EFFECT OF HIGH PRESSURE PROCESSING ON CHEMICAL COMPOSITIONS, ANTIOXIDANTS AND ANTI-INFLAMMATORY PROPERTIES OF "PHULAE" PINEAPPLE PUREE

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ABSTRACT

Pineapple (*Ananas comosus* L.) is a widely consumed tropical fruit valued for its nutritional, functional and antioxidants properties. The *"Phulae"* pineapple cultivar, a specialty fruit from Chiang Rai, Thailand, small size and crisp texture, along with high levels of vitamin C and bromelain. Pineapple puree retains these key bioactive compounds and serves as a convenient form for processing, storage, and incorporation into functional foods and beverages. However, conventional heat treatment (CHT) often degrades these compounds, creating a need for alternative preservation methods. High pressure processing (HPP) offers a non-thermal approach that can maintain nutritional quality, antioxidant capacity, and sensory attributes of fruit-based products. To address this issue, this study compared HPP (HPP; 400 and 600 MPa, 10 min, 25 °C) with CHT at 80 °C for 10 min in preserving the quality of *"Phulae"* pineapple puree, using the puree without any treatment as a control.

This research was to investigate the effects on the quality, antioxidant activity, anti-inflammatory activities and volatile profile of *"Phulae"* pineapple puree. In-depth investigations were carried out to assess antioxidant activities (DPPH, FRAP, ABTS, and ORAC tests), anti-inflammatory activities (bromelain, NO, and HA), volatile component profiles, and bioactive substances (such vitamin C and total phenolic content or TPC). The results showed that HPP effectively preserved total soluble solids or TSS (13.5 °Brix) and color (L^* 46.6–48.0; b^* 21.3–22.0), with only slight deviations from the untreated sample (L^* 49.4; b^* 24.1). Vitamin C was significantly higher in HPP samples (216.9 mg/kg) compared to CHT (167.0 mg/kg). HPP at 400 MPa yielding the highest TPC (73.8 g GAE/kg), DPPH (2.3 mmol TE/kg), and FRAP (4.7 mmol Fe (II)/kg), while ABTS activity peaked at 600 MPa (4.6 g TE/kg FW). Bromelain activity was better maintained under HPP, with bromelain ranging from 3441.7–3733.3 CDU/L, HA inhibition 78.8–79.9%, and NO inhibition 47.0 mmol TE/kg FW, whereas CHT markedly reduced these values to 2025 CDU/L, 77.1%, and 27.4 mmol TE/kg FW, respectively ($P < 0.05$). Correlation analysis indicated that phenolic compounds and bromelain activity were key contributors to the puree's antioxidant and anti-inflammatory functions, while HPP effectively preserved these bioactive components and color integrity compared to heat treatment. Volatile compound analysis identified esters and terpenes as the dominant aroma contributors, and PCA revealed that PC1 and PC2 accounted for 60.4% and 25.3% of the variance, respectively, with HPP-treated samples clustering closely with the untreated control. In conclusion, HPP proved to be a promising non-thermal technology for preserving the nutritional (vitamin C, phenolics), functional (antioxidant capacity, anti-inflammatory activities), and sensory (color, aroma) qualities of *"Phulae"* pineapple puree, offering clear advantages over CHT.

Keywords: High Pressure Processing, *"Phulae"* Pineapple Puree, Physicochemical Properties, Antioxidant, Volatile Compounds

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ABBREVIATION AND SYMBOL

AAPH	2,2-azobis (2-amidinopropane) dihydrochloride
ABTS	2,20 -azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid
$ABTS^{+}$•	2,2-azinobis- (3-ethylbenzothiazoline-6-sulfonate) radical cation
Acyl-CoA	Acyl Coenzyme A
AUC	Area Under the Curve
a^*	Red/green Coordinate
BSA	Bovine Serum Albumin
b^*	Yellow/blue Coordinate
CAR	Carboxen
CDU	Casein Digesting Unit
CERAC	Cerium (IV)-Reducing Antioxidant Capacity
CHROMAC	Chromium Reducing Antioxidant Capacity
CHT	Conventional Heat Treatment
CUPRAC	Cupric Reducing Antioxidant Capacity
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DVB	Divinylbenzene
EDTA	Ethylenediaminetetraacetic Acid
Et al.	Et Alia
Etc.	Et Cetera
FE(II)	Ferrous Ion
Fe^{2+}	Ferrous Ion
Fe^{3+}	Ferric
FL	Fluorescein
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh Weight
G	Gram
GAE	Gallic Acid Equivalents

ABBREVIATION AND SYMBOL

GC-MS	Gas Chromatography Mass Spectrometer
GI	Geographical Indication
HA	Hyaluronidase Inhibitory Activity
HAT	Hydrogen Atom Transfer
HCl	Hydrochloric Acid
HPP	High Pressure Processing
kg	Kilogram
L	Liter
L^*	Lightness
LOX	Lipoxygenase
M	Molarity
MEP	Methylerythritol Phosphate
Mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
mmol	Millimole
mol	Mole
MPa	Megapascal
NaCl	Sodium chloride
nm	Nanometer
NO	Nitric Oxide Radical Scavenging Activity
ORAC	Oxygen Radical Absorbance Capacity
ONOO-	Peroxynitrite Anion
PCA	Principal Component Analysis
PDMS	Polydimethylsiloxane
PET	Polyethylene Terephthalate
POD	Peroxidase
PPO	Polyphenol Oxidase

ABBREVIATION AND SYMBOL

RDA	Recommended Daily Allowance
RI	Retention Index
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
TA	Total Acidity
TAC	Total Antioxidant Capacity
TCA	Trichloroacetic Acid
TE	Trolox Equivalents
TPC	Total Phenolic Content
TPTZ	Tripyridyltriazine
TSS	Total Soluble Solids
SET	Single Electron Transfer
SNP	Sodium Nitroprusside
SPME	Solid-Phase Microextraction
UV-Vis	Ultraviolet-Visible
µL	Microliters
µM	Micromolar
ºBrix	Degree Brix
ºC	Degree Celsius
-OH	Hydroxide Ion
-NH	Nitrogen-Hydrogen Bond
-CH	Methine Group
-SH	Sulfhydryl Group
No.	Number

CHAPTER 1

INTRODUCTION

1.1 Background

Many non-thermal techniques (such as pulsed ultraviolet, irradiation, cold plasma, ultrasonication, ozone, etc.) were created to produce food that meets the consumer's needs and to avoid using thermal processing that can lose its nutrition and color. HPP is a processing technique used to extend the shelf life of foods while maintaining their nutritional value and quality. It is a non-thermal pasteurization method that utilizes extremely high pressures between 100 to 600 Megapascal (MPa) or higher to kill harmful microorganisms, such as bacteria, yeasts, molds, and viruses, that may be present in food. HPP does not involve high temperatures that help preserve the nutritional content, flavors, and textures of the food. Moreover, this processing method helps preserve heat-sensitive nutrients, such as vitamins, minerals, and enzymes, which might otherwise be degraded or destroyed by traditional thermal treatments, preservation of antioxidants, maintaining the integrity of essential fatty acids and bioactive compounds in the food, and might enhance the bioavailability of certain nutrients, making them more easily absorbed by the body. Ready-to-eat meals, fruit juices, dairy goods, seafood, deli meats, and other food items frequently undergo HPP. Its popularity has grown as people look for minimally processed, wholesome, and safe food alternatives. Over time, as people's awareness of their health and interest in making educated dietary decisions have increased, healthy food has evolved. Nutritional, vitamin, and mineral-rich meals are becoming increasingly popular with consumers. Foods with health advantages and those that promote general well-being are given priority. Tropical fruits noted for their sweet and sour flavor include pineapples, which come in a variety of cultivars and types. While different locations and nations may have their own unique pineapple varieties. Pineapple has many health benefits that can reduce the risk of an unhealthy health problem or any diseases due to its compositions such as vitamin C, antioxidant activity, dietary fiber, bromelain, carotenoid, phenolic compounds, etc. (Kongsuwan et al., 2009). It is also an important

commercial fruit in both the domestic and overseas markets. Chiang Rai is one of the provinces in Thailand known for its pineapple cultivation, along with other agricultural products. *"Phulae"* pineapple (*Ananas comosus* L. Merr) refers to the Queen pineapple variety as a geographical indication (GI) of Chiang Rai, Thailand is recognized for its sweet, crispy and aromatic flavor. Pineapples are harvested at their peak ripeness and transported to local markets for direct consumption as fresh pineapple. Chiang Rai's pineapple industry also processes pineapples into various products such as canned pineapple, pineapple juice, dried pineapple, and pineapple jam which has a longer shelf life, making them suitable for both domestic consumption and export. The pineapple industry in Chiang Rai, like any other agricultural sector, faces challenges and opportunities. Factors that may affect the pineapple situation in the region include weather conditions, market demand, export opportunities, disease outbreaks, and government policies related to agriculture. Challenges faced by pineapple producers include fluctuating prices, market competition, climate-related issues, and concerns about sustainable farming practices. In addition, changes in international trade policies and demand in export markets may also impact on the pineapple industry in the region. Processing pineapples into products such as puree provides an effective way to add value, as puree offers several advantages over other processed forms. Unlike canned slices or juice, puree retains much of the fruit's natural pulp, fiber, and bioactive compounds, making it suitable for use in a wide range of applications, including beverages, jams, sauces, and functional foods. Its semi-solid form allows for easier formulation into value-added products, longer shelf life when properly processed and stored, and better preservation of flavor, color, and nutrients, which can help producers maximize economic returns and reduce postharvest losses.

Although HPP has been widely studied, there remain gaps in understanding its nutritional implications, particularly in fruit purees. Insights into these effects can help consumers, researchers, and food manufacturers make informed decisions about using HPP to produce safe, nutritious, and high-quality foods. This study aimed to evaluate the impact of different processing methods, including HPP at various pressure levels and conventional thermal treatment, on the quality of *"Phulae"* pineapple puree. Specifically, we investigated changes in bioactive compounds (such as vitamin C and total polyphenols), antioxidant activities (DPPH, FRAP, ABTS, and ORAC assays),

anti-inflammatory activities (bromelain activity, NO radical scavenging activity, and HA), and volatile compound profiles. Understanding the functional qualities of HPP-treated pineapple puree can not only enhance consumer confidence in HPP technology for processing functional foods but also potentially increase the economic value of the fruit. The findings from this study may serve as a guide for selecting optimal processing techniques that preserve both the nutritional value and sensory appeal of fruit-based products.

1.2 Objectives

The main objectives of this research were:

1.2.1 To study the effect of HPP, and CHT on bioactive compounds, antioxidant activities, and anti-inflammatory activities in *"Phulae"* pineapple puree

1.2.2 To study effect of different processing methods on volatile profiling in *"Phulae"* Pineapple Puree.

1.3 Research Hypotheses

This thesis study examined the two research questions via two experiments, which were applied HPP and CHT to *"Phulae"* pineapple puree

1.3.1 It was expected that using HPP preserved a significantly higher level of bioactive compounds, antioxidant activities, and anti-inflammatory activities in *"Phulae"* pineapple puree compared to CHT.

1.3.2 It was expected that using different processing methods (HPP and CHT) resulted in distinct volatile metabolic profiles in *"Phulae"* pineapple puree, with HPP better retained the characteristic aroma and flavor compounds compared to CHT.

