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EFFECT OF HIGH-PRESSURE PROCESSING ON QUALITY AND SHELF LIFE OF GREEN FRESH JUICE PRODUCED FROM A BLEND OF FRUIT AND VEGETABLE

Ahlam Abdulla Al Hammadi

جامعة الإمارات العربية المتحدة
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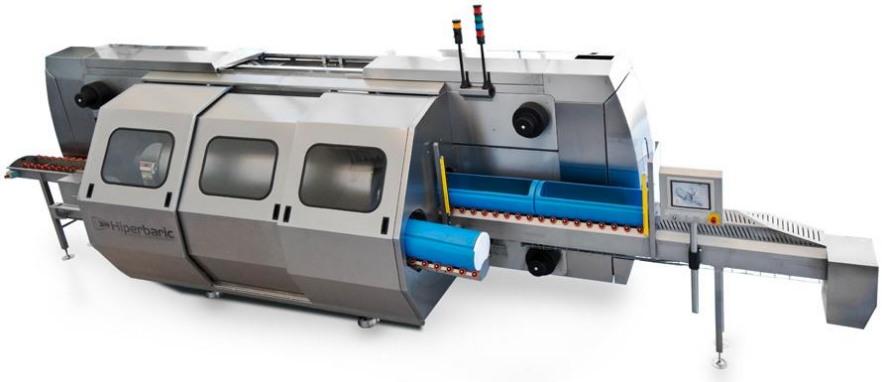

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Ahlam Abdulla Al Hammadi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Food Science

Under the Supervision of Professor Afaf Kamal-Eldin

April 2021

Declaration of Original Work

I, Ahlam Abdulla Al Hammadi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "Effect of High Pressure Processing on Quality and Shelf Life of Fresh Juice Produced from a Blend of Fruit and Vegetable", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal-Eldin, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously been presented or published or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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1) Advisor: Prof. Afaf Kamal-Eldin

Title: Professor

Department of Food Science

College of Agriculture and Veterinary Medicine

2) Member: Dr. Rabih Kamleh

Title: Senior Vice President – QA and R & D

Name of organization: Agthia GROUP, Abu Dhabi, UAE

3) Member: Dr. Sami Ghnimi

Title: Associate Professor

Laboratory of Automatic Control Chemical and Pharmaceutical Engineering

Name of organization: Claude Bernard University Lyon 1, France

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

1) Advisor (Committee Chair): Afaf Kamal-Eldin
   Title: Professor
   Department of Food Science
   College of Agriculture and Veterinary Medicine
   Signature  Date 13/5/2022

2) Member: Raman Kumar Bhaskaracharya
   Title: Assistant Professor
   Department of Food Science
   College of Agriculture and Veterinary Medicine
   Signature  Date 13/5/2022

3) Member (External Examiner): Kjersti Aaby
   Title: Associate Professor
   Department of Food and Health
   Institution: Nofima AS - The Norwegian Institute for agricultural and food research, Norway
   Signature  Date 16/5/2022

This Master Thesis is accepted by:

Dean of the College of Agriculture and Veterinary Medicine: Professor Bhanu Chowdhary

Signature  Date 03/07/2022

Dean of the College of Graduate Studies: Professor Ali Al-Marzouqi

Signature  Date 04/07/2022

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Abstract

Background: The technology of high hydrostatic pressure (HPP) in food processing started to take a huge attention in food industries due to its ability to increase the shelf life of processed products by inactivating food-borne microorganisms and undesired enzymes that cause changes not acceptable by the consumers. Because of the treatments take place at low temperature and because no chemical preservatives are added, advantageous and gives more value to the product and matches consumer demand for healthy products.

Aims: The aim of this thesis was to study the effect of HPP on certain quality parameters of green fruit juice compared to thermal process during shelf-life.

Method: A green fruit juice composed off Fresh rocket leaves and green apple fruits was used for the study. HPP treatments were performed at 200 MPa and 600 MPa for 180 second at 4°C, and the thermal treatment of the green juice was done at 85°C for 120 second in addition to the control. The microbiological, physical, chemical properties of the juices were analyzed at day 0, 3, 6, 14, 21 and 28.

Results: The study confirmed that HPP treatment can maintain the quality of the green juice with very minor changes compared to thermal process. Results of the microbial growth shows a significant reduction in TPC, yeast and molds for HPP treated samples compared to the thermal processed samples. The results of Brix, pH and ascorbic acid for all samples shows no significant different during the storage and among the treatments. However, results of the color analysis were significantly different between both HPP treatments (200 MPa & 600 MPa) and thermal processed sample. For the chlorophyll a, results showed that the impact of the thermal process is high compare to HPP and this indicate that HPP treatment can maintain the chlorophyll a in green juices. The study confirmed that HPP would be preferred non-thermal treatment for treating fresh green juices, however further studies needed to understand the enzymes reaction during the treatment and shelf life.

Keywords: Non-thermal processes, high pressure, green fruit juice, color.

Title and Abstract (in Arabic)

تأثير تقنية الضغط العالي على جودة وصلاحية العصائر الطازجة الخضراء المصنّعة من مزيج من الخضروات والفواكه

الملخص

بدأت تقنية الضغط الهيدروستاتيكي العالي في معالجة وتصنيع الأغذية تحظى باهتمام كبير في الصناعات الغذائية نظرًا لقدرتها على زيادة فترة مدة صلاحية المنتج عن طريق تدمير الكائنات الدقيقة كالمايكروبات والبكتريا والإنزيمات غير المرغوب فيها والتي تسبب تغييرات غير مقبولة من قبل المستهلكين. نظرًا للمعالجة التي تحدث عند درجات حرارة منخفضة بدون إضافة أي مواد حافظة كيميائية ، فإن هذه الميزة تعطي قيمة غذائية أكبر للمنتج وتطابق طلب المستهلك الحصول على منتج صحي. الهدف من هذه الرسالة هو دراسة تأثير المعالجة بالضغط العالي في العصائر الخضراء مقارنة بالعملية الحرارية خلال فترة الصلاحية المفترضة للمنتج. عولجت عينات الضغط العالي عند ضغط 200 ميجا باسكال و 600 ميجا باسكال لمدة 180 ثانية عند درجة حرارة 4 مئوية، وتمت المعالجة الحرارية للعصير الأخضر عند 85 درجة مئوية لمدة 120 ثانية بالإضافة إلى العينات تم فحصها بدون إي معالجة. تم تحليل الخواص الميكروبيولوجية والفيزيائية والكيميائية للعصائر في اليوم 0 و3 و6 و 14 و 21 و28.

أكدت الدراسة أن المعالجة بالضغط العالي يمكن أن تحافظ على جودة العصير الأخضر مع تغييرات طفيفة جدًا مقارنة بالعملية الحرارية. تظهر نتائج الفحص الميكروبيولوجي انخفاضًا كبيرًا في مجموع تعداد البكتريا والخميرة والعفن للعينات المعالجة بالضغط العالي مقارنةً بالعينات المعالجة حراريًا. لم تظهر نتائج تحليل إجمالي السكر الصلب، والرقم الهيدروجيني وحمض الأسكوربيك لجميع العينات أي اختلاف ملحوظ أثناء التخزين أو بين تقنيات المعالجة المختلفة. ومع ذلك، كانت نتائج تحليل اللون مختلفة بشكل كبير بين كل من العينات المعالجة بتقنية الضغط العالي (MPa200 و MPa600) والعينة المعالجة حرارياً. بالنسبة للكلوروفيل أ، تظهر النتائج أن تأثير العملية الحرارية مرتفع مقارنة بـتقنية الضغط العالي وهذا يشير إلى أن المعالجة بتقنية الضغط العالي يمكن أن تحافظ على الكلوروفيل أ في العصائر الخضراء. أكدت الدراسة أنه يفضل تطبيق المعالجة غير الحرارية باستخدام الضغط العالي لمعالجة العصائر الخضراء الطازجة، ولكن هناك حاجة لمزيد من الدراسات لفهم تفاعل الإنزيمات أثناء المعالجة وفترة الصلاحية.

مفاهيم البحث الرئيسية: المعالجة غير الحرارية، تقنية الضغط العالي، عصير أخضر، كلوروفيل أ.

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Dedication

To my beloved parents and family

Table of Contents

List of Tables

List of Figures

List of Abbreviations

HCA	Hierarchical Cluster Analysis
HHP	High Hydrostatic Processing
HPHT	High Pressure High Temperature
HPP	High Pressure Processing
HPT	High Pressure Technology
HTST	High Temperature Short Time
LOX	Lipoxygenases
PCA	Principal component analysis
PDA	Potato Dextrose Agar
PET	Polyethylene terephthalate
PME	Pectin methylesterase
POD	Peroxidase
PPO	Polyphenol oxidase
TSS	Total Soluble Solid
UHPP	Ultra High Pressure Processing

Chapter 1: Introduction

1.1 Overview

It's well known that heat treatment is the main process used in the pasteurization or sterilization of foods with the aim to destroy the microorganisms and ensure the safety of the product for human consumption (Farnworth et al., 2001). However, thermal treatments cause damages to the natural components of some food components and properties, such as heat-sensitive nutrients like vitamins, texture, color, flavor, and taste profile. Nowadays, with the increase of the demand for high quality and less processed foods and more healthy options, food engineers are putting huge efforts to develop novel non-thermal technologies that can improve the shelf life and quality characteristics of the final product at the same time (Gong et al., 2014).

High pressure processing (HPP) or high hydrostatic pressure (HHP) is a new technology in food processing which is categorized as non-thermal processing and started to have wide applications potential and at the moment is one of the most successful alternatives to the thermal technologies used by food industries (Wang et al., 2015). In 1883, Certes was the first in history to link the effects of HPP processing technology and microorganisms. However, the effect of high pressure on foods was first exposed at the end of 19th century by Bert Hite and co-workers in agricultural experiment station at West Virginia University in 1899. High hydrostatic pressure up to 600 MPa was used as a tool to preserve milk, and later on vegetables and fruits in 1914. After these prime studies, no sustained research was published about HPP until 1980's (Elamin et al., 2015).

In mid-80's, the interest in this process was recovered due to the successful growth of commercial HPP alternative to traditional thermal processing and preservation of

foods. The major revolution in HPP came up in Japan in 1992 by introducing to the market the first HPP treated product which was jam. This pressure-treated product exposed to the food markets by one company at the beginning, therefore created the noticeable success of HPP when six other companies followed in the next three years. Over the past 20 years, this credible technology has been already effectively implemented in the food industry, and many studies have been performed to understand the advances of HPP technology, which produced food products that are safe, fresh, nutritious with long shelf life and innovative. This new technology is a commercially applicable technique that can be used for both liquid and solids products such as meat and meat products, dairy products, sauces, beverages and fruits and vegetables (Gezai, 2019). Thus, this innovative technology can offer a great possibility to introduce to the market mildly processed food items which nowadays grab the attention of the consumers.

Both HPP and thermal processing are helping to extend the shelf life of the products by killing the spoilage organisms, yeasts and lactic acid bacteria and improve the safety of foods by destroying vegetative pathogenic microorganisms, e.g., *Escherichia coli* O157:H7, *Salmonella spp*., and *Listeria monocytogenes*. However, HPP is different from thermal processes in being less destructive to the important and sensitive food quality components like vitamins, flavor compounds, and pigments, in which consumers are currently interested (Balasubramaniam et al., 2008). In addition to that HPP improves the safety of foods, it also helps to extend the shelf life of foods by maintaining the desirable attributes associated with “minimally processed” foods while meeting the right food safety standards mainly deactivation of bacteria *Listeria monocytogenes, Salmonella, E. coli* and campylobacter, yeast, and mold (Hsiao-Wen et al., 2017).

Nowadays, food industries are under pressure to find alternate processes which provide a safe product, but with less destructive methods to achieve this goal. Consumers are willing to pay higher prices for these high-quality products if they meet their expectation in team of “freshness”, safety, and health. HPP provides an interesting alternative processing method to meet these requirements. Even though this processing method requires a greater initial financial investment, it pays off in higher quality, higher value, and premium products.

1.2 Project Objective

The aim of the study is to investigate the effect of HPP on a beverage prepared by blending fruit and vegetable juices in terms of quality and shelf life stability. The studied beverage is composed of mixture of fresh green apple juice and fresh rocket leaves (arugula) juice. The study evaluated the product quality of the HPP-treated beverage through:

- Studying the effect of the process on the coloring pigment present in juice (chlorophyll) and the degree of browning upon storage.
- Evaluation of the total microbial load count that will determine its microbiological shelf-life stability during storage (bacteria, yeasts & molds).
- Evaluation of the physiochemical characteristics of the product, namely acidity, total soluble solids, and total phenolic/flavonoid contents.

