, which led to higher anthocyanins degradation rate (25). On the other hand, Maillard reaction took place during the dehydration process as Concord grapes contain reducing sugars (glucose and fructose) as well as amino acids. Though the acidic condition and mild temperature are not favorable for Maillard reaction, the prolonged dehydration time and consequently concentrated substrates contributed to the generation of brown color. As for MVD, though the intensity of energy input and drying time could cause variances in the browning process, their differences were not large enough to cause significant color changes.

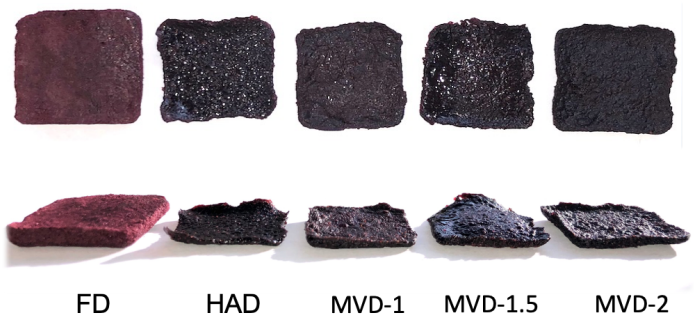


Figure 5. 5 Visual appearance and color of whole Concord grape puree dried by different drying methods: Freeze dried (FD), hot air dried(HAD) and Microwave vacuum dried (MVD) at energy input of 1 w/g (MVD-1), 1.5 w/g (MVD-1.5) and 2w/g (MVD-2).

As can be seen from Table 5.1, FD samples preserved the shape of the grape puree samples as in the ice cubes, as they had the highest thickness ($p<0.05$) and length values. The direct sublimation of water from the inner of frozen grape puree preserved the porous structure and thus caused less thickness and length change. It is well known that HAD can cause sever shrinkage in dehydrated fruit and vegetables because of water removal and heat stress on the cellular structure (26), which was also seen in our results. The HAD samples had the lowest thickness and length values. The non-uniformity in volume decrease also resulted in a large variance in the length of HAD samples. MVD samples had similar thickness and length values as seen in HAD samples.

Table 5. 1 Appearance and textural changes of dehydrated whole grape samples dried by different methods.

	FD	HAD	MVD-1	MVD-1.5	MVD-2
L*	38.85 ± 0.32 c	37.95 ± 0.42 a	37.14 ± 0.34 b	37.58 ± 0.21 ab	37.17 ± 0.46 b
a*	4.42 ± 0.72 a	1.11 ± 0.38 b	1.01 ± 0.09 b	1.14 ± 0.13 b	1.07 ± 0.13 b
b*	0.62 ± 0.29 a	0.10 ± 0.21 b	-0.05 ± 0.05 b	0.16 ± 0.14 b	-0.13 ± 0.06 b
ΔE	-	3.49 ± 0.41 a	3.88 ± 0.14 a	3.55 ± 0.20 a	3.84 ± 0.31 a
Thickness (mm)	5.69 ± 0.14 c	2.72 ± 0.21 a	3.28 ± 0.08 ab	3.72 ± 0.32 b	3.00 ± 0.27 ab

Length (mm)	32.05 ± 0.45 a	30.25 ± 2.31 ab	27.28 ± 0.63 c	28.11 ± 1.53 bc	30.3 ± 0.42 abc
Apparent density (kg/L)	0.27 ± 0.01 a	0.75 ± 0.07 b	0.56 ± 0.12 b	0.54 ± 0.04 b	0.65 ± 0.04 b
Porosity	80.0 ± 0.7 a	43.5 ± 5.1 b	57.3 ± 9.5 b	60.7 ± 2.8 ab	51.3 ± 3.1 b
Hardness (kg)	3.3 ± 1.2 a	16.2 ± 5.4 b	4.3 ± 1.2 a	3.5 ± 1.2 a	24.7 ± 6.0 c