1.4 Research Scope

1.4.1 The effect of different processing including the conventional heat treatment at 80 °C for 10 minute (min) and HPP at 400, 600 MPa for 10 min on physicochemical properties (color, pH, TSS, TA) and bioactive compounds (vitamin C,

polyphenol), antioxidant activity (DPPH, FRAP, ABTS, ORAC assays), and anti-inflammatory activities (NO, HA, Bromelain) in *"Phulae"* pineapple puree were first determined.

1.4.2 The effect of the same processing in part I of *"Phulae"* on volatile profiles was the last analysis and then clarified.

1.5 Research Output

The difference between the processing techniques will be compared on their bioactive compounds, antioxidant activities, anti-inflammatory activities, and volatile profiling.

1.6 Research Outcomes

1.6.1 HPP is an innovative technology to develop functional fruit products and add value to the product as it could preserve puree quality and maintain its nutritional contents.

1.6.2 The information will be useful for the functional food market and further study

1.7 Research Location

This study was conducted at the School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand and Faculty of Agriculture, Kagoshima University, Kagoshima, Japan. The laboratory is equipped with standard instruments for measuring quality attributes, including a colorimeter, a pH meter, a spectrophotometer, and a gas chromatography mass spectrometer.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple Production

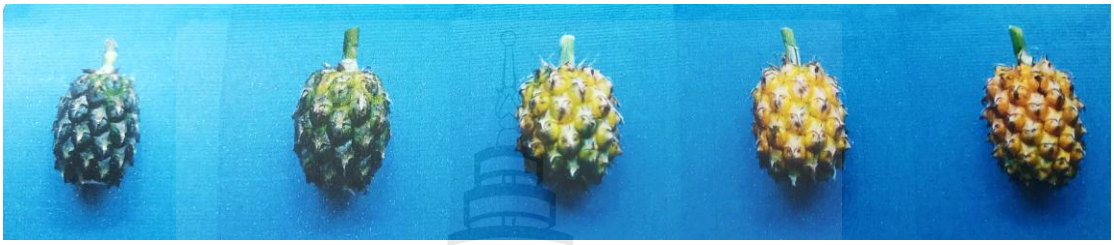
Pineapple (*Ananas comosus* Merr.) is a tropical fruit in the Bromeliaceae family. There are many varieties, such as 'Smooth Cayenne,' 'Red Spanish,' 'Queen,' 'Pernambuco,' 'Sugarloafs,' and 'Cabaiani. The pineapple (Figure 2.1), being a non-climacteric fruit, requires harvesting at a higher stage of maturity to achieve optimal edible quality. Size, the degree of flatness of the fruit's eye, and the yellow color of the fruit's skin typically serve as indicators of maturity (Gross and Saltveit, 2016; Wongs-Aree & Noichinda, 2014). *"Phulae"* pineapples are originally from the Nang Lae district, Chiang Rai province. The *"Phulae"* pineapple belongs to the Queen group, characterized by its small size, thorny leaves, deep eyes, and crispy, dry flesh that is not hard. The fruit has a sweet taste, and the entire core can be consumed.

Source Photographed by the author

Figure 2.1 *"Phulae"* Pineapple

There are five phases of maturation shown as Figure 2.2. The third stage (¾ to less than full gold) is when the harvest takes place. As they age, pineapples grow larger, heavier, and have a higher brix value, but they also become softer. The fruit starts off looking glossy, bluish, and dark green. The pineapple is orange yellow in the hue as it

reaches its final stage of development. With one or two yellowish-green eyes at the base, the eyes of the pineapple are mostly dark green. The "eye" flatness of a pineapple and the degree of skin yellowing determine its maturity. Consumers assess the quality of the fruit based on the color and scent of the skin.



Note Five stages of *"Phulae"* pineapple; Full green; ½ to less than ¾ gold; ¾ to less than full gold; Full gold; Over ripening stage (from left to right)

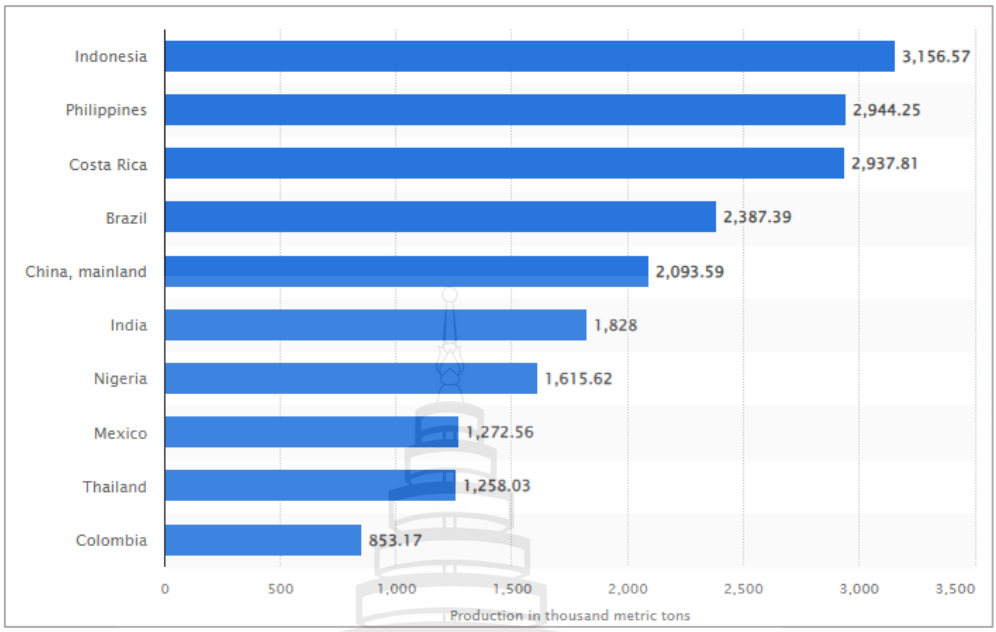
Source Kongsuwan (2010)

Figure 2.2 Maturity stages of *"Phulae"* pineapple.

In 2023, global pineapple production reached approximately 29.6 million metric tons, with Indonesia, the Philippines, and Costa Rica as the top three producers shown as Figure 2.3. Thailand ranked sixth with 1.8 million metric tons (Shahbandeh, 2025). Pineapples typically take 14 to 18 months to mature for harvesting and are versatile in culinary use, found in fresh consumption, canned products, juices, desserts, salads, jams, yogurt, ice cream, and savory dishes (Hossain, 2015; Shahbandeh, 2025). After harvest, pineapples are graded by weight or size, then undergo cleaning, waxing, sorting, packing, and transportation. It's important to store fresh pineapples in cool storage at 8°C with 90 to 95% relative humidity for an extended shelf life, avoiding temperatures lower than 8 degrees Celsius (°C) to prevent chilling injury and postharvest decay (Joy & Ta, 2016). Pineapple, among other tropical fruits like mango, mangosteen, guava, banana, papaya, and avocado, is a major global export. In 2020, global pineapple production reached 27.82 million metric tons, with an expected 2% annual increase to 37 million metric tons by 2030. The largest pineapple-producing region, accounting for 40% of global output in 2021, is Costa Rica, which is the second-largest global producer after the Philippines, responsible for 70% of worldwide exports. Notably, Asia, the world's top producer, primarily consumes its pineapple crop domestically, with Thailand ranking sixth in production at 1.8 million metric tons

(Shahbandeh, 2023). The total output value exceeded USD 500 million in 2020 (FAO). Thailand cultivates three main pineapple types: Spanish, Cayenne, and Queen (Popluechai et al., 2007), with over 25 different cultivars grown across the country, including 'Phuket,' 'Phulae,' 'Nanglae,' 'Intrachitdang,' 'Intrachitkow,' 'Pattavia,' 'Tradsithong,' 'Sawee,' and 'Petburi.' Each region in Thailand may have its unique pineapple cultivars.

In today's market, pineapple products offer consumers a diverse range of options. Beyond fresh pineapples, there is a wide array of pineapple-based products available, including canned pineapple slices and chunks, pineapple juice, dried pineapple snacks, pineapple jams and preserves, pineapple-flavored beverages, and even pineapple-infused alcoholic beverages like pineapple-flavored rum. Additionally, pineapple is a popular ingredient in smoothies, desserts, and tropical-themed dishes, contributing to its widespread appeal. People enjoy pineapple products for their sweet and tropical flavor, their versatility in culinary applications, and their perception as a symbol of refreshment and tropical indulgence. The study of pineapple puree is essential for a multitude of reasons. Firstly, it plays a pivotal role in the food industry, serving as a key ingredient in a wide array of products, from juices and smoothies to jams and desserts. Understanding its composition, flavor, and texture is crucial for maintaining product quality and ensuring consumer satisfaction. Moreover, pineapple puree offers potential health benefits due to its rich nutrient content, making it a subject of interest for nutrition and health research. Additionally, investigations into pineapple puree encompass various fields, including agriculture, food technology, and sensory analysis, contributing to optimized cultivation practices, preservation methods, and sensory appeal. In essence, the study of pineapple puree is indispensable for harnessing its culinary, nutritional, and economic potential across diverse industries.



Source Statista (2025)

Figure 2.3 Leading countries in pineapple production worldwide in 2023

2.2 Benefit of Pineapple to Human Health

The composition of pineapple mostly contains around 80% moisture and 20% total solids, which include sucrose, glucose, and fructose as the main compounds (Hossain et al., 2015). Pineapple is a beneficial source of vitamins, especially vitamins C, A, and B shown in Table 2.1 and 2.2. The major antioxidant capacity compounds could be total polyphenols and flavonoids (Sun et al., 2016). The important enzyme contained in pineapple is called bromelain, which can act as an anti-inflammatory and anti-cancer compound (Butu & Rodino, 2019).

Table 2.1 Nutrients in 100 grams of pineapple

Nutrients	**Amount**
Energy	52 calories
Dietary fiber	1.40g
Carbohydrate	13.7 g
Protein	0.54 g
Iron	0.28 mg

Table 2.1 (continued)

Nutrients	**Amount**
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Phosphorus	11 mg
Zinc	0.10 mg
Vitamin A	130 I. U
Vitamin B 1	0.079 mg
Vitamin B 2	0.031 mg
Vitamin B 3	0.489 mg
Vitamin B 6	0.110 mg
Vitamin C	24 mg

Source Hossain et al. (2015)

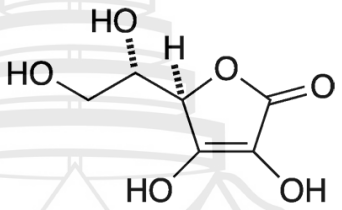
Table 2.2 Antioxidant capacities and bioactive compounds of *"Phulae"* and *"Nanglae"* pineapple

Bioactive compounds and antioxidant capacities	***"Phulae"***	***"Nanglae"***
Vitamin C (mg/100 g FW)	18.88 ±0.03a	6.45 ±0.68b
β-carotene (μg/100 g FW)	3.35 ±0.27a	1.41 ±0.01b
Bromelain (units/ mg protein)	1.66	1.60
Total phenolics (mg GAE/100g FW)	26.20 ±0.49a	20.28 ±1.18b
DPPH (mol TE/100g FW)	118.18 ±8.19b	152.93 ±10.51a
FRAP (mol AAE /100g FW)	165.28 ±2.04b	205.73 ±9.15a

Source Kongsuwan et al. (2009), Setha et al. (2010)

Various fruits and vegetables contain vitamin C, or *L*-ascorbic acid shown as Figure 2.4, a water-soluble antioxidant. Due to its role as a food quality indicator, vitamin C, which contains the antioxidants *L*-ascorbic and *L*-dehydroascorbic acids, is the most widely studied vitamin. Due to oxidation, which is the primary factor in acid

breakdown, these acids' stability depends on the availability of oxygen. The availability of oxidants and the activity of the enzymes that catalyze ascorbic acid oxidation are two factors that affect vitamin C's stability during storage in fruits and fruit-based products. Since greater storage temperatures can increase oxidation rates, ascorbic acid stability has also been correlated with the pH of the samples and with those conditions. Generally, the coldest storage temperatures are optimal for retaining vitamin C (Pérez-Lamela et al., 2021). The current Recommended Daily Allowance (RDA) for vitamin C is 25 mg per day for children ages 4 to 8, 75 mg per day for adult women, and 90 mg per day for adult men.