Propose a HPP treatment condition to help the industry to supply healthier, fresh-like, reputable juice products with long shelf life in terms of the parameters studied.

1.3 Relevant Literature

1.3.1 General Principle and Mechanism of HPP

The process is applied when the food is subjected to high hydrostatic pressure with or without heat generally at pressure ranged between 100 MPa to 600 MPa to achieve microbial inactivation while maintaining the qualities preferred by the consumer (Balasubramaniam et al., 2015). By applying this technology, no damage or distort will affect the foods if the treated product is not blank or having an empty space inside (Balasubramaniam et al., 2015). A lot of studies and researches have been done and proven that this technology can destroy almost all microorganisms and inactivate or activate many enzymes with no or minimum effect on flavors and nutrients that are mainly destroyed by thermal treatments (Elamin et al., 2015). In addition, chemical reactions that cause the destruction of vitamins or produce off flavors can be reduced under high-pressure conditions. The three general principles that explain the behavior of foods under effects of high pressure are the isostatic pressing, Le Chatelier, and microscopic-ordering principles (Balasubramaniam et al., 2008).

Isostatic pressing or Pascal's principle is the first consideration involved in the application of HPP, which pretend that the uniform application of pressure is equal in all directions and the condition should be independent of time and space (Balasubramaniam et al., 2015). It can be established when a fluid is used to transmit the pressure throughout the packaged food. In high pressure applications, the pressure and its effects are instantaneously and homogeneously distributed within the food item, regardless of the shape and size of the food. Due to this unique characteristic, development of this process has been successfully commercialized. However, because air and water differ in compressibility under pressure, the structure and shape of the

foods containing air pockets like marshmallows may be change upon pressure treatment, unless the food is perfectly elastic and consists of closed-cell foam from which air cannot escape. This principle helps to explain why nonporous foods with high-moisture content are not damaged macroscopically by pressure treatment (Balasubramaniam et al., 2008).

Le Chatelier's principle addresses changes to equilibrium as a result of pressure application. It states that any phenomenon such as phase transition, change in molecular configuration or chemical reaction accompanied by a decrease in volume is enhanced by pressure. If pressure changes, the equilibrium shifts in a direction that tends to reduce the change in the corresponding intensive variable (volume). Thus, pressure shifts the system to that of the lowest volume (Balasubramaniam et al., 2015). Microscopic ordering principle states that at constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny and Masson, 1993).

Because of its limited effect on covalent bonds, this technology has the potential to retain the texture, nutrition and sensory attributes of food products. However, it has been reported that 500 MPa pressure at ambient temperature was insufficient to inactivate gram positive bacteria and spores to the extent necessary to meet commercial sterilization requirements (6 log bacteria inactivation). Due to the weakness of high-pressure treatment in destroying pressure-resistant bacteria and spores, especially in neutral food systems such as carrot juice, an additional inactivating factor is needed (Teo et al., 2001).

1.3.2 HPP Equipment and Operation

HPP is generally considered as batch equipment, although semi-continuous equipment is also available. The equipment is typically made up of high strength steel alloys with high fracture toughness and corrosion resistance (Figure 1).

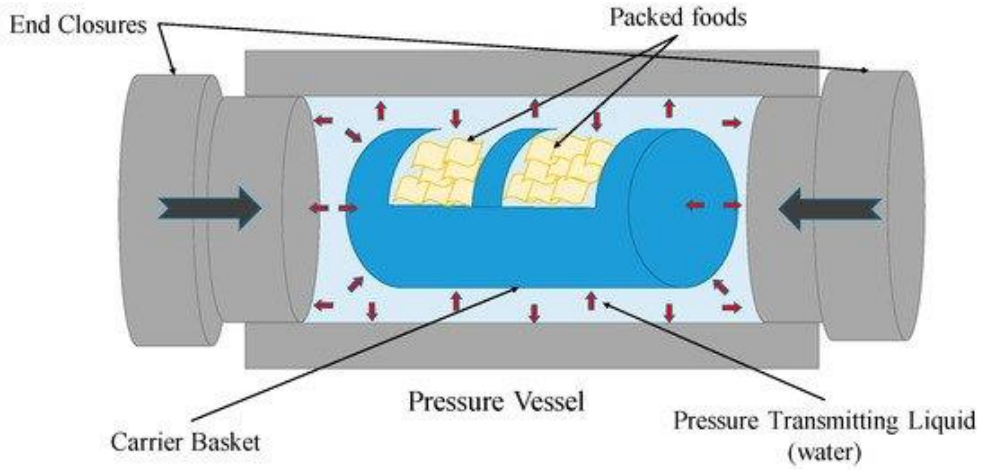

Figure 1: Schematic description of the HPP processing vessel. Source: Balakrishna et al. (2020)

The following are typical components of batch HPP equipment (Ting, 2011):

1. Pressure vessel (thick-wall cylinder).
2. Two end closures to cover the cylindrical pressure vessel.
3. Yoke (structure for restraining end closures while under pressure).
4. High pressure pump and intensifier for generating target pressures.
5. Process control and instrumentation.
6. A handling system for loading and removing the product.

Pressure chambers for food processing, for cost reasons, have a practical limitation at 600 MPa, which is sufficient for most applications. It could be performed as a batch process for both solid and liquid foods or be developed into a semi-continuous process for pump-able foods. A batch process is similar in operation to thermal processing in

a retort system. The food product is typically packed and placed inside a sample loading basket, then it's loaded into the pressure vessel containing pressure-transmitting fluid, mainly water. The pressure vessel and its content are closed with the end closures. A yoke structure slides across the closed vessel to restrain top and bottom closures under pressure. The desired process pressure is achieved through compression of pressure-transmitting fluid using the combined action of a pump and intensifier. During HPP, the product is held for the desired time at the target pressure, the vessel is depressurized at the end of the treatment time, and the product is unloaded (Ting, 2011).

1.3.3 General Applications of HPP Technology

During the past 20 years, HPP technology has been considered as one of the most important innovations in food processing (Elamin et al., 2015). Based on different studies in different food matrices, it has been proven that application of high pressures range between 100 to 800 MPa can be used for different food processing and preservation techniques such as freezing, thawing, blanching, and commercial sterilization (Balasubramaniam et al., 2015).

As mentioned before, high pressure at cold or room temperature can be useful for pasteurizing different kinds of food including meat, salads, fruit juices and vegetables products (Norton and Sun, 2008; Mujica-Paz et al., 2011; Balasubramaniam et al., 2015). However, as pasteurization technologies are not able to inactivate spores, it's very important to keep and maintain the products which are high pressure processed under refrigerated storage and handling (Balasubramaniam et al., 2015).

Utilization of HPP helps in keeping the sensory characteristics besides extending the shelf-life of food products. For example, avocado containing-foods are highly

conserved once HPP is applied, as same as products containing meat where is a study carried by (Hugas et al., 2002) panelists were not able to detect the difference between samples that were HPP and heat-treated.

A. Fruits and Vegetables Products

Many applications of HPP are used nowadays to treat fruits and vegetables-based products. Table (1) present HPP conditions applied for the processing of different fruit juices/nectars (Kadam et al., 2012). Fruits and vegetables products quality includes very good nutritional retention, fresh flavor, enhanced color, and taste, in addition to long shelf life with compared to other pasteurized products (Kadam et al., 2012). Application of HPP technology in the processing of fruit and vegetable products (Avocado and banana purees, tomato, orange and pink grapefruit juices, potatoes and black beans and jams) was shown to prevent discoloration, inhibit of undesirable polyphenol oxidase browning reactions in the presence of low pH and/or leaching, and improve cloud stability and preservation of nutritional and sensory properties during storage. Combination of HPP with temperature and holding time at specific condition helps to achieve desired effects on the texture, color, and flavor of a product. Treatment of HPP at 200 MPa and 300 MPa and pre-warming the samples at 20°C are very good alternative to thermal processing (Calligaris et al., 2012). These treatments were also reported as credible to conventional heat treatments to produce fresh-like banana juice (Wang et al., 2015). The nutritional and sensory characteristics of foods are not affected by HPP processing, and their shelf-life is almost maintained (Kadam et al., 2012). It was reported that a 500-MPa/5 minutes pressure treatment can preserve the most relevant quality attributes of *Aloe vera* gel, including microbiological, nutritional, and antioxidant aspects, without affecting the physicochemical quality during storage intervals of up to 60 days (Reyes et al., 2012).

Table 1: HPP conditions used for processing of different fruits and vegetables juices (Kadam et al., 2012).

Product	Pressure (MPa)	Holding time (min)	Temperature (°C)
Apricot nectar, distillated water	600 - 900	1 – 20	20
White and red grape must	304-811	1 – 5	25
Angelica kaiseki juice	0.01	7	25
Citrus juice	300-375	1 -1.5	0 – 5
Orange juice	350	1	30
Vegetables juices	300-370	10	35
Guava puree	400-600	15	25

Verbeyst et al. (2012) compared thermal and HPP for their effects on bioactive compounds in several plant-based products treating strawberries and raspberries at various temperature pressure combinations. HPP (400, 600, and 700 MPa at 20, 50, 80, and 110°C) did not have a substantial effect on the bioactive compounds (Wang et al., 2015). At a constant elevated pressure and as the temperature increased, the breakdown of anthocyanins and vitamin C occurred. However, no clear trends were observed for the other phenolic substances during the process.

Because HPP technology is mainly limited to disruption of cell walls with limited effects on covalent bonds in low molecular weight compounds and chemical reactions (e.g. enzymatic reactions) may still take place during storage and affect the quality of fruits and vegetables products (Wang et al., 2015). The effect of HPP is limited to its effects on decompartmentalization of the enzymes rather than the chemical reactions themselves. For example, HPP was found to enhance the conversion of sucrose to glucose and fructose in fruits and jams by the action of invertase (Buts et al., 2003).

On the other hand, high-pressure homogenization treatments that combine HPP with sample homogenization are effective in controlling both enzyme activities and spoilage bacteria in juices (Vel_azquez-Estrada et al., 2012; Shireena et al., 2021).

Varela-Santos et al., (2012) studied the effect of HPP on pomegranate juice at 350–550 MPa for 30, 90, and 150 s and the parameters in consideration were microbial quality, physicochemical characteristic and bioactive compounds. Results showed that, neither the pH, Brix, nor titratable acidity were substantially affected by HPP for the first 15 days of storage. However, the color stability depended on the HPP treatments. The spoilage microorganisms naturally present in the pomegranate juice were reduced sufficiently to undetected levels at or above 350 MPa for 150 s, thus extending the microbiological shelf-life for more than 35 days during refrigerated storage at 4°C.

B. Dairy Products

Similar to the case of fruits and vegetables, HPP technology can preserve the flavor, texture and taste of dairy products. It can maintain nutrients of milk without any detrimental effects, extend the shelf-life, and present fresh-like products (Zamora et al., 2012; Wang et al., 2015). Undesirable bacteria were inactivated by applying pressure without the negative effect on cheese flavor that occurs after pasteurization (Varela-Santos et al., 2012; Voigt et al., 2012). HPP treatment has significant effects on milk proteins, e.g. stimulation of the disruption and reformation of casein micelles, and the particle size of fat globules. For whey proteins, HPP leads to changes in protein conformation followed by aggregation mainly through sulfhydryl-disulfide interchange reactions (Sahu and Mallikarjunan, 2012). One study investigated the effect of HPP on the denaturation of lactoferrin and lactoperoxidase present in skim

milk and whey (Mazri et al., 2012). It showed that lactoferrin and lactoperoxidase in milk denatured slowly at 400 MPa, while their denaturation in whey was rapid at pressures above 700 MPa. The treatment performed at 600 MPa at 20°C reduced immunoreactive lactoferrin to approximately 75% and 65% in milk and whey, respectively, as compared with the untreated samples (Wang et al., 2015). These studies also confirm that HPP mainly affects cell and compartment structures rather than the chemical reactions themselves.