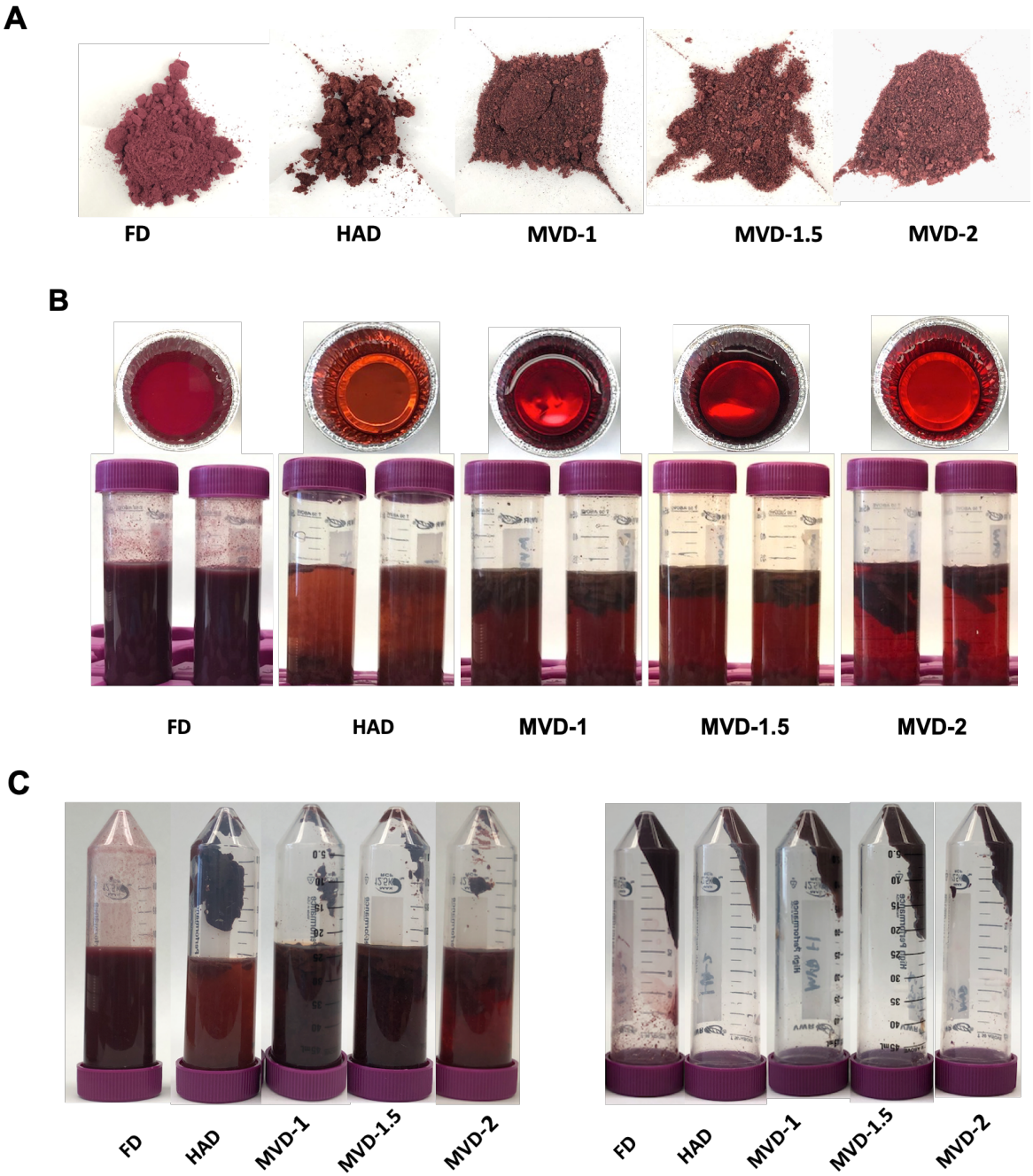
[a]Data are represented as mean ± standard deviation (n=6). Different letters indicate significant differences among samples produced by different drying methods. HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