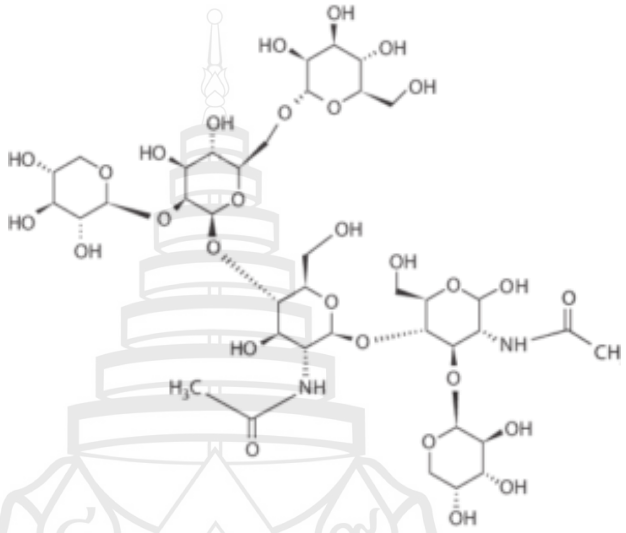


Source American Chemical Society (2021)

Figure 2.4 *L*-Ascorbic acid structure

The capacity of phenolic compounds to donate hydrogen atoms to free radicals explains their role as major antioxidants or free radical terminators (Pérez-Lamela et al., 2021). It is possible for phenolic compounds to control some enzyme activities by removing both active species and electrophiles to stop nitrosation. They can also cause autoxidation and act as an antioxidant. They are in charge of enhancing the immune system, lowering blood pressure, and decreasing inflammation (Rasheed et al., 2012). Flavonoids, another group of phenolic substances mostly found in fruits and vegetables, have significant anti-inflammatory effects. They also have a significant impact on the prevention of several illnesses, including metabolic and neurological diseases (Filippov et al., 2021; Pérez-Lamela et al., 2021). There were 39.4 to 55.2 mg of quercetin per g of total flavonoid content in the extracts of pineapple (Hossain & Rahman, 2011). The stems and young fruits of pineapples contain a crude, water-based substance called bromelain, which is a group of enzymes. Bromelain contains several proteinase inhibitors. Maurer (2001) suggests a connection between the protease activity and the

anti-inflammatory and platelet aggregation-inhibiting effects. Companies market bromelain as a dietary supplement to reduce pain and swelling during surgery or injury, especially in the mouth, nose, and sinuses. They also promote it as a treatment for cancer, osteoarthritis, digestive issues, and aching muscles.



Source Khalid et al. (2016)

Figure 2.5 Bromelain structure

Pineapple contains several compounds with antioxidant activity that help protect the body against oxidative stress and free radical damage. Some of the key antioxidants found in pineapple include vitamin C, which is a potent antioxidant that helps neutralize free radicals and supports the immune system. Another beneficial component is bromelain, a mixture of proteolytic enzymes found in pineapple stems and fruit, which possesses antioxidant properties and is also known for its anti-inflammatory benefits. Additionally, pineapple contains beta-carotene, a precursor to vitamin A, acting as an antioxidant that helps protect cells from oxidative damage. Alongside beta-carotene, flavonoids like quercetin, kaempferol, and rutin are also present in pineapple, contributing to its health benefits. Together with other compounds, these potent antioxidants scavenge free radicals and protect cells and tissues from oxidative stress-induced damage. This antioxidant function is known by a variety of names, such as antioxidant activity, antioxidant capacity, antioxidant power, antioxidant properties, antioxidant potential, antioxidant profile, antioxidant content,

antioxidant components, antioxidant composition, etc. The first two are the most used terms (Pérez-Lamela et al., 2021). The many antioxidant tests can be divided into two categories: those that rely on non-radical redox potential (FRAP, CUPRAC, CERAC, CHROMAC, etc.) and those that do so by scavenging radicals or reactive oxygen species (ROS) (Shahidi & Zhong, 2015).

2.2.1 TPC

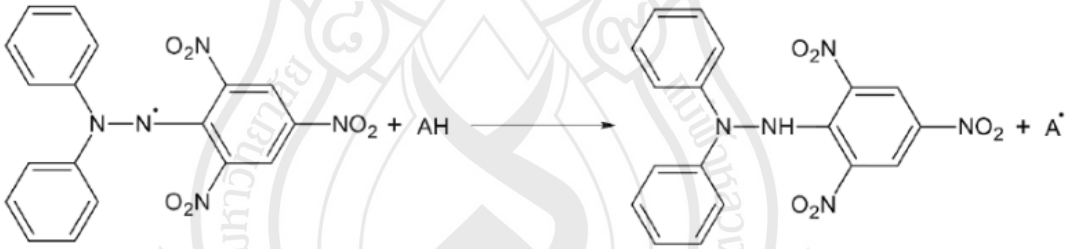
The TPC is a crucial parameter used to estimate the concentration of phenolic compounds in plant-based foods and extracts. Although TPC does not directly measure antioxidant activity, phenolic compounds themselves are potent antioxidants due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The most common method to determine TPC is the Folin–Ciocalteu assay shown in Figure 2.6, which involves the reduction of phosphomolybdic/phosphotungstic acid complexes present in the Folin–Ciocalteu reagent by phenolic compounds through a single electron transfer (SET) mechanism. This reaction results in the formation of a blue chromophore that absorbs light at wavelengths between 750–765 nanometer (nm). The absorbance measured is proportional to phenolic concentration. Typically, the results are expressed in milligrams or grams of gallic acid equivalents (GAE) per 100 g or kg of sample, using gallic acid or other phenolics like catechin or chlorogenic acid as standards. TPC is often used as an indicator of the overall antioxidant potential of food

Source Ford et al. (2019)

Figure 2.6 The reduction of the Folin-Ciocalteu reagent caused by the oxidation of the phenolics in a sample.

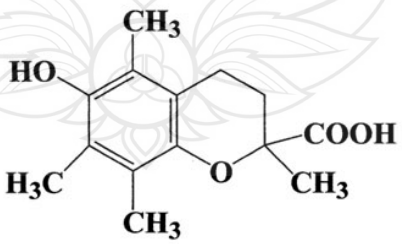
2.2.2 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH assay is a widely used method for evaluating the free radical scavenging activity of natural compounds. DPPH is a stable free radical with a deep violet color due to its unpaired electron, which absorbs maximally at 517 nm. When antioxidants are introduced, they donate hydrogen or electrons to DPPH radicals via hydrogen atom transfer (HAT) or SET mechanisms shown in Figure 2.7. This reaction reduces DPPH to its non-radical hydrazine form, resulting in a color change from violet to pale yellow. The degree of decolorization indicates the scavenging potential of the antioxidant compounds. This method is particularly useful due to its simplicity and rapid execution. The results are commonly reported as IC_{50} values—the concentration required to inhibit 50% of the DPPH radicals—or in terms of Trolox equivalent antioxidant capacity (TEAC), using Trolox as a reference antioxidant (Figure 2.8). The assay is particularly sensitive to antioxidants containing –OH, –NH, –CH, or –SH functional groups.



Source Knez et al. (2025)

Figure 2.7 The reaction involved in DPPH assay

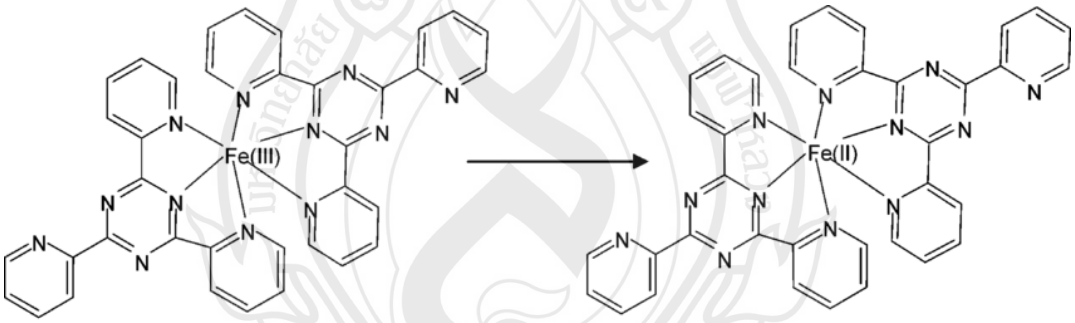


Source Guernelli et al. (2020)

Figure 2.8 Trolox structure

2.2.3 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay measures the antioxidant power of a sample by assessing its ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. This assay is based entirely on the SET mechanism and does not involve free radical reactions. In this method, antioxidants in the sample reduce a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to a blue-colored ferrous (Fe^{2+}-TPTZ) complex shown in Figure 2.9, which absorbs maximally at 593 nm. The intensity of the blue color correlates with the reducing power of the sample. The FRAP assay is conducted under acidic conditions (pH ~3.6) to maintain iron solubility and activity. Results are usually expressed in micromolar Fe^{2+} equivalents or compared against antioxidant standards such as Trolox or ascorbic acid, and are reported per gram, 100 grams, or 100 mL of sample. While FRAP does not account for the kinetics of radical scavenging or account for all types of antioxidants, it is a robust and reproducible method for evaluating the electron-donating capacity of food and biological extracts.



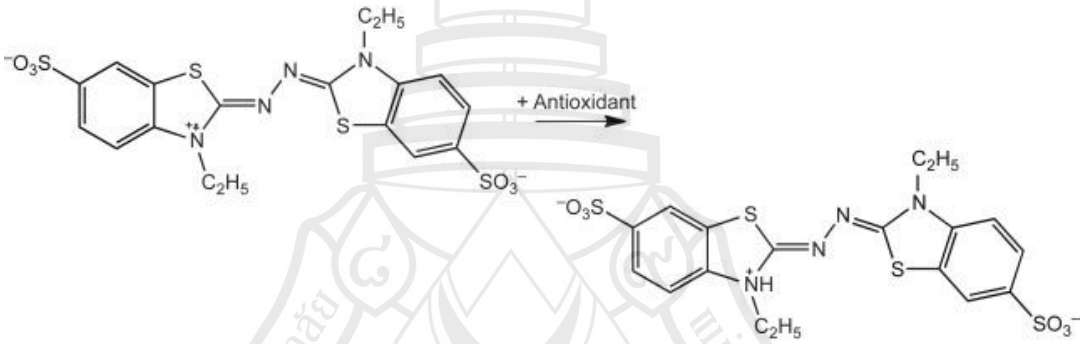
Source Prior et al. (2005)

Figure 2.9 Reaction for FRAP assay.