C. Meat Products

HPP affects the fresh meat quality parameters such as shelf-life, texture and color (Borggaard and Andersen 2004; Bajo et al., 2012; Kadam et al., 2012). It was reported that, HPP completely inactivated *Citrobacter freudii*, *Pseudomonas fluoresecens*, and *Listeria innocua* at 400 - 500 MPa (Simonin et al., 2012). The effects of HPP on color and myoglobin content of minced beef samples was studied and it was found that, the pink color of meat decreased at 200 - 350 MPa and turned to grey brown at 400 - 500 MPa. It was suggested that meat discoloration during HPP is due to whitening effect caused by globin denaturation, heme displacement or release or oxidation of ferrous myoglobin to ferric myoglobin at 400 MPa (Kadam et al., 2012). The most studied and investigated quality parameter was meat texture (tenderization) due to the activity of endogenous proteases leading to weakening of actin–myosin interactions, fragmentation of myofibrils into short segments as a result of Zline disintegration, degradation of elastic filaments consisting of connecting, and weakening of connective tissue (Koohmaraie, 1994). The study of the effects of HPP on meat proteins, enzymes and myofibrillar proteins, partly explains the modification of the texture and tenderness of raw meat that occurs under pressure and the improvement in tenderness after cooking (Simonin et al., 2012)

Earlier studies showed that HPP treatments of muscles at approximately 100 MPa and 30°C generally led to a radical shortening of the muscle (approximately 35%) (Macfarlane 1973; Bouton and others 1977). It has been suggested that the improved tenderness is linked to the effect of pressure on the contraction state of the muscle (Macfarlane, 1973). By combining the effects of muscle contraction and pressure during the treatment, it could lead to breakage of myofibrillar structure, forcing myosin filaments of severely contracted muscles into Z discs, which would explain the tenderizing effect (Macfarlane, 1973).

We can learn from the above findings in milk and meat products that HPP can affect the molecular organization and tissue structures in food materials. This might affect the release of enzymes and extractability of bioactive components from different food matrices, including those of fruits and vegetables.

1.3.4 Effect of Thermal and Non-thermal Food Processing Technologies on the Degradation of Chlorophyll

In previous decades, the main objective of food manufactures was to produce long life and safe foods for consumers while they kept the quality of food as a secondary objective. However, in late 1980s consumer trends started to change with food quality of foods, minimal processing, additive-free, and shelf-stable safe product becoming prominent and taking higher priority order. Therefore, manufacturers used modified thermal processing technologies and introduced new food processing methods (Oey et al., 2008). The HPP technique has received great attention in the food manufacturing industry because it may inactivate enzymes in foods amidst with insignificant effect to the nutritional content and sensorial quality aspects of foods (Oey et al., 2008).

The color of foods is enormously important when from the consumers' point of view because consumer's eyes are attracted to vivid food on the market shelf. The main pigments which are responsible for the color of the food are carotenoids and chlorophylls. Among those, carotenoid has responsible for precursors of the main vitamin which is vitamin A as well as they show antioxidant properties (Canjura et al., 1991). The major chlorophyll constituents found in higher plants are chlorophyll a and chlorophyll b which occurs in approximately 3:1 ratio in the plant tissues. Carotenoids, chlorophylls also improve health by their anticarcinogenic, antimutagenic, and anti-inflammatory activities (Sánchez et al., 2014). In-plant tissues naturally chlorophylls are degraded by enzymatic activity and chemical reactions. During enzymatic action, chlorophylls are degraded to the chlorophyllide (Fang et al., 1998). In addition, thermal degradation highly affect the chlorophyll degradation during thermal processing (Weemaes et al., 1998). During the thermal activity process pheophytin is formed due to the removal of central magnesium of the porphyrin by two hydrogen atoms, which will cause an undesirable color change from bright green to olive brown (Weemaes et al., 1998). The enzymatically degraded product of chlorophyll (chlorophyllide) is converted to the pheophorbide due to the influence of the thermal processing process (Weemaes et al., 1998).

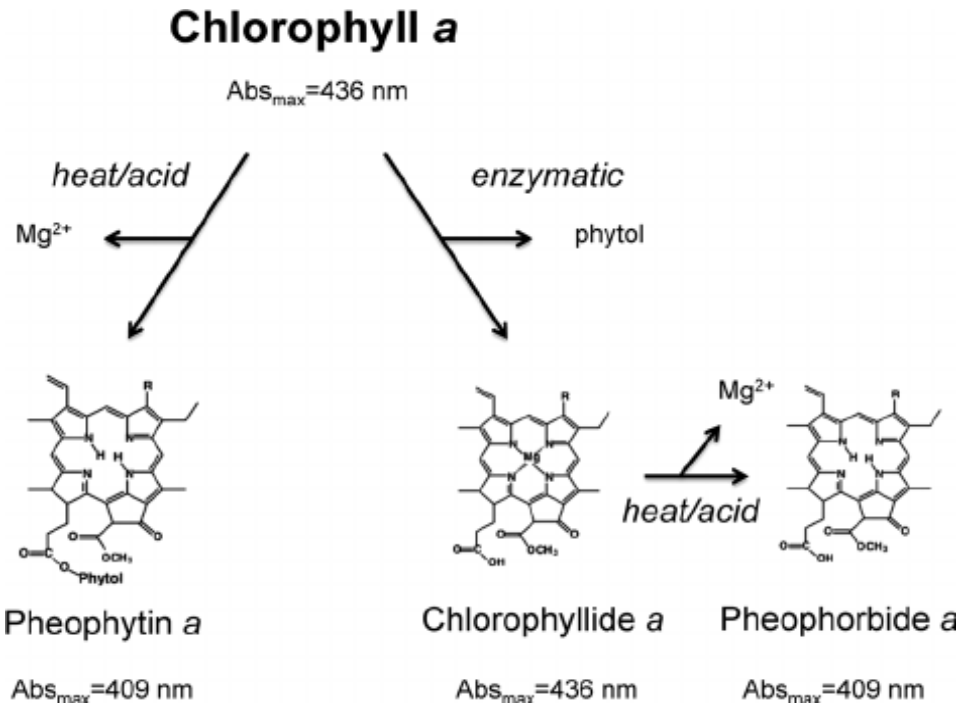

Figure 2: Chlorophyll a and its degradation products after heat treatment and enzymatic treatment. Source: Ostbring et al. (2014)

The effect of thermal and non-thermal food processing techniques on food products are directly affected to the degradation of chlorophyll, carotenoids, and some other important constituent contain in the foods (Sánchez et al., 2014). Plants contain two types of lipid-soluble chlorophylls, namely chlorophyll a and chlorophyll b. with different green colors and degradation kinetics where chlorophyll a being more heat sensitive than chlorophyll b (Weemaes et al., 1999). When heat treatment under 20°C is applied to foods, chlorophylls are not degraded and a slight increment of chlorophyll content might be observed (Sánchez et al., 2014). However, when the temperature is raised to 70 – 117°C, both chlorophylls a and b degraded with chlorophyll b being slightly stable than chlorophyll a (Sánchez et al., 2014). However, as pressure is elevated from 200 to 800 MPa chlorophyll a and chlorophyll b in broccoli degraded by 19.4% and 68.4% respectively (Oey et al., 2008).

Chemical reactions are responsible for the formation of derivatives of chlorophyll which is mainly chlorophyll a and chlorophyll b isomers and pheophytins a and pheophytins b isomers (Sánchez et al., 2014). Both Chlorophyll a and chlorophyll b isomers are do not show color change due to the similarity of the absorption spectra. However, the color change can be observed from bright green to olive-brown due to the formation of the pheophytin (Oey et al., 2008). In the manufacturing industries, various measurements are undertaken to retain the original colors of the foods such as application of metal-complexes neutralization with acids, high-temperature short-time processing (Sánchez et al., 2014).

1.3.5 Impact of High-Pressure Processing (HPP) and High Pressure High Temperature (HPHT) Processing on Chlorophylls in Green Vegetables

At pressure (200 – 800 MPa at room temperatures), both chlorophylls a and b in broccoli juices were stable even when 800 MPa pressure was applied for 4 hours (Oey et al., 2008). Also, a significant difference in chlorophyll degradation couldn't be found under 600 MPa around 5 min treatment time in a sample of spinach (Wang et al., 2012). The increment of the chlorophyll content after HPP treatment indicates the disruption of cells due to the high pressures and the release of chlorophyll content into the surrounding environment which leads to intense green color in the vegetable surfaces (Krebbers et al., 2002). This effect was found in green beans after HPP treatment at 500 MPa for 1 minute (Krebbers et al., 2002). When considering the high-pressure high temperature treatments (HPHT), which operated under 625 MPa pressure for 5 minutes at 70°C, significant degradation of chlorophyll a was observed in green pepper (about 52%), broccoli (27%), and spinach (12%). In this case of HPHT, chlorophyll b remained the same during the process at 70°C while chlorophyll a had lower stability in green vegetables upon different pressure-temperature combinations

(Sánchez et al., 2014). However, increasing the temperatures in HPHT processes above 70°C led to the degradation of both chlorophyll a and chlorophyll b. Upon HPP treatment (625 MPa, 20°C, 5 min), chlorophylls a and b did not degrade but when treated with HPHT treatment at 117°C and 625 MPa for 5 min, degradation of both chlorophyll occurred due to the increment of temperature along with the high pressurization. Degradation of chlorophyll in broccolis subjected to the 121°C for 30 minutes was about 99% for chlorophyll a and about 97% for chlorophyll b (Murcia et al., 2000).

1.3.6 Effect of HPP on Microorganisms

The growth rate of vegetative bacteria is retarded at moderate pressure (200 – 400 MPa) while it can be inactivated at high pressure (above 400 MPa) (Frank, 2005). Although pressure stability is largely dependent on the type of microorganism, it is generally accepted that pressures between 200 and 600 MPa at room temperature are sufficient to cause a substantial reduction of viable vegetative cells. Vegetative forms such as yeasts and moulds are the most pressure-sensitive and are inactivated at pressures between 200 and 300 MPa. Gram-negative bacteria can be inactivated by pressures of about 300 MPa and are less pressure stable than Gram-positive bacteria, which require pressures higher than 400 MPa for inactivation (Frank, 2005). However, exceptions to these general statements can be found such as very pressure-resistant strains of E. coli O157:H7 (Benito et al., 1999), In addition, in contrast to laboratory conditions, microorganisms are often more stable in actual food products possibly due to protection by proteins and sugars. Moreover, synergistic effects between pressure and acidification or addition of anti-microbial substances can be exploited to lower the pressure resistance of microorganisms (Hauben et al., 1997; Garcia-Graells et al., 1998).

It's clearly known that HPP as a novel technology plays a major role in annihilating the pathogenies, food-deterioration microorganisms, besides, lengthen the product's shelf-life keeping the same features (Considine et al., 2008). In general, cocci-shaped gram-positive bacteria are more resistant to HPP treatment in comparison with rod-shaped gram-negative bacteria. In contrast, yeasts and molds are HPP sensitive. The elimination of cells depends on various factors including type of bacterial species, number of treatment batches, pH, adjusted pressure, time and temperature (Adebo et al., 2021).

1.3.7 Effect of HPP on Protein and Enzymes

Similar to the thermal process, protein molecules can be denatured by HPP. Different factors are playing a role in this incident such as protein structure, pressure range, temperature, pH and solvent composition (Palou, 1998). Since HPP effect is mostly on the non-covalent bonds, the secondary, tertiary and quaternary structures can be significantly affected. Functionality of the protein is determined by tertiary structure, so because of this HPP can result in novel functional properties (Tewari et al., 1999).

Some key enzymes in fruit and vegetable processing that are affected by HPP include:

1. Polyphenol oxidase (PPO), which is responsible for enzymatic browning.
2. Lipoxygenase (LOX), which induces changes in flavor, color and nutritional value.
3. Pectinmethylesterase (PME), which is responsible for cloud destabilization and consistency changes.
4. Peroxidase (POD), which gives rise to unfavorable flavors.

POD, which is generally considered to be the most heat stable vegetable enzyme, is at least in some cases also extremely pressure resistant (Lourenço et al., 1990; Yemenicioglu et al., 1997; Weemaes et al., 1998a). In green beans, a pressure

treatment of 900 MPa merely induced slight inactivation of POD at room temperature, while in combination with elevated temperature enhanced the inactivation effect 600 MPa (Quaglia et al., 1996). Upon pressurization, in contrast, PPO may display, depending on its source, either enhancement of catalytic activity or inactivation. Thus, HPP can affect phenolic compound concentrations through its different effects on enzyme activity as well as on its effects on compounds' extractability.

Pressures needed to induce substantial inactivation of PPO vary between 200 and 1000 MPa, depending on the enzyme origin and micro environmental conditions such as medium composition or pH (Weemaes, 1998).