In line with the length measurements, the apparent density of FD sample was significantly lower than HAD and MVD samples as FD sample was larger in volume and more porous (Table 5.1). MVD samples, especially for MVD-1 and MVD-1.5, in which some puffed samples were observed, had lower apparent density than HAD and MVD-2 samples. Puffed samples were created due to the expansion of air trapped in the grape leather due to the vacuum generated in the system. It was reported that microwave power, vacuum level and moisture content had significant impact on the expansion ratio of fruit leather, which could impact the texture quality, such as porosity, and hardness (13). As shown in Table 5.1, FD samples had the highest porosity, followed by MVD-1.5, MVD-1, MVD-2 and lastly HAD samples. MVD-1 and MVD-1.5 treatments were able to generate microwave vacuum puffing in dehydrated grape samples. The texture of the dried samples was determined by mechanical force and evaluated as hardness. The hardness values followed the same tendency as porosity, and even more distinct. MVD-2 and HAD samples had the highest hardness due to volume decrease and case hardening. While the porosity values in FD, MVD-1 and MVD-1.5 samples were higher, their hardness values were much smaller. FD samples can be described as brittle and easy to scatter into powder, MVD-1 and MVD-1.5 samples were brittle, while HAD and MVD-2 samples showed some resistance and deformation before breaking during texture analyses. When evaluated in sensory aspect by tasting (informal; data not shown), all samples were brittle, but FD, MVD-1 and MVD-1.5 samples were more fragile and spongier. FD sample was more easily to be consumed directly or used as an ingredient because of its texture, however, it also imposed difficulties in packaging and transportation as it could be easily crushed into pieces and thus lose its integrity. However, MVD sample had some resistance and could hold its structure during packaging and transportation, while not being hard for chewing.

3.3.2 Rehydration properties

As the dried whole grape snack could also be directly utilized as a drink mix, rehydration properties were also investigated. Porosity, shrinkage and case hardening not only affect the texture but also affect the rehydration properties. The rehydration rate, consistency and appearance are key factors that affect its viability as a drink mix. Firstly, the intact whole dehydrated grape snack was directly used for rehydration by shaking for 20 seconds. FD samples had the best rehydration rate (Figure 5.6C), consistency and appearance (Figure 5.6A & 5.6B). FD samples had the highest porosity which promoted rehydration, while the solubility in HAD

and MVD samples was decreased because of the dense structure. Rehydrated FD samples had uniform dispersion and intense red color (Figure 5.6A & 5.6B), higher soluble contents (Figure 5.6B) and continuous particle distribution (Figure 5.6C), with large proportion of smaller particles (Figure 5.6D). Rehydrated MVD samples had deeper red color and better solubility than HAD samples (Figure 5.6B), though MVD samples still had large pieces floating on the upper portion. HAD samples had lighter red color and a large portion of sedimentation at the bottom (Figure 5.6B). The dried grape snack could also be milled into powder and used as an ingredient in other products, such as muffin and drink mix. After milling, the fine powder of FD samples had the smallest particle size, while MVD and HAD had larger and more coarse particles (Figure 5.6A). HAD particles were the largest and more prone to cling together.



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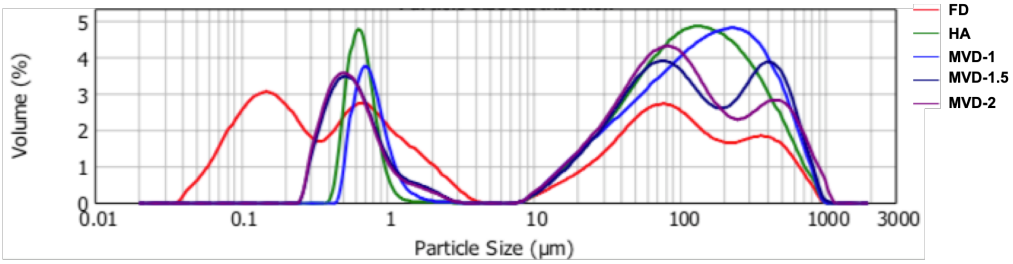

Figure 5. 6 Powder and rehydration appearance of samples produced by different drying methods: powder appearance (A); samples rehydrated for 30 minutes (bottom) and supernatant (top) (B); appearance of rehydrated samples after shaking for 20 seconds (left) and solids portion after centrifugation (right) (C); particle size distribution of rehydrated samples (D).

[a] HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

After rehydration, FD samples had the highest water solubility (Table 5.2), while MVD-1.5 had the highest water absorption index (WAI). There were no significant differences in WAI, though MVD-1.5 samples and FD samples had higher values. The larger proportion of smaller particles after grinding (Figure 5.6D) and the higher porosity (Table 5.1) of FD samples could together increase the water solubility and absorption index. Additionally, the components, especially sugars, present in the dried samples also impacted the ability to bind water molecules. However, the changes in sugar molecules were probably slight because of the moderate temperature used during MVD (Figure 5.3), which was also supported by the insignificant differences in water soluble carbohydrates contents (WSC) among all samples (Table 5.3). Water solubility was greatly affected by porosity, which was supported by the significantly higher porosity and WSI in FD samples. As for the rehydrated solution, the FD sample and some of the MVD samples had insignificant differences in pH, titratable acidity and °Brix values. On the other hand, HAD samples had significantly lower values in titratable acidity and soluble solids content, which was caused by the lower solubility.