2.2.4 ABTS (2,20 -azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical assays

The ABTS assay is another widely used technique to determine the total antioxidant capacity of food extracts, beverages, and biological fluids. This method involves the generation of the $ABTS^{+}$• radical cation by oxidizing ABTS with potassium persulfate shown in Figure 2.10. The resulting blue green $ABTS^{+}$• radical

has maximum absorbance at 734 nm. When an antioxidant is added, it donates electrons or hydrogen atoms to neutralize the $ABTS^{+}$• radical, resulting in a decrease in absorbance. This decolorization is directly proportional to the antioxidant concentration in the sample. Unlike DPPH, ABTS is soluble in both aqueous and organic solvents, making it suitable for assessing both hydrophilic and lipophilic antioxidant compounds. The results are generally expressed in Trolox equivalents (TE), and the assay is sometimes referred to as the TEAC assay. The versatility, sensitivity, and broad applicability of ABTS make it a valuable tool for measuring antioxidant potential in complex food matrices. (Shahidi & Zhong, 2015).



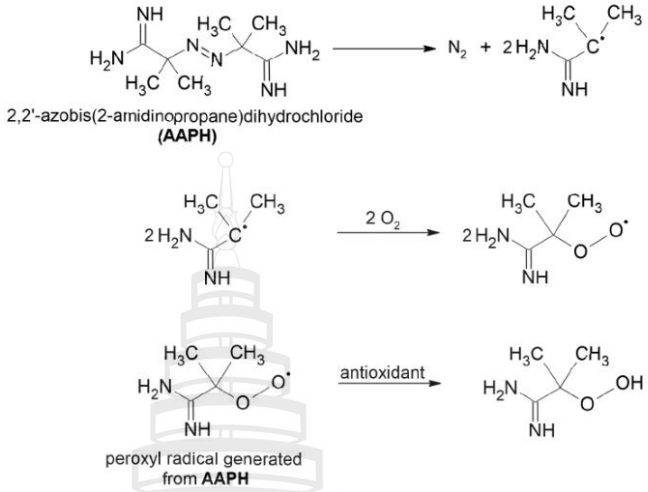
Source Hernández-Rodríguez et al. (2019)

Figure 2.10 ABTS chemical reaction with antioxidant compound.

2.2.5 ORAC (Oxygen Radical Absorbance Capacity)

The ORAC assay measures the ability of antioxidants to inhibit the oxidative degradation of a fluorescent probe, typically fluorescein, by peroxyl radicals. These radicals are generated from the thermal decomposition of AAPH (2,2-azobis(2-amidinopropane) dihydrochloride) shown in Figure 2.11. The degradation of fluorescein results in a decline in fluorescence over time, which is delayed in the presence of antioxidant compounds. ORAC is considered more biologically relevant than other assays because it uses peroxyl radicals, which are among the most prevalent reactive oxygen species in the human body. The results are calculated based on the area under the curve (AUC) of fluorescence decay and are reported as μmol TE per g or 100 mL of sample. The ORAC assay accounts for both the time and degree of inhibition,

making it particularly useful for evaluating the antioxidant capacity of complex mixtures such as fruit purees, juices, and supplements.

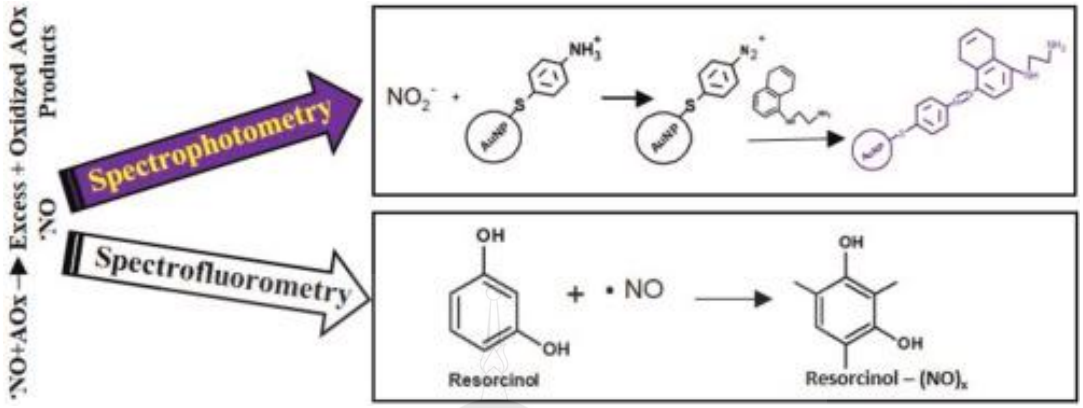


Source Knez et al. (2025)

Figure 2.11 The reactions occurring during the ORAC assay. AAPH—2,2′-azobis (2-methylpropionamidine) dihydrochloride

2.2.6 NO Radical Scavenging Activity

Numerous studies have established the significant role of nitric oxide in several inflammatory processes. Numerous carcinomas and inflammatory diseases such as juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis are associated with the chronic expression of the nitric oxide radical. Sustained levels of this radical production are directly toxic to tissues and contribute to the vascular collapse associated with septic shock. A reaction between the superoxide radical and the extremely reactive peroxynitrite anion (ONOO-) significantly increases the toxicity of NO. Oxygen and the nitric oxide that sodium nitroprusside produces interact to create nitrite shown in Figure 2.12. By immediately outcompeting oxygen in the interaction with nitric oxide, the extract prevents the creation of nitrite (Hazra et al., 2009). Furthermore, the extracellular fluid produces NO, which functions as an inflammatory mediator (Sharma et al., 2007).

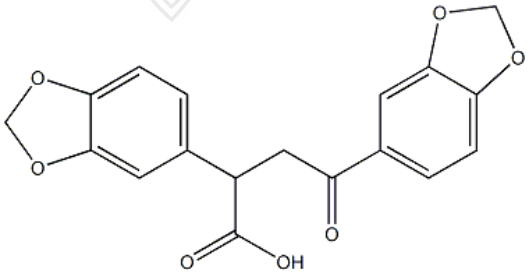


Source Can et al. (2022)

Figure 2.12 Detection of nitric oxide radical and its scavenging activity

2.2.7 Hyaluronidase (HA) Inhibition Activity

HA inhibition activity shown in Figure 2.13 is an enzyme that catalyzes the breakdown of hyaluronic acid, a major component of the extracellular matrix that maintains skin hydration, elasticity, and structural integrity in connective tissues. Overexpression of HA has been linked to several pathophysiological conditions including inflammation, allergic responses, cancer metastasis, and tissue aging. The HA inhibition assay is used to determine the ability of a sample to inhibit HA activity, thereby preserving HA and its biological functions. The assay typically involves incubating the enzyme with HA in the presence or absence of an inhibitor and then quantifying the amount of undegraded HA. A reduction in HA degradation indicates effective enzyme inhibition. The results are reported as percentage inhibition or IC_{50} values. A high HA inhibitory activity suggests strong anti-inflammatory and anti-aging potential, making this assay highly relevant in food, pharmaceutical, and cosmetic research.



Source ChemicalBook (n.d.).

Figure 2.13 *Hyaluronidase* structure

2.3 Volatile Compounds in Pineapple

Volatile compounds in pineapple (*Ananas comosus*) are essential contributors to the fruit's distinctive aroma and flavor, which strongly influence consumer preference, sensory acceptance, and overall product quality. These aroma-active compounds are produced through complex metabolic pathways and are released during fruit development, ripening, and processing. The volatile profile of pineapple, the example shown in Figure 2.14, is chemically diverse, comprising a wide array of esters, alcohols, aldehydes, ketones, organic acids, terpenes, and sulfur-containing molecules. Each of these groups plays a unique role in shaping the fruit's characteristic aroma, with their combined effects resulting in the sweet, tropical, and slightly tangy scent that is highly appreciated in fresh consumption and processed products. Among these volatile classes, esters are the most abundant and significant, particularly in fully ripened pineapples. These esters contribute prominently to fruity, sweet, and pleasant notes and are typically formed through the enzymatic esterification of alcohols with Acyl coenzyme A (acyl-CoA) derivatives. Key esters found in pineapple include ethyl acetate, methyl butanoate, ethyl butanoate, methyl hexanoate, and ethyl hexanoate, which are known for their low odor thresholds and strong fruity impact. The biosynthesis of these esters increases during fruit ripening, correlating with elevated enzymatic activity such as alcohol acyltransferases.

Alcohol is another important group, although present in lower concentrations compared to esters. These compounds contribute to sweet, green, and occasionally floral aromas. Notable examples include 1-hexanol, (Z)-3-hexen-1-ol, and linalool. Many of these alcohols are generated via the lipoxygenase (LOX) pathway, which involves the oxidative cleavage of polyunsaturated fatty acids. Linalool is a monoterpene alcohol known for its citrusy-floral note and occurs in various tropical fruits. Aldehydes, such as hexanal, nonanal, and trans-2-hexenal, are typically derived from lipid oxidation and are especially prominent in the early stages of fruit ripening, imparting green, grassy, or freshly cut aromas. These aldehydes are often rapidly converted into alcohols or acids during ripening, resulting in a shift toward sweeter and less sharp aromas. In addition, ketones like 6-methyl-5-hepten-2-one and β-ionone are

detected in pineapple and contribute to fruity and floral tones. These ketones arise from carotenoid degradation and secondary metabolism pathways and are generally associated with mature or overripe fruit aromas.

Although present in lower amounts, organic acids such as acetic acid, butanoic acid, and hexanoic acid can influence pineapple's sensory attributes by imparting slightly sour, cheesy, or fermented notes, particularly when esters hydrolyze during storage or fermentation. These acids also serve as important precursors for ester formation, linking them indirectly to the desirable fruity aroma profile. Another notable group is the terpenes, which, though typically found in trace amounts, play an important role due to their potent aromas and very low odor thresholds. Compounds like limonene, α-terpineol, and myrcene are synthesized via the mevalonate pathway or the methylerythritol phosphate (MEP) pathway, and impart citrusy, floral, or pine-like notes that enrich the overall aroma complexity. Particularly in tropical cultivars like pineapple, these terpenes contribute to the fruit's unique and appealing flavor profile. Additionally, sulfur-containing volatiles, such as methional, dimethyl sulfide, and 2-methylthiophene, although present in extremely low concentrations, have a profound influence on aroma due to their high volatility and ultra-low odor thresholds. These compounds are often associated with tropical, roasted, or savory nuances, and may also be linked to fermentation or Maillard reactions during processing. The concentration and composition of volatile compounds in pineapple are influenced by multiple factors, including cultivar or variety, ripeness stage, environmental conditions, and postharvest handling. For example, the *"Phulae"* pineapple, a popular cultivar in Thailand, is known for its strong, sweet aroma and is particularly rich in esters and monoterpenes. As pineapples ripen, enzymatic activity shifts the volatile balance: ester synthesis increases significantly, while aldehydes and alcohols decrease due to biochemical conversions and enhanced metabolic flux toward esterification. Postharvest processing methods also exert a strong influence on the volatile profile. CHT, commonly used for pasteurization, can cause significant degradation of heat-sensitive volatile compounds, leading to reduced fruity aroma and the possible formation of undesirable cooked or caramelized notes. In contrast, HPP a non-thermal technique applied at 400–600 MPa has been shown to preserve the integrity of delicate aroma compounds, particularly esters and alcohols, by avoiding high temperatures and maintaining enzyme-substrate

systems in a more stable state. Studies have demonstrated that HPP-treated pineapple juice and purée retain more of their fresh-like aroma compared to heat-treated equivalents. To accurately identify and quantify these volatile compounds, researchers commonly use gas chromatography-mass spectrometry (GC-MS), often paired with solid-phase microextraction (SPME). GC-MS provides excellent separation and precise identification based on mass spectra, while SPME allows for solvent-free, efficient extraction of volatiles directly from the sample or headspace. This combination is especially suited for analyzing fruit matrices where trace-level volatiles play a significant sensory role. Understanding the volatile composition of pineapple, particularly in relation to cultivar, ripening, and processing, is not only important for flavor profiling and quality control but is also essential for guiding industrial practices in juice, purée, and ready-to-eat product development. By maintaining a desirable volatile profile, producers can improve shelf life, enhance consumer satisfaction, and increase the marketability of both fresh and processed pineapple products.