For LOXs, the variation of thermal stability at atmospheric pressure depends on the enzyme source and medium (Oey, 2000). Many studies of pressure inactivation have been reported for tomato, soybean, green bean and pea LOXs. It has been reported that the inactivation is starting in a strict range between 400 and 600 MPa (Heinisch et al., 1995; Ludikhuyze et al., 1998; Tangwongchai et al., 1999; Oey et al., 1999; Oey et al., 2000).

PME demethylates pectin resulting in low-methoxy pectin, which may then form insoluble complexes leading to precipitation of pectin and cloud loss (Goodner et al., 1998; Basak and Ramaswamy, 1996). To inactivate the heat stable PME isoforms heat treatment at 90°C for 1 min is required. However, this thermal treatment may cause changes in the flavor and aroma which reduce the freshness attributes of the juice and result in non-enzymatic browning (Maillard and caramelization reaction), so this the reason why such interest in the use of non-thermal processing technologies for the inactivation of PME in citrus fruit juices. PME from different fruits has been reported to be quite thermo resistant. Temperatures between 80 and 95°C are required to induce significant inactivation and even then PME remains active. Pressure stability has

mainly been investigated for orange PME and to a lesser degree for grapefruit, guava and tomato PME. Threshold pressures for inactivation at room temperature of PME from different sources have been reported to vary largely from 150 to 1200 MPa, depending on the origin and the medium in which the inactivation is carried out (Van Den Broeck, 2000).

1.3.8 Effect of HPP on Vitamins

HPP is used as an alternative to traditional high temperature pasteurization to avoid the detrimental effects including vitamin losses (Hayashi, 1995). Studies have shown that traditional thermal processing of orange juice leads to vitamin losses including loss of vitamin C (Farnworth et al., 2001) and changes in carotenoids (Parish, 1998a), which are important to the color and the nutritional value of the juice. The effect of HPP on the levels of ascorbic acid and beta-carotene are considered to be minimal. Other quality parameters of orange juice, including pH and °Brix, were minimally affected during extended refrigerated storage after the pressure treatments (Parish, 1998a).

No significant effect of HPP on vitamins A, C, B1, B2 and E content of fruit and vegetable products was observed compared to thermal treatment (Bignon, 1996). However, a decrease in vitamin C content in strawberries and guava puree during storage after HPP treatment (400-600 MPa, 15-30 min, 20°C) was found to be much lower compared to the fresh products (Sancho et al., 1999). A more detailed kinetic study of pressure temperature stability of ascorbic acid in buffer, orange juice and tomato juice were performed by Van den Broeck et al. (1998). They found only significant degradation of ascorbic acid when pressures of about 850 MPa was

combined with temperatures between 60 and 80°C, and more in tomato and orange juice than in buffer.

1.3.9 Effect of Food Processing on Phenolic Compounds

Plant foods and their products contain different polyphenolic compounds with antioxidant and anti-inflammatory effects (Pandey, 2009). The hydroxy groups attached to the benzene rings of these compounds can be linked to different substituents through ester linkages and/or glycosides (Castañeda-Ovando et al., 2009). Synthesis of polyphenolic compounds as secondary metabolites could occur through shikimate/phenylpropanoid and malonate pathways (Shahidi et al., 2015). Then the product of the phenylpropanoid pathway enters the flavonoid pathway and synthesized complex phenolic compounds such as kaempferol, quercetin, myricetin, etc.

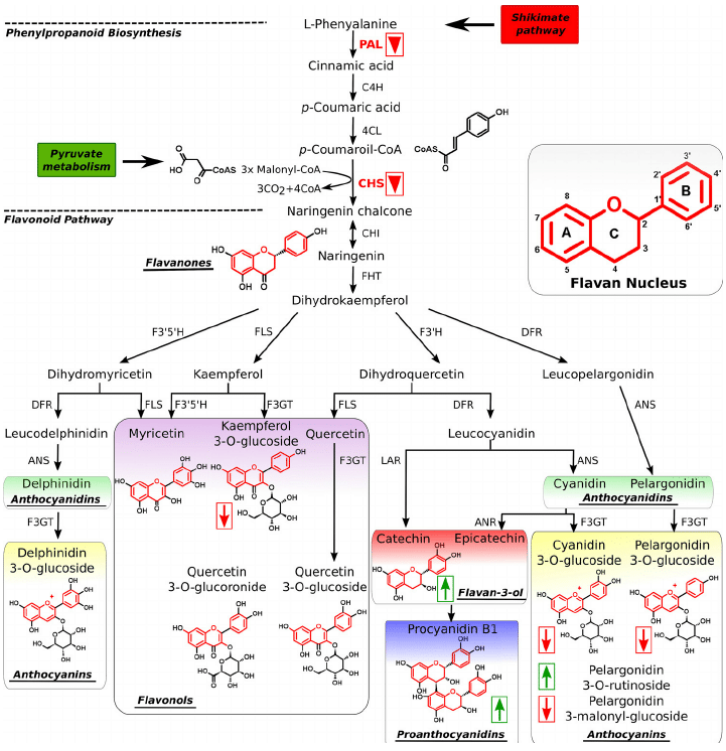

Figure 3: A schematic representation of the phenylpropanoid and flavonoid biosynthesis pathway. Source: (Casañal et al., 2013).

Processing of food can be defined as the techniques which are applied to convert preliminary ingredients into processed foods that are transformed into edible and better-preserved forms better suited for human consumption (Ifie et al., 2018). Processing techniques can be employed in either a domestic or industrial environment with the main purpose to extend the shelf life without changing the composition or the quality of the product. Bioactive compounds have an enormous effect on food processing because those are bio-accessible in the gastrointestinal tract and transformed to different metabolites before approaching to target tissues. Furthermore, among bioactive compounds, evaluation of changes of phenolic compounds during the food processing is vital to human health due to the mentioned antioxidant and other particular properties (Ifie et al., 2018).

A. Anthocyanins

Anthocyanin is a bioactive compound which is a derivative of phenolic constituents and present in vegetables and fruits. This shows different colorations due to the highly conjugation of phenyl rings (Ramawat et al., 2013). Despite of higher varieties of anthocyanins compound in nature most common anthocyanins which are readily available in fruits and vegetables are cyanidin 50%, peonidin 12%, delphinidin 12%, pelargodinin 12%, malvidin 7% and petunidin 12% (Ramawat et al., 2013). There are different factors which needs to consider about the stability of anthocyanins namely temperature, metal ion concentration, pH, oxygen level, sugars and enzymes. Depending on the pH of the medium, anthocyanins can exist in four stages i.e. at pH 1, flavylium cation is predominated, at pH 2-4, quinoidal blue species dominate while pH 5-6, carbinol pseudo-base species and Chalcone exist together whereas pH is above 7, anthocyanin degrades according to the substituent groups (Ramawat et al., 2013). During food processing operations are conducted such as cutting, extraction of juice

and dicing, allow enzyme and substrate to be mixed by distorting the cell arrangement (Ifie et al., 2018). In enzymatic hydrolysis process, sugar group which is attached to the carbon number 3 is get hydrolysis, hence unstable chalcone product is obtained, and ultimately chalcone is broken down to 2,4,6-trihydroxyphenylacetaldehyde and benzoic acid. Therefore, to reduce the rate of breakdown of pigment (anthocyanin) blanching procedure can be applied (Ifie et al., 2018). Another way of affecting to the stability of anthocyanin is direct oxidative degradation or enzymatic degradation such as oxidation by polyphenol oxidase (PPO). Polyphenol oxidase cannot degrade anthocyanins barely because anthocyanins are not a substrate for the PPO. Therefore, quinone derivatives which are formed of oxidation of phenolic compound by PPO is needed for the oxidation of anthocyanin. Moreover, degradation products which are formed through these oxidative reactions are unstable and formed colorless compounds. Therefore, to reduce the rate of oxidative degradation of anthocyanin in fruits and vegetables, Sulphur dioxide is added as an antioxidant. Apart from that, thermal degradation due to the applied heat of food processing also affects the degradation of anthocyanin. However, the degraded product is varying according to the applied heat (Ifie et al., 2018). During the process, the pyrylium ring opening and chalcone formation is indicated the initiation of the degradation process as well as hydrolysis of sugar group and aglycon moiety is confirm the initiation step of degradation (Ifie et al., 2018). The end products of terminal degradation of chalcone can be identified as phenolic compounds and phloroglucinaldehyde. When anthocyanins are associated with other non-colored organic compounds, the stability of anthocyanins is increased, and that process is called co-pigmentation. Co-pigmentation ad polymerization reactions are responsible for the stability of wine

color. The color is a crucial consideration when foods are on market shelves (Ifie et al., 2018).

B. Phenolic acids

In plants major phenolic acids compounds are hydroxybenzoic and hydroxycinnamic acid derivatives which are vital for active defense mechanisms of plants and the development of flavor of fruits (Ifie et al., 2018). The main phenolic acid derivatives which are abundantly found in the berry family are gallic acid, ellagic acid and p-hydroxybenzoic acid. The derivative of cinnamic acid is abundant in potatoes, apples, pears which is called chlorogenic acid while in grapes caftaric acid is dominant. Ferulic acid is abundant in cereals whereas ester derivative of ferulic acid is dominant in citrus fruits (Debelo et al., 2020). Temperature, enzyme and oxygen concentration are highly affected to the stability of phenolic acid compounds. For instance, puree juice which could be prepared from the strawberries needed to store under 4°C. When enhanced the content of ellagic acid can result for the hydrolysis of ellagitannins amidst aging and processing (Ifie et al., 2018). When cooking of legumes using a pressure cooker resulted in releasing phenolic acids from plant matrixes. Furthermore, when orange juice was processed via thermal pasteurization and pulsed electric fields, phenolic acid contents in orange juice enhanced apart from syringic acid and neoeriocitrin (Ifie et al., 2018). Likewise, after thermal processing or high-intensity pulse electric treatment, a similar trend was shown for an increment of most phenolic acid derivatives which are identified in juice soya and milk beverages. However, when jam production from blueberries, ellagic acid level of berries was reduced by 20% due to the antioxidant activities occur during the production process as well as heat applied during the manufacturing process of food resulted in the reduction of chlorogenic acid content in potatoes (Ifie et al., 2018). The reduction is dependent on the strength of heat applied;

therefore, oven-baked potatoes reduced the chlorogenic acid content than the microwaved treated potatoes. Ferulic acid esters hydrolyzed upon the heating and storages which result for the hydrolysis of ester and form free acids ultimately undergo decarboxylation and formed 4-vinyl guaiacol which incorporates unpleasant odor to the final product (Debelo et al., 2020). Hence, constant monitoring on these by-products along with the production flow is vital for organoleptic and nutritional point of view. In cereals, hydrothermal process is responsible for the releasing of phenolic acids from plant cell wall (Debelo et al., 2020). In another study shown that fermentation of wheat resulted in an increment of ferulic acid content and other phenolic acid content due to the action of hydrolytic enzymes (Debelo et al., 2020). Hence, care should be taken to avoid the reduction of the bioactive compounds while increasing the shelf life of products.

C. Flavonoids

Flavonoids are polymeric compounds which are having a hydroxyl group at the third position of the carbon skeleton and abundantly found in leaves and outer parts of higher plants. Considering the food quality parameters most dominant compound is flavan-3-ols which responsible for the bitterness and astringency of foods (Debelo et al., 2020). During food production, significant changes of the structure of phenolic compounds can be seen. For instance, during black tea production catechins are acted as oxidative enzymes i.e. phenol oxidase and peroxidase, which form the flavins during the fermentation process (Ifie et al., 2018). However, these oxidative products and catechins are the dominant concerns for astringent character and taste for the black tea. Similarly, catechin and epicatechin are decreased During the fermentation of cocoa beans due to the polyphenol oxidation to produced high molecular weight insoluble tannins. In contrast, (-)-catechin level can be increased when the roasting

temperature of cocoa beans exceeds 70°C, because of the epimerization of (-)-catechin (Ifie et al., 2018). Onions and asparagus being rich with flavanol derivatives such as quercetin 3, 4′-O-diglucoside, and quercetin 4′-O-glucoside. Chopping of asparagus can cause a significant decreased in rutin and didn't affect to the quercetin 3, 4′-O-diglucoside and quercetin 4′-O-glucoside content in onions when chopping (Ifie et al., 2018).