Table 5. 2 Water absorption index, water solubility index, and pH, soluble solids, titratable acidity of rehydrated samples produced by different drying methods.

	FD	HAD	MVD-1	MVD-1.5	MVD-2
WAI (%)	113 ± 8 a	103 ± 1 a	105 ± 3 a	116 ± 4 a	100 ± 4 b
WSI (%)	73 ± 2 a	59 ± 5 b	62 ± 1 b	61 ± 3 b	64 ± 2.0 b
pH	3.38 ± 0.05 ab	3.39 ± 0.05 a	3.37 ± 0.05 b	3.38 ± 0.05 ab	3.34 ± 0.05 c
SSC (°Brix)	7.5 ± 0.1 a	6.9 ± 0.1 b	7.3 ± 0.1 ab	7.0 ± 0.1 b	7.2 ± 0.1 ab
Tartaric acid (g/L)	5.7 ± 0.1 a	4.9 ± 0.1 b	5.2 ± 0.1 b	5.2 ± 0.2 ab	5.3 ± 0.0 ab

[a] WAI: water absorption index; WSI: water solubility index; SSC: soluble solids content; FD: freeze-dried samples; HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

3.4 Bioactive compounds and antioxidant activity

The retention of bioactive compounds and their antioxidant activity is one of the most important factors in evaluating the different drying methods used in this study. As shown in Figure 5.7, FD samples had the highest total phenolic and total anthocyanins content, and consequently highest antioxidant capacity. There were no significant differences between FD and MVD-1.5 samples in terms of total phenolics content on day 1 and during storage (Figure 5.7A), while HAD had the lowest values. During storage, no significant changes in total phenolics were found in FD and MVD-1 samples, while significant increases were found in the other groups. The significant changes were probably caused by changes of phenolic compounds during storage. As for total anthocyanins, FD samples had significantly higher values than the rest of treatments on day 1 and during storage. For MVD samples, drying at 2 W/g preserved more anthocyanins due to the shorter drying time. On the other hand, anthocyanins content generally did not differ significantly for MVD-1 and MVD-1.5 samples during storage. The stability of anthocyanins is greatly impacted by the magnitude and duration of the thermal treatment (25). Thus, HAD samples had significantly lower anthocyanins content than FD and MVD-2 samples on day 1. During storage, anthocyanins content in all groups generally did not change significantly, except for a few sampling points probably due to high sample variance. For antioxidant content, FD samples had significantly higher percentage of inhibition of free radicals, followed by MVD-2, and lastly HAD samples. Drying at 1.5 W/g caused more variations in the samples which could be seen in Figure 5.7. Therefore, MVD-2 might be a more appropriate method in terms of preserving antioxidants as well as saving time.