Classification	Compound Name	Content ($\mu g \cdot kg^{-1}$)
Esters	Methyl hexanoate	99.54
	3-(Methylthio)propanoic acid methyl ester	19.43
	Hexanoic acid isopropylester	9.64
	Methyl octanoate	326.97
	Octanoic acid isopropyl ester	19.06
	Methyl decanoate	130.15
	Isopropyl decanoate	8.77
Total		613.56
Terpenes	(Z)-β-Ocimene	140.09
Total		140.09
Aldehydes	5-Hydroxymethylfurfural	110.80
	Decanal	8.63
Total		119.43
Ketones	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	7.70
Total		7.70
Lactones	δ-Octalactone	12.49
	Geranyl acetone	79.98
Total		92.47
Alcohol	1-Nonanol	7.89
Total		7.89
Hydrocarbons	1,3,5,8-Undecatetraene1	46.34
Total		46.34
Grand total		1019.78

Source Chang-bin et al. (2014)

Figure 2.14 Volatile compounds identified in pineapples

2.4 Processing Method of Puree Product

Fruit processing commonly employs conventional heat treatment techniques, such as pasteurization and commercial sterilization, as standard methods to ensure microbial safety, enzyme inactivation, and extended shelf life of fruit-based products. These processes are widely used in the juice and purée industries to reduce the risk of foodborne illness and spoilage by destroying pathogenic and spoilage microorganisms. However, the application of heat, especially at high intensities or for prolonged durations can lead to significant degradation of quality attributes, making it a double-edged sword. Over-processing due to excessive heat exposure can cause the loss of important physicochemical properties, such as pH balance, soluble solids content, and titratable acidity, which can in turn alter the sensory perception and stability of the final product (Leneveu-Jenvrin et al., 2020). Rheologically, heat can affect the viscosity and flow behavior of purées and juices by breaking down pectins, starches, or other hydrocolloids, often resulting in undesirable textural changes such as thinning, separation, or sedimentation. One of the most profound consequences of heat treatment is the inactivation or denaturation of enzymes, which is a critical step in slowing down spoilage and oxidation. Enzymes such as polyphenol oxidase (PPO) and peroxidase (POD), which are responsible for browning and degradation of phenolics, are typically targeted during thermal processing. However, complete inactivation is not always achieved with mild pasteurization, leading to residual activity that may continue to affect the product during storage. On the other hand, overheating may cause the complete denaturation of desirable enzymes like bromelain in pineapple or invertase in other fruits, which could otherwise have health-promoting or functional properties.

Heat treatment also has notable effects on the nutritional composition of fruits. Thermolabile compounds such as vitamin C (ascorbic acid), folate, and various B-complex vitamins are particularly sensitive to heat and may undergo rapid degradation during processing. In some cases, losses of vitamin C can exceed 50–70%, especially when fruits are subjected to temperatures above 90 °C for several minutes. Similarly, polyphenolic compounds and other antioxidants, which play a critical role in the health benefits of fruits, can be reduced due to thermal oxidation and polymerization. This

leads to a decline in the total antioxidant capacity (TAC), making heat-processed juices and purees nutritionally inferior compared to their fresh counterparts. Another key quality factor influenced by heat is color. Many pigments in fruits, such as anthocyanins, carotenoids, and chlorophylls, are highly unstable under heat. Anthocyanins, for example, which are responsible for red, purple, and blue hues in fruits like berries and grapes, degrade rapidly when exposed to high temperatures and oxygen, leading to browning or dull color. Carotenoids, though more stable, may isomerize or oxidize, affecting both their bioavailability and visual appeal. These changes can significantly diminish consumer acceptance, as color is one of the first sensory cues used to evaluate food quality. Aroma and flavor, too, are profoundly affected by thermal treatment. Many volatile aromatic compounds in fruits such as esters, alcohols, aldehydes, and terpenes are heat-sensitive and may evaporate, degrade, or transform into off-flavors during processing. This leads to a flattening or cooked character in the final product, diminishing the fresh, fruity, and vibrant notes that consumers associate high-quality juice or purée. For example, esters responsible for the fruity aroma in pineapple and mango are particularly vulnerable to thermal degradation, while the formation of Maillard reaction products during heating may introduce undesirable caramelized or burnt flavors.

Heat treatment methods are generally divided into two categories: pasteurization and sterilization, each having distinct processing goals and consequences. Sterilization involves more extreme conditions, usually exceeding 100 °C, to destroy all viable microorganisms including bacterial spores. It produces self-stable products suitable for ambient storage but often at the cost of substantial nutrient and sensory degradation. In contrast, pasteurization is milder (typically 60–95 °C), aiming to eliminate pathogenic bacteria and reduce spoilage organisms, while preserving more of the food's natural quality. However, pasteurized products are still microbiologically unstable over time and thus require complementary preservation techniques, such as acidification, refrigeration, preservatives, or packaging in modified atmospheres, to ensure safety and shelf life (Pérez-Lamela et al., 2021). Thermal processing plays an essential role in ensuring food safety and prolonging shelf life, it often does so at the expense of key sensory, nutritional, and functional qualities. There are some examples of thermal product shown in Table 2.3. The degradation of bioactive

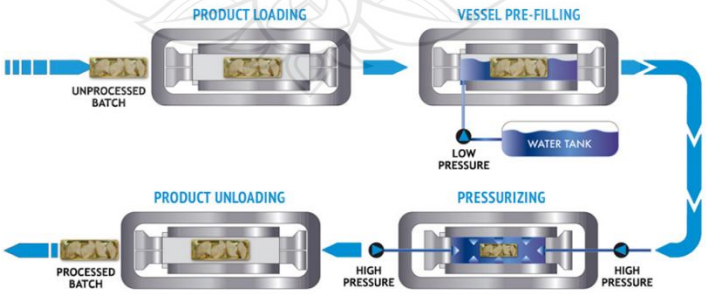
compounds, loss of natural aroma and flavor, and changes in color and texture pose major challenges for manufacturers aiming to meet modern consumer expectations for minimally processed, health-enhancing, and fresh-like products.

Table 2.3 Example of fruit products treated with heat treatment

Samples	**Condition**	**Results**	**References**
Pineapple Juice	55 to 95 ℃, 80 min	Changes in Hunter parameters, an increase in value and a decrease in b value	Rattanathanalerk et al. (2005)
Pineapple Juice	95 °C, 3 min	Decrease in original color, bioactive components, antioxidant activity, and volatile compounds	Wu et al. (2021)
Pineapple Juice	80 °C, 10 min	The juice that has been thermally pasteurized was more negatively impacted.	Chia et al. (2012)
Pineapple puree	70 to 90 ℃, 500 min	Changes in Hunter parameters, an increase in value and a decrease in b^* and L^* value	Chutintrasri and Noomhorm (2007)
Blueberry puree	40 to100 ℃, 20 min	Decrease of PPO activity while increase temperature	Zhang et al. (2021)
Pawpaw pulp	60 ℃, 30 min	Activate 70% PPO, undesirable changes in taste, color, and aroma	Zhang et al. (2017)
Banana puree	90 ℃, 2 min	Decrease in ascorbic acid and a value	Xu et al. (2016)

2.5 HPP

HPP is an emerging technology that has started industrial applications in Japan since 1992 (Hayashi, 1992). In the food industry, HPP was applied with the pressure between 100 and 600 MPa, where food was sealed in the packaging first, and for laboratory equipment, the maximum used is 1400 MPa (Marszałek et al., 2019). Their natural, fresh, and raw-like characteristics (sensorial, nutritional, and functional) have led to a surge in the market (Song et al., 2022). HPP has the potential to maintain the nutritional value and sensory qualities of fruits and their products. The product's quality and safety are affected due to its limited impact on the covalent bonds of low molecular mass components. Food items are compressed by constant pressure applied from every direction, and when the pressure is released, the products return to their original shape. Enough moisture in a food product minimizes the process, and uniform application of pressure in all directions ensures no macroscopic harm to the product. Because the transmission of pressure to the core is not mass/time dependent, the products undergo compression regardless of their size and structure. According to Ke et al. (2021), the primary process involves applying pressure to a product while using liquid as a pressure transmission medium. The mechanically applied pressure to the liquid (often water) that served as the transmission medium affects the result. Throughout the procedure, the vessel carrying the transmission medium and the product will maintain the pressure at a consistent rate shown in Figure 2.15. This facilitated the rapid and uniform distribution of pressure throughout the meal, leading to a more homogeneous final product.



Source Tomerlin et at. (2019)

Figure 2.15 High Pressure Processing Diagram

2.5.1 The Uses of HPP to Maintain the Quality of Produce

HPP as an emerging technology which started the industrial application in Japan since 1992. In food industry HPP was apply the pressure between 100 and 600 MPa where food was sealed in the packaging first and for laboratory equipment the maximum used is 1400 MPa (Marszałek et al., 2019). There is an increase in the market due to their natural, freshness, and raw-like characteristics (sensorial, nutritional, and functional) as reported by Song et al., (2022). HPP could preserve the nutritional values and sensory qualities of fruits and fruit products. It affects the quality and the safety of the product as its limited impact on the covalent bonds of low molecular mass components. However, the product after treated with HPP needs to store at low temperature to prevent the microbial growth, sensory and nutritional compounds loss (Castro & Saraiva, 2014). HPP is one non-thermal method for getting rid of enzymes and microorganisms where temperature is not the key determining factor (Barba et al., 2012). The need for high-quality convenience foods by consumers helped to advance HPP technologies. According to scientific and commercial evidence, HPP can yield products that are microbially safe, stable, and have better quality characteristics (Barba et al., 2015). With little impact on the sensory, physical, and nutritional qualities of the product, HPP might improve safety and lengthen shelf life (Abera, 2019). One of the key benefits of this technology is its new feature, which allows for the elimination of microorganisms at room temperature or lower.

2.5.2 The Effects of HPP on Microorganisms

In a non-thermal method known as HPP, enzymes and microorganisms are both eliminated while maintaining relatively constant pressure. The customer desire for premium convenience foods promoted the growth of HPP technologies. HPP have been demonstrated to yield products that are microbially safe, stable, and have better quality characteristics on both a commercial and scientific level (Barba et al., 2015). With minimal effects on the sensory, physical, and nutritional qualities of the product, HPP might improve safety and extend shelf life (Abera, 2019). One of the key benefits of this technology is its new feature, which allows for the elimination of microorganisms at room temperature or lower (Rastogi et al., 2007). Due to this attribute, the product could be of higher quality than one produced through a thermal technique. Some bioactive compounds that are heat labels will also be preserved by low temperature

processes. Many studies showed the effectiveness of HPP to reduce the microbial in food. The application situation, the kind of microbe, the kind of food, and the actual structure of the food all have some impact. HPP, which uses 300–500 MPa for 1–5 minutes to inactivate vegetative pathogens, and high pressure sterilization, which uses 1–10 minutes at an initial temperature of 70–90 °C and a processing temperature of 110–120 °C to inactivate bacterial spores, are two high pressure processing methods that can reduce the microbes on food. Compared to bacteria, yeast and mold are less pressure-resistant, although most of them can become inactive by 400 MPa pressure (Daher & Pérez-Lamela, 2017).