Thermal application on foods for more than 60 minutes resulted in the total reduction of flavanols. When the broccoli was processed via boiling, the amount of quercetin derivatives was reduced whereas steaming responsible to retain a higher amount of quercetin derivatives. Furthermore, flavanol content is not affected by the pasteurization in citrus juice (Ifie et al., 2018). Moreover, the temperature of soy processing was increased to 100°C, isoflavone beta-glucoside was the most common flavone derivative. When the time duration of the fermentation is increased during the production of fermented soy foods malonyl glucosides content is reduced while aglycones content is increased (Ifie et al., 2018).

To sum up, despite of being heating process affected to phenolic compounds very diversely, fermentation gave a positive effect to the structural composition of phenolic compounds, which may turn impact on bio efficacy and bioavailability of these compounds.

1.3.10 Effect of HPP in Phenolic Compounds and Antioxidant Activity

Several studies have been shown HPP treatments increase total phenolic contents and antioxidant activity in plant foods (Queiroz et al., 2010). Application of high pressure is known to enhance the extractability of bioactive compounds from fruits and vegetables (Ergin et al., 2011; Vaida et al., 2016; Shafat et al., 2019). This is achieved

because HPP disrupts the tissue structure and waken the non-covalent bonds between the matrix and bioactive components (Queiroz et al., 2010).

1.3.11 Advantages and Limitations of HPP Treatment

Looking to the market, HPP started to gain its popularity although the volume of production is relatively small. The reason is not only because it's used as a preservative process, but also due to its minimal effect on food quality and its ability to change the functional properties of foods. Other major advantages of HPP as mentioned earlier, are its ability to inactivate pathogenic microorganisms in foods at room temperature and its potential to extend the shelf-life of food products without changing any of the sensory parameters and nutritional values. Finally, other advantages are the HPP process reduces processing time, consumes less energy, and practically has no effluents.

However, the main limitation of this non-thermal process is the costs which is high capital cost compare to other process used for the same objectives. Also, because water is required to destroy the microorganisms, HPP technology cannot be used for dry foods such as spices; or for foods which contain enmeshed air, such as strawberries, as Fellows (2009) explained. Moreover, it was observed that treating milk with HPP was not as effective as in other food systems, and this might be due to the high content of fat and protein which seems to protect the microorganisms against the pressure. On the other hands, the low pH in fruit juices could act as an addition inhibitory factor that enhancing the effectiveness of the HPP (Fan and Sampedro, 2010, p.38).

In conclusion, studies have proof that high pressure processing has minimum impacts on the nutritional properties and sensory parameters of food compared to other food processing methods. However, more studies are needed to conclude a clear statement regarding the quality and shelf life of low acid products.

1.4 Summary

From all the studies which have been done on HPP technology, it's concluded that using this technology for food preservation without any chemical additives or preservatives has the capability to provide the right requirements for the consumers in terms of "minimally processed" foods, and also its ability to ensure safety and significantly extended refrigerated shelf life which has opened new market opportunities particularly in the area of "natural" preservative free food products. High pressure processing can be applied in food industry on production scale for the products which are particularly need a good retention of flavors and maintain the sensitive nutrients with good quality during the shelf life. In addition, this new technology is also applied to inactivate microorganisms without any or less heat application which keeps the product without any noticeable change in taste or appearance.

On the other hand, few limitations that might be a challenging to some of the food industries. Although it destroys the vegetative microorganisms, when it's applied alone it will not inactivate spores and some food enzymes. Also, optimization of process might depend on the food matrix itself, as some studies showed that some enzymes are activated at certain pressure, while it's inactivated when different pressure it applied.

Chapter 2: Methods

2.1 Research Design

2.1.1 Juice Samples Preparation

Fresh rocket leaves (Arugula) were purchased from a local market (UAE). The leaves were washed properly to remove all sand and then cut to small pieces (approx. 3 cm). They were then soaked in the sanitizer in chilled water (80-120 ppm) for 45-60 minutes with changing the solution every 30 minutes before squeezing in a juicer machine (Kuvings Slow Juicer, Korea). Green apple fruits were washed, sanitized, cut into 4 quarters, and collected in chilled water to avoid oxidation. Cut green apple fruits were feed into the Juice extractor. The blend was prepared by mixing 65% to 35% fresh green apple juice to fresh rocket leaves juice. Percentage of 0.2 of ascorbic acid was added in the juice collection tank and mixed very gently until dissolved.

2.1.2 High Pressure Processing Treatment Condition

HPP treatment of the juice blend was carried out using an industrial batch model high pressure food processor with a capacity of 55 L (Hiperbaric 55, Spain). Two different pressures (200 and 600 MPa) were used to treat the samples for 3 minutes holding time. Juices were filled into Polyethylene terephthalate (PET) bottles size of 200 mL and placed into the vessel for processing at 4°C, with distilled water as the pressure-transmitting fluid.

2.1.3 Thermal Processing

Fresh blended green juice sample was pasteurized at 85°C for 120 second by using multi-purpose processing vessel (Armfield, UK). Treated samples were filled under the laminar flow in sterilized glass bottles and stored at 4°C for further analysis.

2.1.4 Refrigeration

After both HPP treatment and the thermal treatment were done, samples were refrigerated and stored at 4°C for shelf-life study. Samples of thermal treatment were taken for analysis at 0, 3, 6 and 9 days, while samples of HPP treatment were collected at day 0, 7, 14, 21, 28 and 35 days. On each analysis day, samples were taken at random and analyzed for quality changes. Control samples was analyzed only at day 0 due to their microbial and chemical spoilage.

2.2 Quality Assessment

2.2.1 Microbiological Analysis

To detect viable natural microorganisms in the juice, total plate count method was used. With pipette, one 1 ml of juice sample was added to 2 petri dishes. In each plates 15-20 ml PCA & VRB media were added accordingly and mixed properly and allowed to set. Aseptically, pipet 0.1 ml of sample on pre-poured, solidified PDA agar plates and spread inoculum with a sterile, bent glass rod. The set plates are incubated as following, PCA for the TPC at 35°C for 48hrs in inverted positions, VRB for coliforms 35°C for 24 hrs in inverted positions, and PDA for yeast and molds at 25°C for 5 Days in upright positions.

2.2.2 Physicochemical Characters

Total soluble solid (TSS) was determined using refractometer (ATAGO, Japan) at 25 ± 1°C and the results were reported as °Brix. pH was measured at ambient temperature (25 ± 1°C) using a pH meter (Metrohm, 913 pH meter, Switzerland).

The color which include L*, lightness (0=black, 100=white), a* (-a=greenness, +a=redness) and b* (-b=blueness, +b=yellowness) of the fresh green juice were obtained using HunterLab colorimeter coupled with an optical sensor (HunterLab, U.S.A.). The instrument was calibrated as follows; L*= 94.22, a*= -1.2, b*= 0.3. Chroma (C), color intensity (E), hue angle (H*), degree of whiteness (%WI), color index (CI) , browning index, yellow index, were calculated using the equations below (Das et al., 2004; Falade and Ayetigbo, 2015; Maskan, 2001; Rhim et al., 2006).

$$C = (a^{*2} + b^{*2})^{0.5}$$

$$E = (L^{*2} + a^{*2} + b^{*2})^{0.5}$$

$$H^{*} = \tan^{-1}(b^{*}/a^{*})$$

$$WI\ (\%) = 100 - [(100 - L^{*})^{2} + ((a^{*})^{2} + (b^{*})^{2})]^{0.5}$$

$$\text{Colour index (CI)} = \frac{a^{*}}{b^{*}}$$

$$Browning\ \text{Index} = \frac{100(X - 0.31)}{0.71}$$

$$X = \frac{(a^{*} + 1.75L^{*})}{(5.465L^{*} + a^{*} - 3.012b^{*})}$$

$$Yellow\ \text{Index} = \frac{142.86b^{*}}{L^{*}}$$

2.2.3 Phytochemicals

Catechins were extracted from two grams samples in 3 ml of 80/20 methanol/water solution. Catechins was determined by HPLC-UV using a high-resolution silica-based 2.2 μm Acclaim® C18 reverse-phase column (4.6 mm x 25 cm), type Spherisorb ODS-2 5 μm, 100 A° at room temperature with spectrophotometric UV detector at 280 nm. Spectral recording for identification purposes is facilitated by using a photodiode detector with a spectral range from 200 nm to 400 nm.

Carotenoids determined using HPLC-UV on a reversed-phase C18 column with UV detection (450 & 480 nm). The chromatography column was conditioned for at least 15 min with water 0.2% H3PO4 (V/V)/methanol/acetonitrile 96/2/2 (v/v/v). The injection volume was 20 μL of methanol/water 80/20 (V/V) (4.8). Separations were carried out using a mobile-phase gradient as follows. A solvent system of acetonitrile-methanol-dichloromethane-water 85:5:5:5 (v/v/v/v) was applied from 0 - 8 min, changed to 60:8:30:2 (v/v/v/v) by a 5-min linear gradient (An et al., 2008). This composition was maintained for 15 min and returned to the beginning in 15-min. At a flow rate of 1 ml/min, mixtures of astaxanthin, lutein, zeaxanthin, lycopene and B-carotene were eluted within 22 minutes.

For the Chlorophyll a determination, sample were concentrated by filtering a known volume through a membrane filter (47 mm, 5.0 μm pore size). The pigments were extracted from the concentrated sample in an aqueous solution of acetone. The chlorophyll a concentration was determined spectrophotometrically by measuring the absorbance (optical density - OD) of the extract at various wavelengths. The resulting absorbance measurements were then applied to a standard equation (Dunton, 2004).

$$\textit{Uncorrected Chlorophyll a (µg/L)} = \frac{[11.64\,(Abs663) - 2.16\,(Abs645) + 0.10\,(Abs630)]E(F)}{V(L)}$$

Where F = Dilution Factor

E = The volume of acetone used for the extraction (mL)

V = The volume of water filtered (L)

L = The cell path length (cm)

Ascorbic acid analysis was determined using HPLC. The HPLC (Hitachi D-2000 Elite system manager) equipped with two pumps L-2130, auto injector / auto sampler L-2200 syringe loading sample injector valve's fitted with 10µl sample loop of 200 vials and UV-VIS detector L2420. The Chromatographic separation was achieved using column oven L-2300 and column intersil ODS-3 C18 (GL Sciences Inc. Tokyo Japan 5µm, 250×4.6 mm). Flittering assembly (Model Rocker-300 Thaiwan) and ultrasonic cleaner Ceia (Model CP-104 Italy) were used for solvents filtration and degassing

2.2.4 Statistical Analysis

The one-way analysis of variance (ANOVA) was conducted with Duncan multiple-comparison test to compare the significance among samples, using SPSS package (SPSS 26.0 for Windows, SPSS Inc, Chicago, IL, USA) Principal component analysis, as well as hierarchical clustering analysis, was performed on the physicochemical, phytochemical and microbial data of the juice using XLSTAT (Addinsoft, New York, USA) version 2017.

Chapter 3: Results and Discussion

3.1 Microbial Activity

The microbial activity of the fruit and vegetable raw juice blend before processing was 2.71±0.01 log CFU/mL for total plate count, 2.09±0.01 log CFU/mL for yeast and mould count, and not detected for E. coli and coliform count (Figure 4). The treatment of the raw juice thermally and with high-pressure processing significantly reduced the microbial activity of the fruit juice ($p<0.001$).