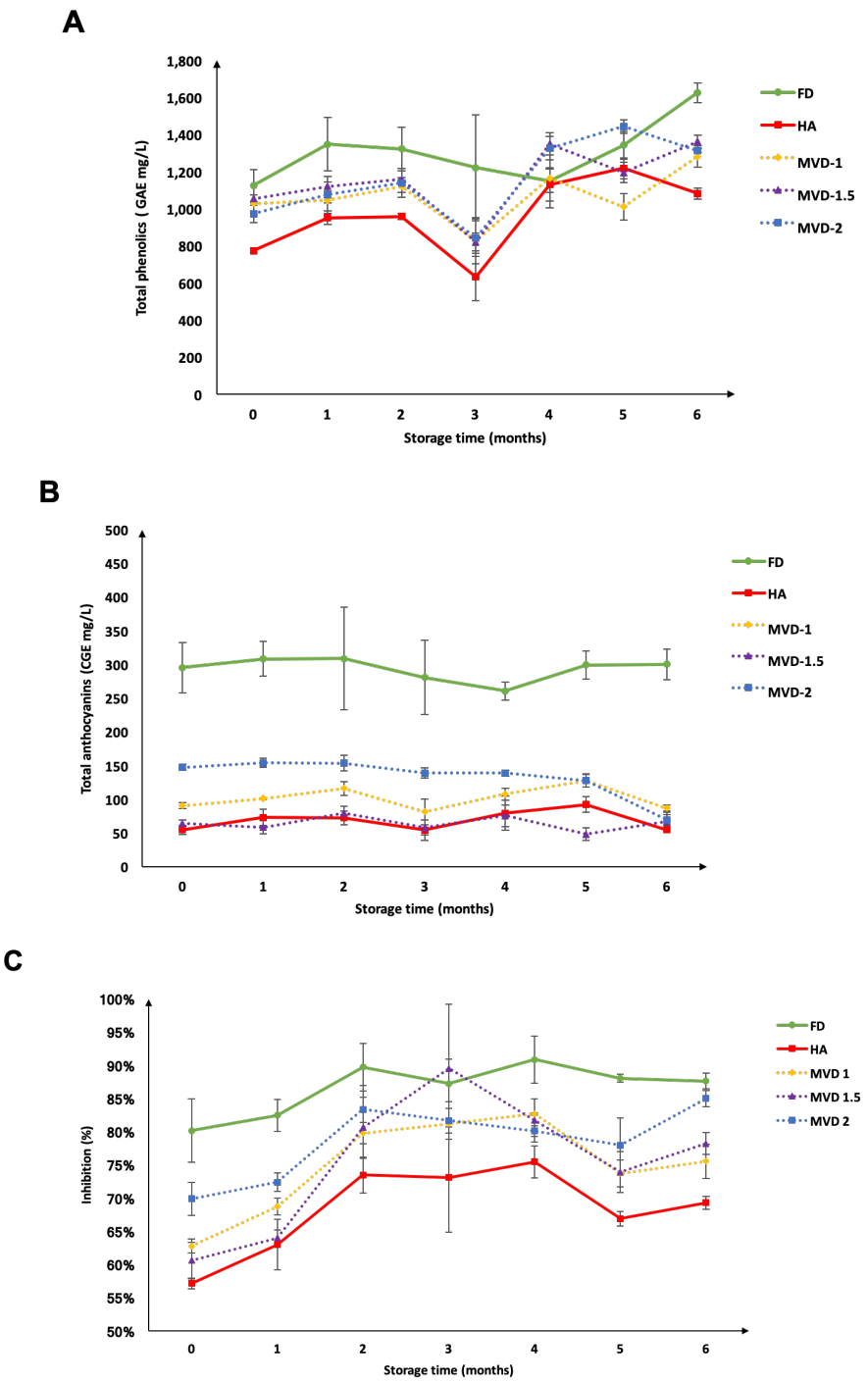

Figure 5. 7 Changes of total phenolics (A), total anthocyanins (B) and antioxidant activity (C) of dehydrated whole Concord grape puree stored at room temperature for a shelf-life study of 6 months.

[a] FD: freeze-dried samples; HAD: hot air-dried samples; MVD-1: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.0 W/g; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g; MVD-2: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 2.0 W/g.

3.5 Proximate composition

Inclusion of seeds in the grape puree samples significantly increased the crude fiber, total fatty acids, rumen unsaturated fatty acids (RUFAL) and some minerals contents, such as calcium, magnesium, zinc and manganese (Table 5.3). This is expected as grape seeds contain about 40% fiber, 16% fatty acids and 11 % protein (2). On the other hand, FD and MVD-1.5 samples had significantly higher levels of crude protein and copper contents than other groups. The high temperature and long drying time in HAD might cause the denaturation and depletion of proteins, due to Maillard reactions. No significant differences were found among different groups in terms of water soluble carbohydrates and sodium contents. The crude fiber content and RUFAL in MVD-1.5 sample was almost 5 times higher than the FD without seeds samples, while this number for total fatty acids was about 4 times. Therefore, compared to the traditional dried grapes sold on the market, which excludes the seeds, the dehydrated whole Concord grape snack could provide more nutraceuticals, such as fiber and unsaturated fatty acids. This novel whole Concord grape product, that includes skin and seeds, can meet costumers' demand for antioxidants-rich products with balanced food matrix. Most importantly, it can be easily consumed as a natural food instead of supplement capsules such as grape seed extracts currrenlty sold in the marketplace, while reducing environmental problems caused by the management of juice waste.