2.5.3 The Effects of HPP on Physicochemical Properties

Fresh fruit and vegetables should not typically be exposed to such high pressures since they may permanently harm the cell structure. The cell structure and texture of some fruits and vegetables were changed after being treated with pressure (Bolumar et al., 2016). The impact of pressure on food ingredients is what causes the structural changes in food systems that HPP creates. Molecular interactions such as hydrophobic interactions and electrostatic connections that sustain the spatial distribution of macromolecules or biopolymers make them sensitive to modification by HPP such as the changes of protein and starch. Mostly apply to processed fruits and vegetables, meat, and dairy products.

2.5.4 The Effects of HPP on Nutrition Value and Bioactive Compounds

HPP is known for its ability to maintain and, in some cases, increase the nutritional properties of food. Many studies shown in Table 2.4 focused on the positive impact of HPP on the bioactive component in diverse products. The phenolic component, phenolic acid, and total flavonoid were among the beneficial compounds in fruit that a review by Gamlath in 2011 indicated HPP might preserve. The HPP increases the total phenolics component in various fruits, such as the strawberry, blackberry, and onion, in addition to retaining some beneficial compounds in such fruits (Gamlath, 2011). The hydrophobic interactions in the cell membrane and cellular walls are disrupted, which results in an increase in phenolic compounds. The interruptions increase the pace of substance transfer and make it easier for the solvent to reach the cell (Prasad et al., 2009). Studies that measured the impact of HPP on various compounds produced a variety of results because HPP exhibits a high degree of

variability in the bioaccessibility of the same and different compounds depending on the magnitude of pressure, processing time, and processing temperature (Evrendilek, 2018). Antioxidants are compounds that play a crucial role in protecting the body from oxidative stress and free radicals. Many antioxidants are sensitive to heat and can be damaged during conventional food processing. HPP's gentle treatment helps retain these valuable antioxidants, contributing to the overall nutritional quality of the food (Huang et al., 2020; Khan et al., 2018).

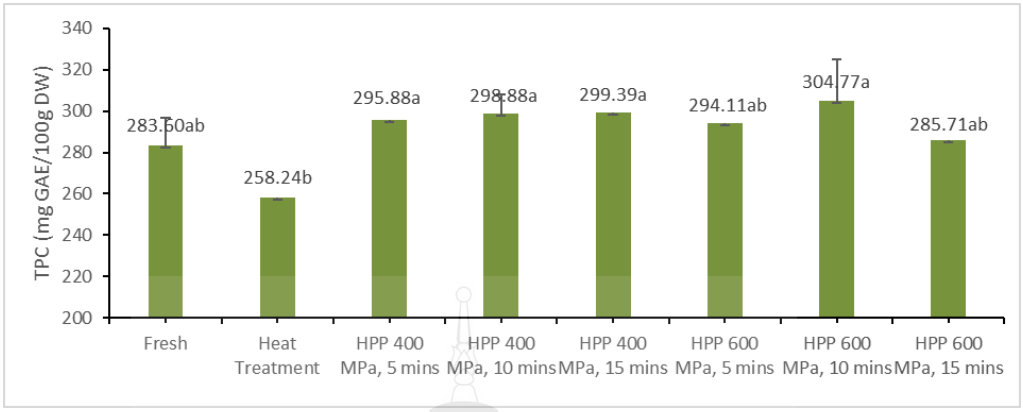
Table 2.4 Example of fruit products treated with HPP

Samples	**HPP treatment**	**Results compared to experimental control**	**References**
'Nanglae' Pineapple Juice	400 and 600 MPa, 5 min	maintain appearance, TSS, during cold storage	Chuensombat et al. (2019)
Pineapple puree	300 MPa, 5 min	improved the sensory quality compared with the commercial one	Marcellini et al. (2007)
Pineapple Juice	500 MPa, 10 min	retain the original color, antioxidant activity, bioactive compounds, volatile compounds	Wu et al. (2021)
Acidified Granny Smith apple puree	400 MPa, 5 min, 20 ℃	retaining vitamin C, ascorbic acid, and phenolics	Landl et al. (2010)
Orange juice-milk beverage	400 MPa, 9 min, 26.6 ℃	retaining color and ascorbic acid	Barba et al. (2012)
Red grapefruit juice	550 MPa, 10 min, 25 ◦C	higher amount of vitamin C, DPPH and FRAP when compared with heat treatment (110 °C, 8.6 s).	Gao et al. (2015)

Table 2.4 (continued)

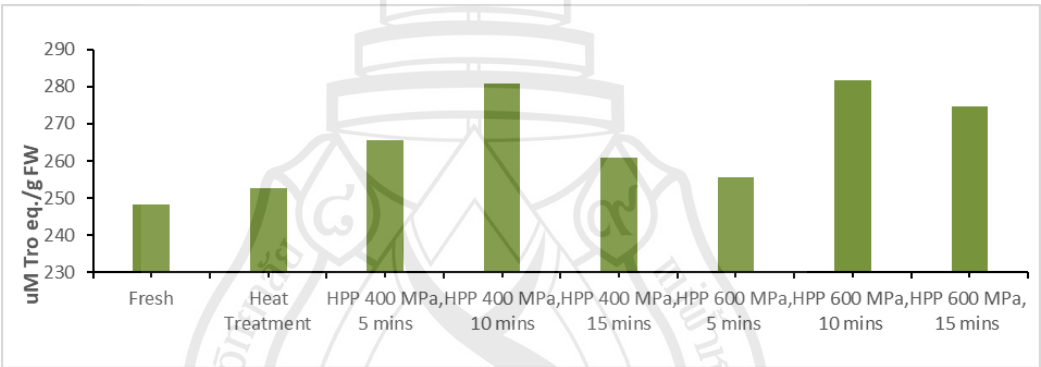
Samples	**HPP treatment**	**Results compared to experimental control**	**References**
Apple juice	400 MPa, 15 min	retain antioxidant activity (DPPH, FRAP)	Deng et al. (2019)
Mixed fruit and vegetables smoothie	630 Mpa, 6 min, 22 ◦C	the initial values of nutritional quality indicators increase and decrease during storage	Fernández (2019)
Jujube pulp	400, 500 and 600 MPa, 20 min	higher antioxidant capacity	Shen (2016)
Strawberry purée	300 and 500 MPa, 1, 5 and 15 min, 0 ◦C and 50 ◦C	higher polyphenols content and color parameters	Marszałek (2017)

The findings presented by Defriana.A. in Figure 2.16-2.17 demonstrate that the sample labeled as *"Phulae"* pineapple puree, subjected to HPP at 400 and 600 MPa for 10 minutes, exhibited significantly higher Nitric Oxide (NO) radical scavenging activity when compared with other treatments. It signifies that the food contains substantial levels of antioxidants capable of effectively neutralizing harmful nitric oxide radicals in the body. Specifically, the recorded values for TPC were 298.88 and 304.77 mg GAE/100 g DW, respectively. HPP aims to preserve the nutritional content, flavor, and sensory attributes of the food. Consequently, 400 and 600 MPa for 10 minutes of HPP parameters represent the most favorable condition for enhancing the antioxidant potential of pineapple puree. The selected pressure and duration (400 MPa for 10 minutes and 600 MPa for 10 minutes) be able to manage the preservation of these desirable properties with the maintenance of microbiological safety. Given these results, it is a promising candidate for further investigation and application in subsequent studies within the field.



Source Defriana (n.d.)

Figure 2.16 The results of TPC of "*Phulae"* pineapple



Source Defriana (n.d.)

Figure 2.17 The results of NO radical scavenging activity of *"Phulae"* pineapple

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Preparation of *"Phulae"* Pineapple Puree

"Phulae" pineapples were harvested at full maturity stage contains TSS at 13–15-degree brix (°Brix) from the orchard in Nang-Lae District, Chiang Rai province, located (19.95°N), Lat. (99.87°E.) and at altitude of 390 m in Thailand. Fresh *"Phulae"* pineapples were washed with tap water. The fruits were manually peeled, cut into small pieces, extracted by using a blender (Viva Collection HR2088/91, Philips, China) at the speed of 38,000 RPM for 30 second-, and 80-mL puree was packed into 100 mL screw-cap polyethylene terephthalate (PET) plastic spouted pouch (8 × 12 cm.) shown in figure 3.1.

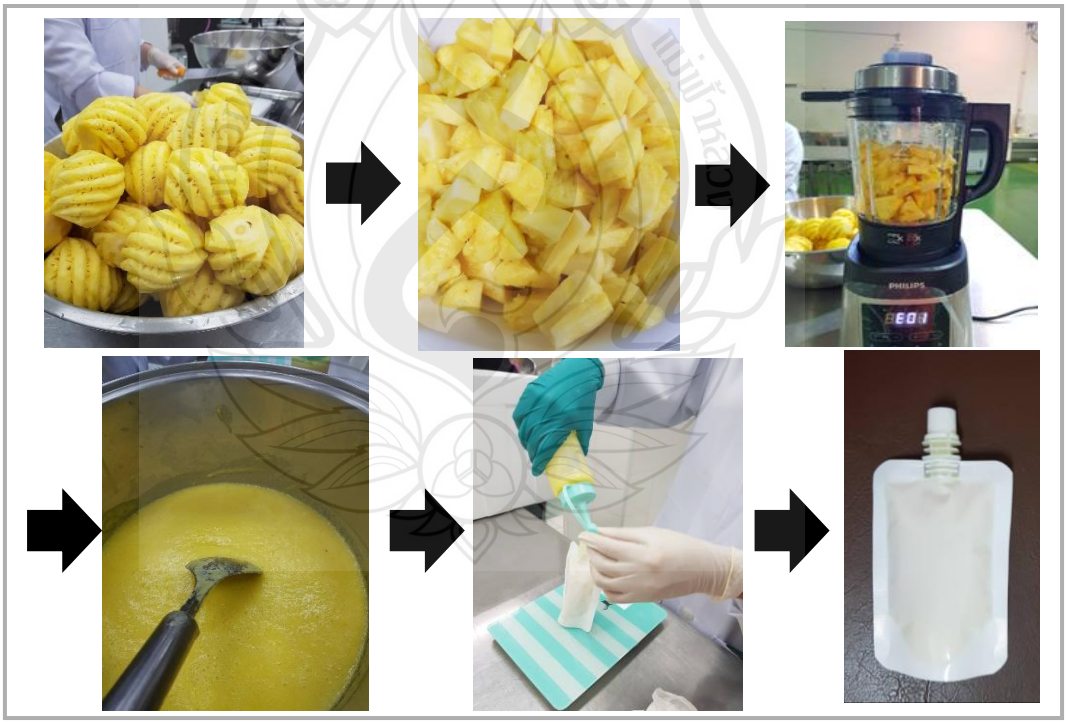


Figure 3.1 Puree preparation

3.2 HPP and CHT

The packed puree was treated under different conditions including 400 and 600 MPa with 10 minutes in a HPP apparatus (Bao Tou KeFa High Pressure Technology Co., Ltd., China) with a capacity of 3.0 L at room temperature (25°C). For each HPP treatment, the 15 samples were processed shown in figure 3.3. The fluid that transmitted pressure in this investigation was reverse osmosis (RO) water; the time spent applying or releasing pressure was not included in the treatment duration. The *"Phulae"* pineapple puree was stored at a freezer temperature of -20°C until used.