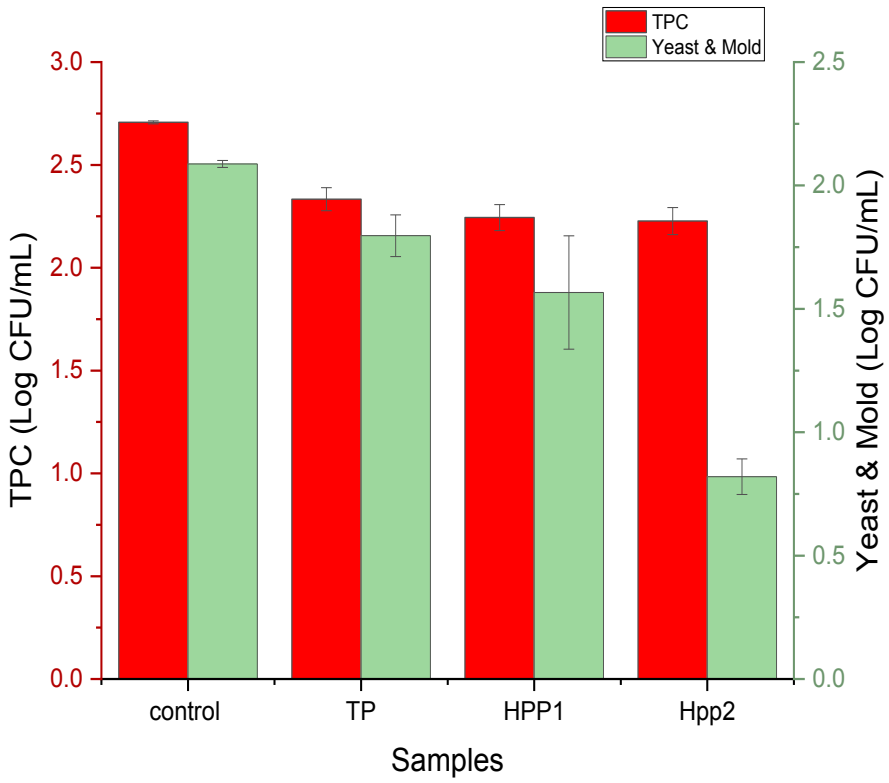

Figure 4: Microbial activity of the juice mixes immediately after processing (n=3)

*Note: HPP1 = high pressure processing at 2000 bar; HPP2 = high pressure processing at 6000 bar; TP = Thermal processing

The reduction by both treatment techniques could be associated with the destruction of the microbial cells. This result was in conformance with the result obtained by Afrasiabi and Maghsoudlou (2015), Oliveira et al (2018) and Linhares et al (2020) who reported a decrease in the microbial activity of broccoli puree, acai juice when subjected to both thermal and non-thermal treatment. In all the juice samples, *Escherichia coli* and coliforms were not detected and could be attributed to good hygienic practice during the production of the juice mixes.

Results of the effect of storage on the microbial activity of the juice are presented in (Figure 4) total plate count and (yeast and mould count). For thermally processed juice, the total plate count as well as the yeast and mould count increased throughout the storage period while for the HPP treatments, changes are less evident. The microbial load or count reported in this study was found to be less than that of Linhares et al. (2020) who report above 4 log CFU/mL for thermal and HPP treated juice. The value, however, fell short of the value reported by Oliveira et al. (2018) who reported a reduction of less than 1 log CFU/mL of acai berry produced domestically using a household blender.

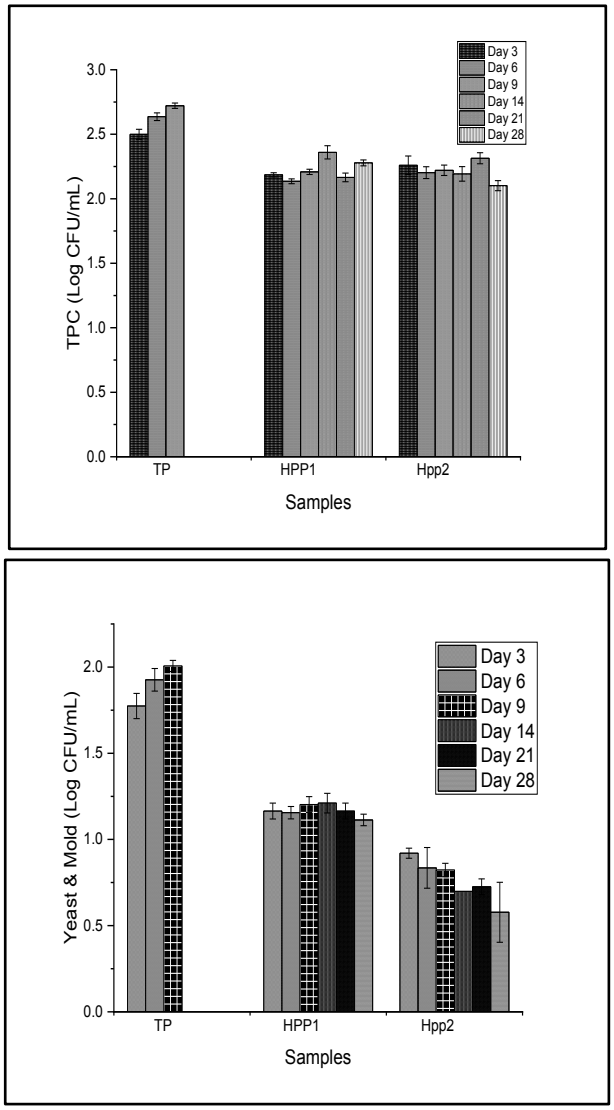

Figure 5: Effect of storage on the microbial content of the juice mix. Right: total plate count, Left: Yeast and mould count (n=3)

*Note: HPP1 = high pressure processing at 2000 bar; HPP2 = high pressure processing at 6000 bar; TP = Thermal processing

3.2 Total solids (ºBrix), pH, and Color of Juices

Table 4 presents the values for ºBrix, pH, and color of the control and the heat- and HPP-treated juices. The ºBrix of the fruit and vegetable juice ranged between 9.72 to 9.74 Brix with no significant difference among the samples. Similarly, Tiwari et al. (2008) in their research reported a non-significant effect of non-thermal processing HPP and ultrasonication) on the ºbrix of strawberry juice. They are of the opinion that,

these processes had minimal effect on the breakdown of macromolecules such as carbohydrate which could invariably increase the sugar concentration of the juice and hence increases the ºbrix of the fruit and vegetable juice blends. The pH of the fruit and vegetable juice mix ranged between 3.28 to 3.36 with no differences among the juice mix samples. Both the thermal and HPP caused an insignificant change in the pH of the juice mix. According to Tiwari et al. (2008), HPP may not excite much energy need for the degradation of different organic acid found in the juice mix.

The colour of a product is one of the most important indicators in the acceptability of the products by the consumers. The colour attributes of the juice mixes were evaluated using a digital colorimeter and parameters such as *L*, a** and *b** were obtained while derived parameter such as colour intensity (E), delta chroma (C), whiteness index WI) hue angle (H*), and colour index (CI) were calculated using the CIE parameters are also shown in Table 4. The color of the fruit and vegetable juice blend shows that L* which is an indication of how bright the juice blend is ranged between 21.08 for HPP1 and 26.64 for the control sample. The HPP1 had less brightness (L*) when compared to the control sample while the juice blend treated thermally had more brightness owing to a higher L* value. This might be due to some degradation of the chlorophyll pigments in the heat-treated sample or to inactivation of polyphenol oxidase enzyme.

Table 2: Physiochemical properties of the fruit and vegetable juice mixes (n=3 for each treatment)

Sample	Brix	pH	L*	a*	b*	H	WI	C*	E*	CI
control	9.74±0.02^{a}	3.34±0.06^{a}	23.10±0.12^{b}	-7.96±0.03^{b}	29.55±0.42^{a}	-69.85±1.05^{a}	17.23±0.41^{b}	30.60±0.66^{a}	38.34±0.14^{b}	-0.27±0.01^{b}
TP	9.74±0.02^{a}	3.36±0.06^{a}	26.64±0.09^{a}	0.88±0.01^{a}	28.90±0.28^{b}	-73.16±0.94^{b}	21.15±0.79^{a}	28.91±0.49 ^{a}	39.32±0.27^{a}	0.03±0.00^{a}
HPP1	9.73±0.02^{a}	3.28±0.03^{a}	21.08±0.17^{d}	-7.92±0.06^{b}	26.72±0.30^{d}	-82.88±0.83^{c}	16.30±0.52^{c}	27.87±0.94 ^{a}	34.94±0.19^{d}	-0.30±0.01^{b}
HPP2	9.72±0.01^{a}	3.33±0.05^{a}	21.95±0.23^{c}	-8.87±0.08^{c}	27.78±0.31^{c}	-73.62±0.1.21^{b}	16.68±0.37^{c}	29.16±0.83 ^{a}	36.50±0.33^{c}	-0.30±0.01^{b}

TP: Thermal Processing, HPP1: High pressure processing at 2000 bar, HPP2: High pressure processing at 6000 bar. H: hue angle; WI: white index; C: Chroma; E*: total color change; CI: color index. Values within each column not sharing a common super script are statistically significant ($P<0.05$)

These result disagree with Gao et al. (2016) who reported that both thermal and HPP processing caused insignificant color changes in strawberry juice. Afrasiabi and Maghsoudlou (2015) in their report on the effect of HPP on the brightness (L*) of broccoli puree reported an increase in the brightness of the puree, which was attributed to the formation of a transparent structure.

The results of the degree of reddish and greenish (a*) of the juice mix range from -8.87 in HPP 2 processed juice to 0.88 in thermally treated juice (Figure 6). A negative sign indicates a high level of greenness while a positive value indicates redness of the sample. According to the result, thermal processing of the juice resulted in the juice sample being redder when compared to other processing techniques as well as the control sample. High-pressure processing was found to reveal the greenness of the juice sample compared to other processing techniques. The greenness of the HPP2 samples could be attributed to an increased chlorophyll content of the juice as a result of cells disruption which in turns led to the release of chlorophyll into the surrounding environment of the HPP2 processed juice.

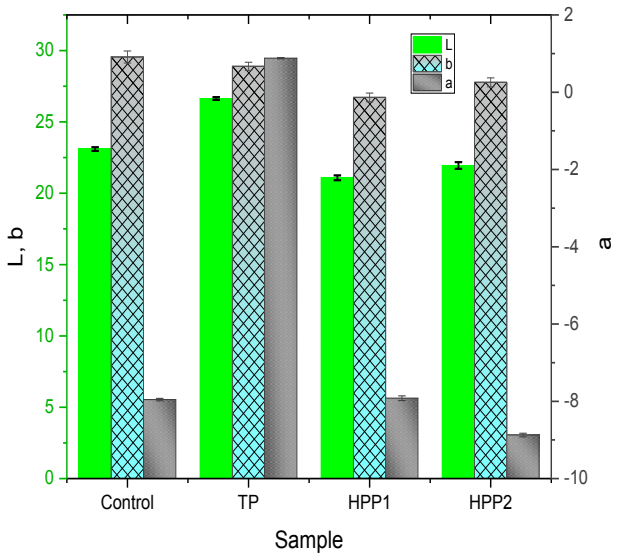

Figure 6: Comparison of the color characteristics of the juice mixes after the different treatments (n=3).

*Note: Hpp1 = high pressure processing at 2000 bar; Hpp2 = high pressure processing at 6000 bar; Tp = Thermal processing

The degree of yellowish and bluish of the juice samples is represented by b* and the result indicated that the b* value of the juice mix ranged between 26.72 and 29.55. The yellowness of food reflects the food degradation by light, chemical and temperature change. Both processing techniques (i.e. thermal and high-pressure processing) led to the reduction in the degree of yellowness of the sample. Juice samples processed at HPP pressure of 2000 bar had the least b* value. The result of the Chroma (C*) indicate the color intensity of the juice as perceived by the panelist as well as the total color difference (E*) which shows the color difference between the control sample and thermally or high-pressure samples is shown in Table 4. The result revealed that all the processing techniques resulted in the reduction of the color intensity of the juice when compared to the control sample. However, only juice samples subjected to HPP recorded the least total color change among the fruit and vegetable juice mix. The total color change is regarded as being distinct if E* > 3. The values obtained in this study

were above the maximum set and could be attributed to the effect of different preservation techniques on the fruit and vegetable juice blends. According to Pathare et al. (2013), the total color change (E*) and Chroma (C*) are considered the most sensitive color parameter when measuring the color degradation of fruit juice due to response to temperature fluctuation during storage. The hue angle (h*) is the qualitative attribute of food color in which the food is being defined as reddish, greenish etc. The degree to which the hue angle is formed defined the color of the food. When the hue angle is 0 or 360, the food being red, a hue angle of 90, 180 as well as 270 indicates a yellow, green and blue hue, respectively. According to the presented results, the hue angle of the juice mix ranged between -69.85 and 82.88 with significant difference ($p < 0.05$) among the fruit and vegetable juice mixes. The white index (WI), indicated that the juice mix subjected to thermal processing had the highest white index while the least was observed to be juice mix subjected to high-pressure processing at 2000 bar with no significant difference ($p < 0.05$) for HPP juice. The high white index found in thermally processed juice could be attributed to the inactivation of polyphenol oxidase which can cause non-enzymatic browning of the juice mix.