Table 5. 3 Proximate composition analysis of dehydrated whole Concord grape puree.

	FD	FD w/o seeds	HAD	MVD-1.5
Crude Fiber (%)	7.13 ± 5.02 a	2.2 + 0.08 b	9.33 ± 0.65 a	10.57 ± 0.26 a
Crude Protein (%)	4.40 ± 0.00 a	3.60 + 0.00 b	3.60 ± 0.22 b	4.13 ± 0.17 a
Total Fatty Acids (%)	1.83 ± 0.11 a	0.47 + 0.00 b	1.68 ± 0.08 a	1.81 ± 0.00 a
RUFAL (%)	1.55 ± 0.09 a	0.30 + 0.00 b	1.41 ± 0.07 a	1.52 ± 0.00 a
WSC (%)	67.4 ± 2.21 a	53.97 + 12.05 a	67.2 ± 2.11 a	67.37 ± 1.62 a
Ash (%)	3.44 ± 0.09 b	5.33 + 0.07 a	2.98 ± 0.12 c	3.22 ± 0.08 bc
Calcium (%)	0.09 ± 0.00 a	0.05 + 0.00 b	0.08 ± 0.01 a	0.09 ± 0.00 a
Phosphorus (%)	0.12 ± 0.00 a	0.08 + 0.00 c	0.11 ± 0.00 b	0.11 ± 0.00 b
Magnesium (%)	0.06 ± 0.00 a	0.04 + 0.00 b	0.06 ± 0.00 a	0.06 ± 0.00 a
Potassium (%)	1.58 ± 0.02 b	1.85 + 0.02 a	1.41 ± 0.06 c	1.53 ± 0.05 bc
Sodium (%)	0.01 ± 0.00 a	0.01 + 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 a
Iron (ppm)	17.67 ± 0.94 ab	24.0 + 1.63 a	17.0 ± 0.82 b	21.0 ± 3.56 ab
Zinc (ppm)	5.67 ± 0.47 a	4.67 + 0.47 a	5.00 ± 0.00 a	5.00 ± 0.00 a
Copper (ppm)	6.00 ± 0.00 a	6.00 ± 0.00 a	5.00 ± 0.00 b	6.00 ± 0.00 a
Manganese (ppm)	13.33 ± 0.47 a	10.00 ± 0.00 b	14.33 ± 0.47 a	13.33 ± 0.47 a

[a] RUFAL: rumen unsaturated fatty acids; WSC: water-soluble carbohydrate; FD: freeze-dried samples; HAD: hot air-dried samples; MVD-1.5: hot air dried 4 hours and sequentially dried by microwave vacuum dryer at energy level of 1.5 W/g.

4. CONCLUSIONS

This study compared the drying efficiency and quality of three drying methods in developing a dehydrated whole Concord grape snack. It is evident that freeze-dried samples produced the best quality while hot air drying resulted in the least preferred attributes. It is feasible to directly dry the whole grape puree in the microwave vacuum dryer (MVD) after reducing about 33.4 % of the moisture content by a 4-hour hot air drying. Using microwave vacuum drying improved the drying efficiency and some product qualities. MVD samples had better qualities compared to hot-air dried samples, especially when using them as ingredients. Inclusion of seeds in this dehydrated snack produced a more nutritious product as it contains more fiber and unsaturated fatty acids. At last, considering the high energy input and time-consuming process of the freeze-drying method, sequentially using microwave vacuum drying after hot air drying could be a better choice. In order to improve product qualities, more studies are needed to optimize the drying parameters, to produce more puffed products, and to find alternative concentrating methods to reduce moisture content. For example, investigating the feasibility of using low temperature vacuum evaporation to decrease the moisture content in the first stage instead of hot air drying could better preserve the color and nutrients.