The control samples (untreated) consisted of 15 samples, while the conventional heat-treated samples were processed at 80 ± 5 °C for 10 minutes according to Suthiluk et al. (2023). For the heat treatment, 15 samples were prepared, and 80-mL puree was packed into 100 mL screw-cap PET plastic spouted pouches (8 × 12 cm), as shown in Figure 3.2. All *"Phulae"* pineapple puree samples were then stored at –20 °C until analysis.

Figure 3.2 Conventional Heat Treatment (CHT)

Figure 3.3 Preparing the puree before treated with High pressure processing (HPP)

3.3 Determination of Physiochemical Properties in *"Phulae"* Pineapple Puree

3.3.1 Measurements of color parameters

The five grams of puree was transferred into a clear plastic bag and spread to a thickness of 0.3 mm. The values of the surface color of bags of *"Phulae"* pineapple puree are measured using a colorimeter (Model: CM-600d, Konica Minolta, Tokyo, Japan). A white standard board is used for calibration. Color values were expressed by CIELAB as L^*, a^*, and b^* values.

3.3.2 Determinations of pH, TA, TSS

Physicochemical properties, including pH, TSS, and total acidity (TA), were analysed. The pH was measured by using a pH meter (Binder, Scientific Promotion Co., Ltd.). The pH meter was calibrated with pH 4.0 and 7.0 standard buffer solutions before use. TSS or °Brix measure the sugar content of the sample using a digital hand refractometer (ATAGO, Japan) and is referred to as the degrees Brix. TA was measured by using an acidity meter (ATAGO, Japan).

3.4 Determination of Bioactive Compounds and Antioxidant Activities in *"Phulae"* Pineapple Puree

3.4.1 Determination of Vitamin C content

The ascorbic acid concentration was calculated with slight modifications according to Kowitcharoen (2018). Each sample, which was subsequently extracted by homogenizing with a 5% meta-phosphoric acid solution. 15 minutes of centrifuging at 12,000 g and 4 °C. Through Whatman filter paper No. 4, the supernatant was filtered. Four milliliters of the extracted sample was combined with 2 ml of 0.02% indophenol, 4 ml of 2% thiourea, and 2 ml of 2% 2, 4-dinitrophenylhydrazine solution, and the combination was then incubated for three hours at 37 °C. After that, 1 ml of 85% H_2SO_4 was added, and a microplate reader (Multiskan Go, ThermoFisher Scientific, Japan) was used to detect the absorbance at 540 nm. Milligrams of ascorbic acid per kg of extract (mg /kg extract).

3.4.2 Determination of TPC

The determination of TPC using the method of Cao et al. (2012), with certain modifications and assessed via a colorimetric assay using Folin-Ciocalteu phenol reagent. One gram of the sample extract was diluted with 9 mL of distilled water, after which 1,250 µL aliquots of the diluted solution were transferred in duplicate to tubes containing an equal volume of 10% (v/v) Folin-Ciocalteu's reagent. Subsequently, 1,000 µL of 7.5% (w/v) sodium carbonate solution was added with agitation. The tubes were thereafter maintained at room temperature for 60 minutes prior to measuring absorbance at 765 nm using a microplate reader (Multiskan Go, ThermoFisher Scientific, Japan). The polyphenol concentration in samples was determined using a standard curve of gallic acid with a range of 20-100 µg/mL. The TPC was quantified GAE g/kg fresh weight (FW).

3.4.3 Determination of antioxidant capacity using the DPPH Assay

The determination of antioxidant capacity using the DPPH Assay using the method of (Roy et al., 2010). The sample 1 g was diluted with 19 mL of distilled water. The diluted extract (50 µL) was mixed with an aliquot of 1,950 µL of 60 µM DPPH radical in methanol. The reaction mixture was vortex-mixed and let to stand at room temperature in the dark for 30 minutes. Absorbance at 517 nm was measured using a microplate reader (Multiskan Go, ThermoFisher Scientific) and methanol as a blank. The control and standard were subjected to the same procedures as the sample except that, for the control, only distilled water was added, and, for the standard, the extract was replaced with 0-500 µM Trolox standard. The percentage inhibition values were calculated from the absorbance of the control and of the sample. The calibration curve was plotted between Trolox concentration (µM) and % inhibition. The DPPH radical scavenging activity of *"Phulae"* pineapple puree was expressed in terms of mmole TE/kg FW).

3.4.4 Determination of antioxidant capacity using the FRAP assay

The FRAP assay was determined according to Malta & Liu (2014). The sample 400 µL was added with 2.6 ml of FRAP solution which was prepared by mixing of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM hydrochloric acid (HCl), and 20 mM FeCl3 in a ratio of 10:1:1 (v/v/v). Then incubated at 37°C for 30 minutes and measured absorbance at 595 nm by using a microplate reader (Multiskan Go,

ThermoFisher Scientific, Japan). Ferrous sulfate equivalent was used as standard and distilled water as blank.

3.4.5 Determination of antioxidant capacity using the ABTS assay

The method for the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) test was slightly modified by Samakradhamrongthai (2020). The stock solutions were the 7.4 mM ABTS•+ solution and the 2.6 mM potassium persulfate solution. The two stock solutions were combined in equal parts and left to react for 12 hours at room temperature in the dark to create the working solution. To measure the absorbance of 1.17±0.02 units at 734 nm using the spectrophotometer, the solution was then diluted by mixing 1 mL of ABTS•+solution with 60 mL of methanol. With each test, a fresh ABTS•+solution was made. Fruit extracts (150 mL) were exposed to 2850 mL of the ABTS•+ solution in a dark environment for two hours. At 734 nm, the absorbance was then measured. The standard curve was linear between 25 and 600 mM Trolox. The units used to express the TE/kg of fresh mass are millimeters (mM). For additional dilution to be necessary, the measured ABTS value must be above the linear range of the standard curve.

3.4.6 Determination of antioxidant capacity using the ORAC assay

Following the modified ORAC assay described by Prior et al. (2003) and Shibata et al. (2021), a stock fluorescein (FL) solution (Stock #1) was prepared by dissolving 0.0225 g of FL in 50 mL of 0.075 M phosphate buffer (pH 7.0). A secondary stock solution was obtained by diluting 50 µL of Stock #1 into 10 mL of phosphate buffer. Subsequently, a 320-µL aliquot of this secondary solution was further diluted in 20 mL of phosphate buffer, from which 200 µL was dispensed into each well. This preparation resulted in a final FL concentration of 14 µM per well, equivalent to 7.5 nmol of FL per well. A Trolox stock solution (500 µM) was stored at −20 °C until use. For the standard assay, 20 µL of Trolox calibration solutions (6.25, 12.5, 25, and 50 µM) prepared in 0.075 M phosphate buffer (pH 7.0) were added to designated wells and incubated at 37 °C for 10 minutes. After this pre-incubation, 75 µL of AAPH was added to each well, and the plate was further incubated at 37 °C for an additional 2 hours. The assay was performed in a 96-well black plate, fluorescence intensity was recorded using a SpectraMax mini plate reader equipped with SoftMax Pro 7.2 software, with the temperature set at 37 °C. Readings were taken at 485 nm (excitation) and 535 nm

(emission) every 2 minutes for a total duration of 90 minutes. The AUC was determined from the fluorescence intensity data using the following equation:

$$AUC = 1 + \frac{f2min + f4min + f6min + \cdots f88min + f90min}{f0min}$$

The ORAC value was expressed as mmol of Trolox equivalents (TE) per kg of sample (mmol TE/kg).

3.5 Determination of Anti-inflammatory Properties in *"Phulae"* Pineapple Puree

3.5.1 Determination of bromelain enzyme activity

The enzyme activity was determined by quantifying the low-molecular-weight digestion products, which are soluble in trichloroacetic acid (TCA), resulting from the proteolytic action of the enzyme. The proteolytic activity of bromelain was assessed following the method described by Devakate et al. (2009). The assay was conducted using a 5 mL solution of 0.75% casein, prepared in an anhydrous disodium phosphate buffer (50 mM, pH 7), with the pH adjusted by gradually adding 0.1N HCl. The solution was preincubated at 37 °C for 10 minutes before introducing the enzyme, which had been diluted to a final volume of 1 mL using an activating buffer. The activating buffer consisted of 20.7 mM L-cysteine in 6 mM disodium ethylenediaminetetraacetic acid (EDTA) at pH 7. The proteolytic reaction was terminated after 10 minutes by adding 5 mL of 30% (w/v) TCA, and the mixture was incubated at 37 °C for 30 minutes. The solution was then cooled to room temperature and filtered twice using Whatman No. 42 filter paper. The absorbance of the resulting filtrate was measured at 280 nm using a UV–Vis spectrophotometer (U-2900, Hitachi High-Technologies Corporation). The enzymatic activity was quantified based on a standard calibration curve of absorbance versus tyrosine concentration (g/L) reported as casein digesting unit (CDU).

3.5.2 Determination of total protein content

Total protein content was determined using a protein assay kit (Dojindo Molecular Technologies, Inc.) based on the Bradford Coomassie Brilliant Blue method (Bradford, 1976). The colorimetric reaction was measured at 595 nm using a SpectraMax mini plate reader operated with SoftMax Pro 7.2 software. Bovine serum albumin (BSA) was used as the protein standard. A stock solution of BSA (4 mg/mL) was prepared in distilled water and subsequently diluted to various concentrations. The absorbance of these dilutions was measured at 595 nm to generate a standard curve of absorbance versus BSA protein concentration (µg/mL). For sample preparation, the samples were mixed with buffer (0.5 M Tris-HCl, pH 7.5, sodium chloride (NaCl), 1 mM EDTA) and centrifuged at 12,000 rpm for 10 minutes at 4 °C. The total protein content was then calculated and expressed in milligrams (mg), following the method described by Ketnawa et al. (2012).

3.5.3 Determination of NO radical scavenging activity

The nitric oxide radical scavenging activity was analyzed using the method of Win and Setha (2022) with modification. The nitric ions were generated from the interaction between oxygen and aqueous sodium nitroprusside (SNP), quantified by Griess Illosvoy reaction. 800 µL of 10 mM sodium nitroprusside was added to 40 µL of diluted extract. The reaction solution was then let stand at room temperature for 2 hours and 30 minutes. Then, 400 µL of 0.33% sulfanilamide in 20% glacial acetic acid was added after 200 µL of the reactive solution was transferred to a fresh tube. For five minutes, the sample tubes were left at room temperature. The test tube was then filled with 400 µL of 0.1% N-(1-naphthyl) ethylene-diamine dihydrochloride, and it was allowed to stand at room temperature for 30 minutes. A spectrophotometer was used to test the combination at 540 nm. To establish a standard curve, a Trolox dilution series from 0.2 to 0.5 mg/ mL was utilized. The results were represented in mmol TE/ kg FW.