The effects of days of storage on the ºBrix and pH of the juice blend are presented in Table 5. Thermally processed juice mixed was observed to cause insignificant slight decrease in the ºBrix as the storage days' progresses from day 0 to day 3, slight increase at day 6, and stability as the storage days increased to day 9. This could be because the thermal processing coupled with the low refrigeration storage prevent the juice microflora from degrading some macromolecules in the juice which could invariably lead to the increase in the ºbrix of the juice mix. HPP at 2000 bar caused an insignificant decrease in the degree Brix of the juice mix as the storage days progresses

from day 0 to day 3. However, there was an insignificant increase in the ºbrix after storage for six (6) days and this remains constant throughout the storage period. According to Gao et al. (2016), HPP of fruit or vegetable at 2000 bar or lower may not result in the degradation of organic matter which could lead to the change in ºbrix of the juice. The increase of the HPP pressure to 6000 bar resulted in an insignificant increase in the ºBrix of the juice which becomes slightly predominant at day 14 (9.80). The result obtained in this study commensurate with the report of Gao et al. (2016) who reported insignificant changes in the physicochemical properties of cupped strawberry subjected to high-pressure processing.

The result which depicts the effect of storage on the pH of the juice is shown in Table 5. As it could be seen from the results, both thermal and HPP processing as well as storage did not exert any significant changes in the pH of the juice mix. Adil et al. (2015) reported no significant changes in the pH of apple and grapefruit juice blend subjected to sonication and high hydrostatic pressure processing. The finding of this study could be due to storage for days at low temperature (4°C) prevents the degradation of the organic acid and other metabolites present in the juice blends by spoilage or pathogenic microorganisms.

Table 3: Effect of storage on the Brix and pH of the juice mix (n=3 for each treatment)

	Day 3	Day 6	Day 9	Day 14	Day 21	Day 28
			Brix			
TP	9.72±0.02[a]	9.73±0.02[a]	9.73±0.01[a]			
HPP1	9.71±0.01[a]	9.72±0.02[a]	9.72±0.02[a]	9.75±0.02[a]	9.72±0.02[a]	9.71±0.01[a]
HPP2	9.74±0.01[a]	9.74±0.02[a]	9.75±0.02[a]	9.80±0.03[a]	9.72±0.02[a]	9.74±0.02[a]
			pH			
TP	3.30±0.02[a]	3.33±0.06[a]	3.30±0.02[a]			
HPP1	3.31±0.02[a]	3.35±0.02[a]	3.35±0.02[a]	3.34±0.01[a]	3.35±0.01[a]	3.34±0.01[a]
HPP2	3.30±0.02[a]	3.35±0.01[a]	3.36±0.01[a]	3.34±0.01[a]	3.34±0.01[a]	3.35±0.02[a]

TP: Thermal Processing, HPP1: High pressure processing at 2000 bar, HPP2: High pressure processing at 6000 bar

The effects of storage days on the color characteristics of the fruit and vegetable mixes processed by heat-treatment or HPP are presented in Table 6. The effect of days of storage on thermally processed juice blend revealed that there were no significant changes in the degree of lightness (L*) as the storage of the juice processes from day 0 to day 3. However, an increase in the storage days to day 6 caused a significant change in the lightness of the juice blend. On storage day 9, there was a minimal insignificant increase in the lightness of the juice. The increase in the brightness of the juice as a result of the thermal process could be attributed to the degradation of the color compound present in the juice blend.

The color attributes a* and b* were found to increase slightly as storage days increases. The increase in the redness of the juice due to thermal processing could be a result of the degradation of chlorophyll to pheophytin and/or Millard reaction formed during the thermal processing. Also, during the storage of the juice, there could be isomerization of anthocyanin compound in the juice, and hence, the increase in the redness (+a*) of the juice. Storing the juice mix processed at 2000 bar using HPP increased the brightness of the juice, the initial reduction in the blueness as the storage days increase from day 0 to day 3. There was an increase in the blueness as the storage days extended to day 6 but further decrease progressively during the extension of the storage beyond six days of storage. This could be because enzyme and microorganism were incompletely inactivated during high-pressure processing resulting in the initiation of chemical reaction which could either be enzymatic or non-enzymatic in the juice mix and hence the decrease in blueness.

Table 4: Effect of storage on the colour of the juice mix

	Day 3	Day 6	Day 9	Day 14	Day 21	Day 28
			L*			
TP	26.95±0.12[a]	28.02±0.54[a]	28.19±0.17[a]			
HPP1	22.23±0.47[b]	23.55±0.51[b]	24.31±0.33[b]	26.84±0.42[a]	26.70±0.80[a]	26.54±0.22[a]
HPP2	23.41±0.47[b]	22.40±0.39[b]	23.40±0.39[b]	26.61±0.44[a]	26.41±0.13[a]	26.31±0.28[a]
			a*			
TP	1.23±0.06[a]	1.63±0.09[a]	1.88±0.03[a]			
HPP1	-6.69±0.01[b]	-7.23±0.19[b]	-6.97±0.13[b]	-4.28±0.17[a]	-3.56±0.08[a]	-3.26±0.17[a]
HPP2	-7.32±0.05[c]	-7.13±0.17[b]	-7.14±0.17[c]	-4.31±0.15[a]	-3.09±0.12[b]	-3.44±0.10[a]
			b*			
TP	29.14±0.41[a]	29.66±0.86[a]	30.14±0.24[a]			
HPP1	27.69±0.53[b]	28.93±010[a]	28.98±0.12[b]	25.24±1.25[a]	28.27±1.62[a]	29.29±0.78[a]
HPP2	29.25±0.74[a]	27.56±0.55[b]	27.88±0.22[b]	26.36±0.88[a]	28.35±1.07[a]	29.20±0.28[a]

TP: Thermal Processing, HPP1: High pressure processing at 2000 bar, HPP2: High pressure processing at 6000 bar.

This result is consistent with the report of Oey et al. (2008) who reported the discoloration of HPP-processed food products when stored at 3°C. As for stored juice processed at 6000 bar using high-pressure processing, there was a progressive decrease in the degree of the blueness of the juice blend. The degree of yellowness or greenness of HPP juice mixes was observed to vary with the pressure of the process. For stored fruit and vegetable juice mix processed at 2000 bar, it was observed that yellowness of the juice increases as the length of storage increases while juice mix subjected to 6000 bar of pressure had its degree of yellowness initially increase when stored for three days. The yellowness however decreases as the storage days further increased beyond three days. The decrease in the yellowness as the days of storage increases could be a result of cell disruption during the high-pressure processing thereby leading to the leaching of chlorophyll a into the juice matrix and hence increase the greenness of the juice mix. This finding commensurate with the report of Cao et al. (2011) who studied the effects of hydrostatic pressure on the quality of strawberry pulp.

3.3 Phytochemical Properties of The Juice Mixes

The phytochemical properties of the juice mixes (ascorbic acid, chlorophyll, carotenoids, and polyphenols) as affected by thermal and HPP are shown in Table 7. The ascorbic acid of the juice mix ranged between 213 and 242 mg/L. As shown in the result, the thermally treated juice blend had the lowest ascorbic acid content while juice blend subject to 6000 bar HPP had the highest ascorbic acid contents. The low value for ascorbic acid in the thermally treated juice blend could be a result of its thermal instability and degradation. The degradation of ascorbic acid during thermal treatment could occur due to the formation of furfural when food containing ascorbic acid is subjected to thermal processing or treatment.

Table 5: Phytochemicals properties of the fruit and vegetable juice mixes

Sample	Ascorbic Acid (mg/100g)	Carotenoids (mg/Kg)	Chlorophyll a (mg/L)	Polyphenols (mg/Kg)
TP	213±3.6^{b}	21.7±2.0^{b}	5.347±0.322^{c}	322±10.6^{c}
HPP1	217±8.7^{b}	30.0±0.6^{a}	10.403±0.354^{b}	354±6.4^{b}
HPP2	242±2.4^{a}	24.1±3.8^{b}	9.495±0.421^{a}	421±15.6^{a}

TP: Thermal Processing, HPP1: High pressure processing at 2000 bar, HPP2: High pressure processing at 6000 bar.

The heat led to a sugar-ascorbic acid reaction whose intermediate product is 3-deoxy-l-pentosone (a furaldehydes) which further degrade to furfural. The formation of furaldehydes is enhanced or catalyzed in the presence of low acid. Tiwari et al. (2008) also in their research reported a reduction in the ascorbic acid of thermally treated orange juice. On the other hand, HPP- treated juice was found to have higher ascorbic acid retention compared to thermally processed juice. Juice mix treated at 6000 bar resulted in higher ascorbic acid or vitamin C retention compared to juice treated at 2000 bar. Many research such as Ahmed and Eun (2018); Barrett and Lloyd (2012) had reported an increase in ascorbic acid retention during high pressure or high hydrostatic pressure processing of fruit or juice. Other reason could be due to the inactivation of ascorbate oxidase, an enzyme responsible for the decay or degradation of ascorbic acid.

Thermally treated juice also had the lowest carotenoids content while juice mix treated at 2000 bar had the highest content. The reduced carotenoids content of thermally treated juice could result of oxidation and chemical decomposition of the carotenoids (Bonnie and Choo 1999). Oliveira et al. (2018) reported similar trends for non-

thermally treated acai juice. On the other hand, HPP treated juice mixes were observed to possess higher carotenoids contents compared to thermally treated juice blend. In their study, De Souza Carvalho et al. (2020) reported an improvement in the carotenoid content of acai and buriti juice after high hydrostatic pressure treatment. They suggested that the increase could be associated with the release from the plant cell structure retained bioactive chemicals which occurs during tissues/cells disruption.

The chlorophyll a composition of the juices varied from 5.347 in thermally treated juice to 10.403 mg/L in 2000 bar HPP- treated juice ($p<0.05$). The low level of chlorophyll a in the thermally-treated juice could be a result of the disintegration of the natural cellular structure thereby leading to the amenability of chloroplast pigment into a various enzymatic and non-enzymatic reaction which finally resulted in the formation of a brown coloration (Gaur et al., 2006). HPP- treatment of the juice mixes on the other hand resulted in higher chlorophyll a concentration than thermal processing. This may be due to the disruption of cells and release of chlorophyll content into the surrounding environment which leads to intense green color in the vegetable surfaces.

Polyphenols play an active role in human health as it acts as antioxidant chelating free radicals and reduce the risk of cardiovascular and other diseases. The polyphenol contents of the fruit and vegetable juice mix subjected to the different treatments are presented in Table 7. The result revealed that thermally treated juice had the lowest polyphenol compared to the HPP- treated samples. Polyphenol s are heat-labile compounds and are subjected to decomposition during thermal processing as evident in the thermally treated juice. The findings of this study were in contrast with the report

of Cao et al. (2011) who reported an increase in the phenolic compounds of thermally treated strawberry juice, which can be due to increased release from fruit tissues. Klopotek et al. (2005), on the other hand, reported a reduction in the total phenolic compound of strawberry juice subjected to heat treatment. HPP- treated juice, especially the one treated at 6000 bar had high polyphenol compared to thermally treated juice. This could be associated with the inactivation of polyphenol oxidase and peroxidase, enzymes that catalyzed phenol oxidation. Both polyphenol oxidase and peroxidase had been tagged as cogent enzymes responsible for the degradation of phenol in processed fruit and vegetables. The total phenols of grape juice were reported to increase as a result of HPP-treatment (Corrales et al., 2008). Other reason for its increase could be the extractability of antioxidant compounds such as protein, anthocyanins, amino acids and other compounds that possess hydroxyl phenol group after HPP.

The effects of storage on the content of the four phytochemicals (ascorbic acid, carotenoids, chlorophyll, and polyphenols) in the fruit and vegetable juice blend are presented in Table 6. The ascorbic acid content of the juice mixes was affected by the length of storage; as the length of storage progresses, there was a reduction in the ascorbic acid of both thermally and HPP-treated juice blends. During storage, ascorbic acid degradation follows the same pathway and yield the same products of degradation as obtained during thermal processing. However, instead of being thermally catalyzed, the degradation of ascorbic acid is being catalyzed by the acid content of the juice resulting in the acid hydrolysis of the lactone ring of the ascorbic acid. After acid hydrolysis, the ascorbic acid is decarboxylated and dehydrated leading to the formation of 3-deoxy-l-pentosone which in turns degrade to furfural. The process is can also be

accelerated in the presence of oxygen (Kurata and Sakurai, 1967). Also, a possible reason for the degradation of ascorbic acid during the storage of high pressure treated juice could be as a result of metal ion losses which catalyze the degradation of ascorbic acid due to the formation of complexes with chelating agent and had been reported to be favored by high-pressure processing.