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CHAPTER 6

CONCLUSIONS AND FUTURE WORK

Concord grape is economically important and its health benefits have been proven in many previous studies (1,2). However, the decrease in grape consumption has impacted farmers and grape processors. The investigation of novel processing technologies to produce nutritious and fresh-like grape products is important to meet current consumers' demand. In this research, innovative food processing technologies, especially non-thermal technologies, are investigated to assess their feasibility in producing safe and nutritious Concord grape products. The main findings and conclusions for this dissertation are:

Chapter 2: The efficacy of high pressure homogenization (HPH) in inactivating non-pathogenic and spoilage microorganisms was evaluated. HPH (300 MPa, single pass, 10 °C) was able to achieve a greater than 5-log reduction of *Escherichia coli* (*E.coli* ATCC 25922) and a greater than 4-log reduction of a spoilage microorganism (*Saccharomyces cerevisiae*) in Concord grape juice. HPP treated juices were able to maintain similar physicochemical properties, bioactive compounds, antioxidant activity and polyphenoloxidase (PPO) enzyme activity to that in the heat-treated (71.1 °C, 3 sec) juice samples. HPH-300 MPa treated samples were microbiologically stable for up to 15-week storage under refrigeration.

Chapter 3: High Presure Processing (HPP) and Pulse Electric Field (PEF) + HPP juice samples were microbiologically stable for 5 months at 4 °C. PEF was effective in extracting significantly higher amounts of color pigments and phenolic components. PEF+HPP treated juice had the highest color intensity, total phenolic content, total anthocyanins content, antioxidant activity, aroma liking, appearance liking, flavor intensity and purchase intent. Decrease in phenolic compounds were seen during storage, especially for non-thermally treated samples because of the high PPO and POD enzyme activities.

Chapter 4: A feasible method to create a whole Concord grape puree that includes seeds and skin was developed and evaluated. Proximate composition analysis revealed that significantly higher crude fiber, protein, total fatty acids and linoleic acid contents were found in the whole grape puree. HPP (600 MPa, 3 min, 5 °C) was able to extend the refrigerated shelf-life to at least 5 months. HPP samples had comparable phenolic contents to mild heat-treated samples after 4-months of refrigerated storage. HPP treated puree had significantly higher overall liking and purchase intent due to its fresh-like appearance and better consistency, compared to the thermally treated puree, while providing similar taste profile.

Chapter 5: Proper processing methods in producing a shelf-stable dehydrated whole Concord grape snack by combining hot-air drying and microwave vacuum drying were developed. The drying efficiency and quality of three different drying methods in developing the dehydrated whole grape snack were also compared. The best drying method for producing this whole grape snack is by freeze-drying. However, using microwave vacuum drying after reducing about 33.4 % of the moisture content by a 4-hour hot air drying improved textural properties and antioxidants contents, compared to the traditional hot air drying alone. Considering the high energy input and time consumption in freeze-drying, further optimization of concentration procedures and parameters of microwave vacuum drying warrant additional research, as it could be the preferred method.

Evaluation of HPH in microbial reduction, preserving quality and shelf-life of Concord grape juice

Results from Chapter 2 provide parameters for using HPH in the preservation of grape juice. However, as the low pH of Concord grape juice can also put stress on the survival of microorganisms, the implementation of the HPH parameters demonstrated in this study (>250 MPa, single pass) in other fruit juices should be validated before utilization. The inactivation efficiency of HPH on juices can also be depended on attributes of different juices. Maresca et al. (3) reported that the efficacy of HPH in inactivating microorganisms did not differ neither in 3 different fruit juices nor in water. However, it is also reported that the viscosity of juices could affect the efficacy of HPH on inactivation of spoilers and pathogens (4).

Additionally, sensory study of the HPH treated juice in comparison with the heat-treated juice is needed to clearly understand consumers' acceptance and preferences. If fresher attributes were noted by consumers, further characterization of volatile compounds in juice samples with different treatments are needed to better elucidate the flavor profile. It is also desirable to include the information of processing technologies at the end of the sensory study to perceive consumers' acceptance towards HPH treated juice. It is reported that consumers had a better acceptance towards HPP than PEF (5), in which circumstance familiarity played a major role in determining their acceptance.

To decrease the energy and maintenance cost, investigation of multi-passes at lower pressure levels in the HPH treatment is favored to further understand the HPH effect on microbial and enzyme inactivation. It is generally recognized that as the number of HPH passes increase, the increase in fractional disruption of microorganisms is small, especially when pass numbers exceed three (4). Therefore, the number of passes that can achieve the maximum reduction while being efficient should be exploed in future studies. Moreover, combination of HPH with other hurdles, such as heat and additives, could also be explored to achieve a higher disruption rate, as well as inactivation of oxidative enzyme activities.

At last, the type of both pathogen and spoilage microorganisms investigated in this study are limited. A more comprehensive validation study would be needed to ensure the safety of HPH processed fruit juices, such as including *Salmonella* and a cocktail of 5 different pathogenic *E.coli* strains. Future studies on pertinent pathogenic and spoilage strains, especially in other fruit juices, are needed to further elucidate the efficacy and parameters needed for utilizing HPH in processing fruit juices.

Changes of flavor and aroma profile in non-thermally treated juice/puree in comparison with heat treated juice/puree

In the sensory results of chapter 3 and chapter 4, the flavor/aroma ratings in non-thermally treated juice and puree are higher than heat treated counterparts. Similarly, in an evaluation of consumers' perception of apple juice processed by non-thermal technologies (HPP and PEF) and thermal treatment, consumers rated non-thermally processed juice as "fresh, natural and balanced flavor", while cooked flavor was described in thermally treated juice (6). The fresh flavor and aroma are the most valued attributes of non-thermally treated products, which represented the fresh-like attributes. Thus, it is of significance to further investigate the changes of flavor profiles after different treatments, such as identification and quantification of volatile components.

It is also recommended to use HPLC to further identify and quantify individual flavonoids that are presented in thermally and non-thermally treated Concord grape juice, to better understand the changes of these health-promoting compounds after different treatments.

Additionally, a comparison study of the efficacy of PEF and enzyme in extraction bioactive compounds, color pigments, as well as increase in yield should be conducted to better understand the advantages and limitation of PEF in Concord grape juice production. As the oxidative enzyme activites were high in the non-thermally treated grape juice, suitable countermeasures should be explored to decrease the quality degradations during storage. The combination of traditional practices, such as mild heat, in inactivating oxidative enzyme activities and increase the efficiency of PEF and HPP are needed to better understand their applications in industrial settings. Application of other non-thermal technologies, such as ultraviolet light, high intensity light pulses and ultrasound in combination with pulsed electric field are also recommended to increase efficiency and decrease energy cost as reported in other fruit juices (7, 8).

For the whole Concord grape puree, the reasons for textural and flavor changes observed after different processes and during refrigerated storage need to be further investigated and elucidated. HPP treated whole grape puree had better consistency than thermally treated puree. Inclusion of the experiments on pectin methylesterase activity would be appropriate in the future study. Moreover, the nutritional quality of this novel product should be carefully studied by utilizing biological markers or animal studies. For example, the effect of soluble and insoluble tannins on digestibility and gut microbiome should be evaluated to better assess the feasibility and the nutritional benefits of inclusion of seeds in the puree. At last, differences in aroma compounds in thermally and non-thermally treated puree should be identified.

Optimizing drying parameters to create a dehydrated whole Concord product with better texture and color

Though MVD did not produce the best quality, it did improve the texture and preserved more nutrients compared to the traditional hot air drying. Future works on optimizing drying parameters to produce more puffed products are needed. Alternative concentrating methods, rather than hot air drying, can be investigated to improve the texture and rehydration properties, as well as preserving more nutrients.

In drying the whole Concord grape puree, using hot-air drying in the first stage to decrease the moisture content could cause degradation of nutrients. To solve this problem, vacuum evaporation can be used to remove the moisture at a lower temperature and thus preserve the color and nutrients. Parameters used for vacuum evaporation and microwave vacuum drying need to be further investigated to provide a dehydrated product with better quality. Most importantly, in microwave vacuum drying stage, optimizing the operations and energy input levels is necessary to create puffed whole grape snack, presenting interesting texture and higher rehydration rate. A sensory study is also needed to understand consumers' acceptance towards this novel dehydrated grape snack.

Unlike the whole grape puree, direct tasting the dehydrated grape snack required chewing for a few seconds. This may lead to higher requirements for the texture requirement than the whole Concord grape puree as the inclusion of seeds might cause unfamiliar sensations which could decrease the acceptance of the dehydrated grape snack. Therefore, a sensory study on this dehydrated grape snack is needed in order to optimize the formulation, processing and final storage conditions.

Despite concentration before using microwave vaccum dryer, formulations that could result more puffed products may also be explored to develop novel grape products. Inclusion of dairy products, such as cheese and whey protein, as well as other plant based proteins, such as pea protein, in the formulation of the dehydrated whole grape puree snack instead of using

methylcellulose or maltodextrin, can not only eliminate the concentrating step, but also creating a snack that has a more balanced food matrix.

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