There was a significant increase in the carotenoids content of the juice mixes following thermal and HPP treatment at the end of the storage days. According to Tiwari et al. (2008), carotenoid degradation during storage could occur as a result of partial inactivation of oxidative enzymes such as peroxidase, polyphenol oxidase, carotenoids cleavage dioxygenases and β-glucosidase. These enzymes had been reported to catalyze the oxidation of carotenoids to lactones, epoxides and endoperoxides.

The result revealed that there was initial degradation or reduction in the chlorophyll a concentration of juice for both treatments (thermal and high-pressure processing). This could be due to the partial inactivation of chlorophyllase and peroxidase. Peroxidase is also responsible for the degradation of chlorophyll during storage. During the degradation of chlorophyll by peroxidase, hydrogen peroxide catalyzes the oxidation of phenolic compounds resulting in the formation of phenoxyl radicals, which may catalyze the oxidation of chlorophyll a into a colourless compound as evidence at the end of the storage period of the fruit and vegetable juice blends.

Table 6: Effect of storage on the phytochemical composition of the juice mix

	Day 3	Day 6	Day 9	Day 14	Day 21	Day 28
			Ascorbic acid			
TP	219.28±3.85^{b}	192.92±15.35^{b}	178.26±25.23^{a}			
HPP1	196.40±12.87^{b}	193.94±17.20^{b}	204.39±19.55^{b}	213.98±3.03^{b}	206.55±11.96^{b}	192.40±2.61^{b}
HPP2	236.81±2.40^{a}	240.77±1.44^{a}	240.06±8.83^{a}	228.66±2.03^{a}	216.83±4.48^{a}	237.92±5.77^{a}
			Carotenoids			
TP	29.99±1.71^{a}	31.21±0.42^{a}	29.30±2.33^{a}			
HPP1	29.30±1.13^{a}	28.02±1.86^{b}	27.42±0.88^{b}	26.34±0.76^{b}	33.05±3.61^{a}	36.39±6.38^{a}
HPP2	27.05±1.48^{b}	27.12±1.86^{b}	28.64±0.59^{a}	34.94±5.56^{a}	23.73±3.15^{b}	38.75±9.69^{a}
			Chlorophyll a			
TP	5483.06^{c}	ND	ND			
HPP1	10189.50^{a}	10427.32^{a}	10388.12^{a}	ND	ND	ND
HPP2	9881.60^{b}	10077.18^{b}	9962.98^{b}	ND	ND	ND
			Polyphenol			
TP	340.00±28.28^{b}	269.00±15.56^{c}	257.50±10.61^{c}			
HPP1	340.44±14.76^{b}	372.50±24.75^{b}	292.50±17.68^{b}	269.50±14.85^{a}	232.50±17.68^{a}	387.10±19.66^{a}
HPP2	421.00±15.56^{a}	413.50±7.78^{a}	390.00±14.14^{a}	234.55±6.30^{b}	235.50±10.61^{a}	382.30±17.39^{a}

TP:Thermal Processing, HPP1: High pressure processing at 2000 bar, HPP2: High pressure processing at 6000 bar.

In thermally treated juice, it could be observed that the polyphenol decreased with an increase in the storage days. This could be due to some activities of polyphenol oxidase and peroxidase as a result of partial inactivation during heat treatment and hence, catalyzed the oxidation of the polyphenols. The polyphenol concentration of high pressure treated juice decreased as the storage days progresses to 21 days, however, at storage days above 21 days, the polyphenol content significantly increases. The initial decrease could be due to the activities of enzymes capable of degrading polyphenol. Further increase in the polyphenol content at 28 days of storage could be a result of the activities of some microorganisms present in the juice during storage. These microorganisms might catalyze the breakdown of some macromolecules and sugars present in the juice into organic acid which may facilitate the extraction or release of insoluble polyphenols associated with the juice matrix (Ye et al., 2014; Adebo and Medina-Meza, 2020).

3.4 Multivariate Analysis of The Physicochemical, Phytochemical and Microbial Activity of the Juice Mix

To deduce the relationship between the treatment techniques and their corresponding physicochemical, phytochemical and microbial properties of the juice, a chemometrics approach was used in the extraction of information from the original data. Unsupervised principal component analysis (PCA) was used and it revealed that three principal components (PCs) were able to describe the entire variation of the experimental data of the juice mixes. The first PC accounted for 59.49% while PC 2 accounted for 29.90% of the variation in the experimental data Table 9. To view the dispersion of the sample within the PCs, a biplot (Figure 8) was used. The biplot revealed that both HPP1 and HPP2 were grouped on the negative axis of PC1 which

denote similarity among these two juice blends. The control and thermal treated juice were grouped on the negative and positive axis of PC 2, respectively, which shows dissimilarity among the juice. Aside from the principal component analysis, the extract samples were subjected to hierarchical cluster analysis (HCA) for similarities among the samples. As shown in (Figure 8), the HCA suggested three (3) clusters in which cluster 1 was mainly characterized by control, cluster 2 mainly characterized by thermally treated juice. Finally, HPP 1 and HPP 2 juices were juice samples characterized in Cluster three (3).

Table 7: Factor loading and the Eigenvalue of the experimental data

	F1	F2	F3
ºBrix	0.809	0.279	0.518
pH	0.733	0.448	-0.512
L	0.688	0.725	-0.028
a	0.443	0.880	0.172
b	0.977	-0.040	-0.207
H	0.819	-0.099	-0.565
WI	0.561	0.827	0.034
C*	0.797	-0.480	-0.368
E	0.910	0.371	-0.187
CI	0.505	0.850	0.153
Ascorbic acid	-0.736	0.651	-0.187
Carotenoids	-0.843	0.533	0.076
Chlorophyll	-0.954	0.295	-0.055
Polyphenol	-0.782	0.572	-0.248
TPC	0.829	-0.541	0.141
Y&M	0.733	-0.089	0.675
Eigenvalue	9.518	4.785	1.697
Variability (%)	59.489	29.904	10.607
Cumulative %	59.489	89.393	100.000

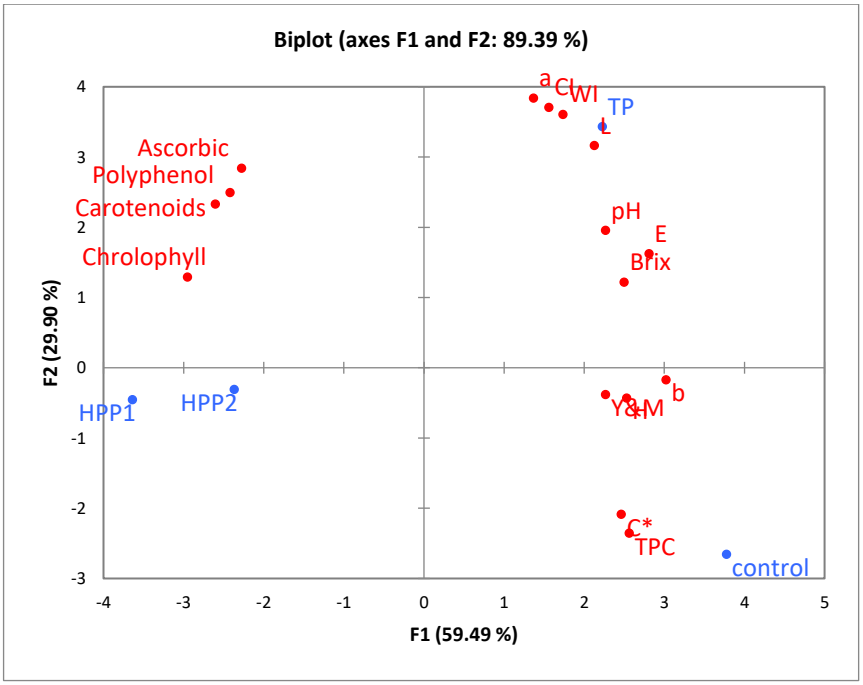

Figure 7: Biplot of the principal components 1 and 2

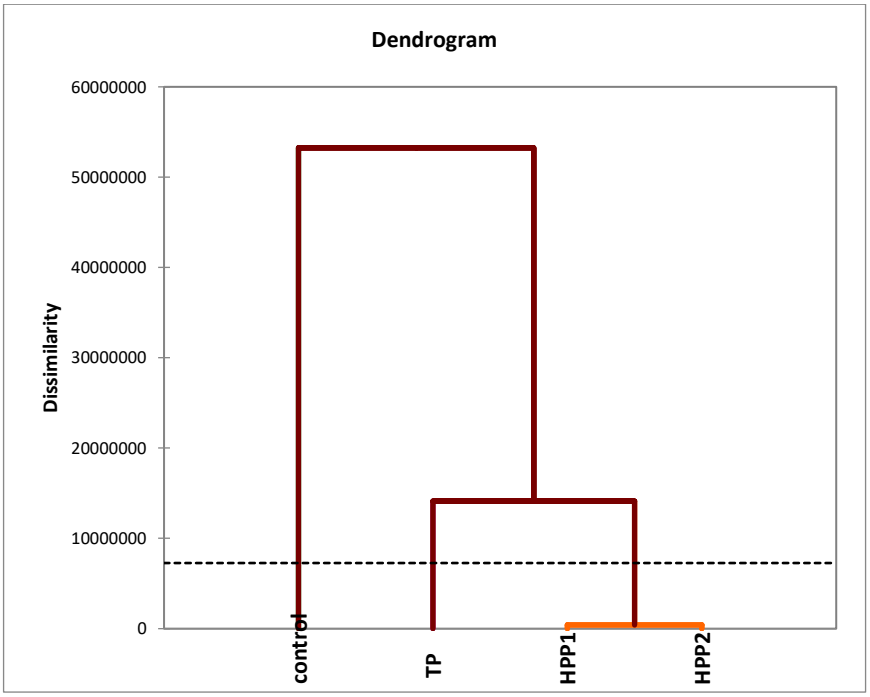

Figure 8: Hierarchical cluster analysis of the juice samples

Chapter 4: Conclusion

The main objective of the research was to study the effect of high-pressure processing on fresh green juice compared with thermal processing and analysis the quality parameters during the storage time. With regard to the physicochemical parameters, there was no significant difference in brix and pH among the samples and during the storage period. However, thermal treatment showed significant impact on the color of the treated samples compared to the HPP treatment and control. As per results, juice samples which subjected to high-pressure processing recorded the least total color change among the fruit and vegetable juice mix. As shown in the above results of chlorophyll a, the high concentration on HPP samples could be a result of disruption of the cells due to the high pressure and released chlorophyll pigment into the product. This can prove that HPP treatment are able to maintain the green color of the product. For the ascorbic acid, it's clear from the obtained results that samples treated with thermal process had the lowest level of ascorbic acid and HPP samples treated at 6000 bar had the highest. It's clearly observed that degradation of ascorbic acid due to HPP treatment is minimal compared to thermal process. The same findings were obtained for carotenoids, chlorophyll a and polyphenol. Samples treated thermally had the lowest level compare to HPP treated samples and among the storage time even though degradation was happing slowly. The findings of the antioxidant are supporting other studies mentioned earlier and reported that HPP treatment can maintain the antioxidant in the product treated. Regarding to the microbial growth in juices, both treatments significantly reduce microbial activity of the juice. However, growth of TPC and yeast and moulds were observed for all the processed samples. From the results obtained from all parameters, HPP technology can be recommended to treat green juices or

products in general due to its ability to maintain the chlorophyll a pigment with minimal or almost no detection of any changes

4.1 Recommendations

Different pressure needs to be tested (425, 450, and 475 MPa) and if the same results can be achieved it will help the industries to save time and money. More time selections must be tested with changing the pressures. In addition, further analysis of the enzymes behavior at different pressure and holding times will help to understand more the real factors that affect the quality of any product. More tests have to be done to know reasons for increasing or decreasing of some nutrients such as chlorophyll, antioxidants and TPC after non-thermal treatments. Also, a comparison between different non-thermal processes can help the industries to choose the most profit process with maintain the right parameters which consumer is looking for.

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United Arab Emirates University

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The technology of high hydrostatic pressure (HPP) in food processing started to take a huge attention in food industries due to its ability to increase the shelf life of processed products by inactivating food-borne microorganisms and undesired enzymes that cause changes not acceptable by the consumers. The aim of this thesis was to study the effect of HPP on certain quality parameters of green fruit juice compared to thermal process during shelf-life.

Ahlam Al Hammadi received her MSc in Food Science from the Department of food science, College of Agriculture & Veterinary Medicine at UAE University, UAE. She received her BSc from the College Agriculture & Veterinary Medicine at UAE University, UAE.

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