3.5.4 Determination of HA inhibitory activity

The determination of HA described by Samejima and Park (2019) and Zhou et al. (2016), 100 µL of hyaluronidase (Type IV-S, derived from bovine testes; Sigma, St. Louis, MO, USA) was prepared in buffer to a final concentration of 5 mg/mL and incubated at 37 °C. Subsequently, 200 µL of compound 48/80 (Sigma) was added to the buffer at a final concentration of 0.5 mg/mL and incubated at 37 °C for 20 minutes.

Freeze-dried samples were reconstituted in distilled water, vortexed, and centrifuged at 10,000 RPM for 10 minutes before being introduced into the reaction mixture, followed by incubation at 37 °C for an additional 20 minutes. Hyaluronic acid sodium salt (extracted from rooster comb; Wako, Osaka, Japan) was then added at a final concentration of 0.4 mg/mL, and the reaction mixture was further incubated at 37 °C for 40 minutes. To terminate the reaction, 200 µL of 0.4 M NaOH and 200 µL of borate buffer (pH 8.0) were added, and the mixture was subjected to boiling water for 3 minutes before being rapidly cooled on ice. Subsequently, 3,000 µL of p-dimethylaminobenzaldehyde (Wako) solution was introduced, followed by incubation at 37 °C for 20 minutes. The absorbance was then measured at 585 nm, and the enzyme inhibitory activity (%) was calculated.

3.6 Determination of Volatile Compounds in "*Phulae*" Pineapple Puree

The headspace-SPME GC-MS technique, as previously published by Wu et al. (2021) with slight modifications, was applied to extract and analyze the volatile compounds. 20 mL headspace glass vials containing 5 g of pureed pineapple were filled with the mixture. The volatile compounds present in the headspace were extracted and absorbed using an SPME fiber (Supelco, Bellefonte, PA, USA) coated with 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) for 30 minutes at 40 °C. The gases were extracted, and then they were thermally desorbed in the injection inlet of a GC-MS system (7890A/5975C, Agilent Technologies Co., Ltd.; Santa Clara, CA, USA) for six minutes at 270 °C. Splitless mode was used for sample injection into the GC-column. A HP-5MS capillary (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) was used to separate volatile chemicals. The carrier gas used was helium, flowing at a constant 1.0 mL/minute rate. The GC-oven's temperature was first set at 40 °C for two minutes. After that, it increased to 220 °C at a rate of 5 °C/minute, then ramped to 260 °C at a rate of 10 °C/minute and was maintained there for an additional two minutes. The mass spectra were obtained using electron impact ionization mode, with the ion source operating at 230 °C. Agilent

ChemStation software (Agilent Technologies, Waldbronn, Germany) was used for peak area integration and chromatogram visualization. The n-alkane (C8–C20) obtained from Sigma–Aldrich, Germany was exposed to determine the volatile compounds' Retention Index (RI). The volatile components were tentatively identified by comparing the mass spectra of the samples with the retention index (RI), authentic references, and the data system library (NIST 20). The RI is calculated by the following formula.

$$\text{Index} = 100\left[\frac{(\text{tR} - \text{tRz})}{(\text{tR}(\text{z}+1) - \text{tRz})} + \text{z}\right]$$

Where tR, tRz, and tR(z+1) are the elution times of the unknown constituent, pre-eluted normal alkane, and posteluted normal alkane, respectively; z is the number of carbon atoms in pre-eluted hydrocarbon RI.

3.7 Statistical Analysis

Data were expressed as means ± standard deviation as triplicate determinations. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using Statistic Analysis System version 20.0 software (IBM SPSS Statistics 20.0). Differences at $P < 0.05$ were considered statistically significant. All measurements were determined in triplicate. The correlations between different antioxidants and anti-inflammatory activities among the treatment groups of puree pineapple were carried out at $p<0.05$ (*) and $p<0.01$ (**). The statistical analysis of volatile concentration data was log-transformed and Pareto-scaled before analysis using MetaboAnalyst 6.0 (www.metaboanalyst.ca (accessed on 12 August 2024)), to detect clusters and potential outliers, multivariate statistical analysis using PCA was employed. The intensity of each peak was first normalized to the summed total peak intensity of the chromatogram. The data matrix was then transformed using the Pareto scaling method.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of HPP on Physicochemical Properties in *"Phulae"* Pineapple Puree

4.1.1 Color Parameters

The CIELAB color space (*L*, a*, b**) was employed to evaluate the visual attributes of pineapple puree after different processing treatments, as it offers a standardized and perceptually uniform method for describing color changes. In this system, *L** reflects the lightness of the sample, with values ranging from 0 (absolute black) to 100 (pure white), while *a** and *b** indicate color dimensions along the red-green and yellow-blue axes, respectively. Positive *a** values denote a red hue, whereas negative values correspond to green. Similarly, positive *b** values indicate yellow, and negative values represent blue hues (Durmus, 2020). From table 4.1, it shows that processing significantly influenced the *L**, *a**, and *b** values of the pineapple puree ($P < 0.05$). The untreated sample exhibited an *L** value of 49.41 ± 0.39, which decreased to 45.08 ± 0.76 in the CHT-treated sample, indicating a noticeable darkening of the puree due to thermal exposure related to the appearance of the puree shown in Figure 4.1. In contrast, the HPP-treated samples, particularly at 600 MPa, maintained higher *L** values (46.63 ± 0.81 at 400 MPa and 48.02 ± 0.83 at 600 MPa), suggesting better retention of brightness and visual freshness. This preservation of lightness implies that HPP processing minimizes pigment degradation and browning reactions commonly associated with heat. As for the *a** values, all treatments showed negative values, indicating a dominant green hue across samples. However, slight variations occurred depending on the treatment. According to Pandiselvam et al. (2023), increasing pressure up to 500 MPa resulted in a gradual increase in *L** (lightness) of pineapple puree, while *a** and *b** values were largely unaffected by pressure but significantly influenced by temperature. The rise in *L** is attributed to pressure-induced cell wall disruption and improved light scattering, whereas *a** and *b**, which reflect intrinsic

pigment composition (e.g., carotenoids), remain stable under pressure but are sensitive to thermal degradation or isomerization. The CHT treated sample had an *a** value of -2.34 ± 0.21, slightly more positive than the untreated control (-2.41 ± 0.23), suggesting a slight reduction in green intensity caused by pigment oxidation or structural breakdown of green chlorophyll derivatives. Meanwhile, HPP treatments resulted in similar *a** values to the untreated sample (-2.40 ± 0.21 for 400 MPa and -2.71 ± 0.15 for 600 MPa), with HPP at 600 MPa even enhancing the green attribute slightly compared to all other treatments.

Figure 4.1 The appearance of the purees after the treatments. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min.

This result showed that HPP may better limit oxidative reactions. In terms of *b** values, which reflect the intensity of yellowness, the untreated sample recorded the highest *b** value at 24.11 ± 1.10. A significant decline was observed after CHT, dropping to 19.69 ± 0.89, demonstrating that thermal processing led to a loss of yellow coloration indicating that thermal processing resulted in a loss of yellow coloration due to the breakdown of carotenoids or the precipitation of insoluble matter affecting light reflection (Suthiluk et al., 2023). Moreover, heat treatment can induce thermal isomerization of carotenoids, converting the naturally predominant all-transforms into cis isomers. These cis isomers possess lower color intensity and are more prone to oxidative degradation, leading to reduced visual yellowness and overall pigment stability in heat-treated samples (Song et al., 2018) On the other hand, HPP treated samples showed higher *b** values than CHT (22.04 ± 0.87 at 400 MPa and 21.27 ± 0.90 at 600 MPa), though still lower than the control. These results confirm that HPP better retains the yellow pigmentation of pineapple puree compared to thermal methods,

especially at the moderate pressure level of 400 MPa. HPP can preserve color attributes which are lightness and yellowness more effectively than CHT, which aligns with prior studies on various fruit products (Goraya et al., 2024).

4.1.2 pH, TA, TSS

The physicochemical properties of untreated "*Phulae*" pineapple puree were analyzed to establish reference values for evaluating the effects of subsequent processing treatments which are HPP and CHT. The untreated puree displayed a pH of 3.96 ± 0.01, TSS of 13.54 ± 0.01 °Brix, and TA of 0.44 ± 0.01%. These values match those reported by Kongsuwan et al. (2009) for ripe *"Phulae"* pineapples, confirming that the fruit used in this study was at the right stage of ripeness and had the typical characteristics of this variety. These starting values provided important reference points for evaluating how much the different processing methods affected the fruit's physical and chemical properties. Following treatment, significant differences were observed among the samples ($P < 0.05$), especially between the control and CHT-treated puree, while HPP-treated samples showed relatively minor deviations. For instance, the pH of the untreated puree decreased slightly to 3.94 ± 0.01 and 3.92 ± 0.01 in samples subjected to HPP at 400 MPa and 600 MPa, respectively, indicating a minor but statistically significant acidification. This slight pH reduction agrees with earlier findings on other fruit matrices, such as mango and papaya purees, where high-pressure treatment led to increased acid content due to cell membrane disruption, resulting in leakage of organic acids into the juice matrix (Gómez-Maqueo et al., 2020; Yuan et al., 2018). In this study, the TA of HPP-treated samples increased from 0.44 ± 0.01% (control) to 0.48 ± 0.01% (400 MPa) and 0.46 ± 0.01% (600 MPa), further supporting the hypothesis that high hydrostatic pressure compromises cellular integrity and promotes the release of acidic constituents from vacuoles or other intracellular compartments. The increase in TA, without a proportionally large decrease in pH, also reflects the buffering capacity of organic acids naturally present in pineapple, such as citric and malic acids. These acids contribute to both TA and flavor, and their release due to mechanical or pressure-induced rupture of parenchyma cells can explain the results observed. Additionally, the lack of major difference between HPP at 400 and 600 MPa suggests that within this pressure range, the structural degradation of cell membranes may have reached a saturation point, whereby further pressure does not

yield proportionally more acid release, aligning with the pressure thresholds observed in other tropical fruit studies (Bi et al., 2020; Thu et al., 2017). In contrast, CHT led to more pronounced changes in all measured physicochemical parameters. The pH of the CHT-treated puree decreased slightly to 3.90 ± 0.01, which, although statistically different ($P < 0.05$), remained within the acidic range characteristic of pineapple products. However, the TA increased more noticeably to 0.51 ± 0.01%, the highest value among all samples. This change is likely due to thermal disruption of cellular membranes, causing more extensive liberation of organic acids than HPP, as suggested by previous thermal processing studies on citrus and guava juices (Gómez-Maqueo et al., 2020). The thermal energy applied during CHT may also promote chemical changes such as Maillard reactions or thermal degradation of polysaccharides, indirectly influencing acid content and pH by generating acidic degradation products. The TSS of the untreated puree (13.54 ± 0.01 °Brix) remained relatively stable after HPP, with values of 13.56 ± 0.01 °Brix at 400 MPa and 13.58 ± 0.01 °Brix at 600 MPa ($P > 0.05$). This lack of significant change implies that HPP does not considerably affect the solubilization or degradation of sugars and soluble solids within the puree matrix. Such stability has been reported in other fruit systems as well, including passion fruit (Niu et al., 2022), where the pressure-induced rupture of cells may lead to some release of sugars, but not at levels sufficient to statistically alter °Brix readings. Additionally, the absence of enzymatic browning or thermal degradation under non-thermal HPP conditions helps maintain the original sugar profile, thus contributing to the preservation of natural taste and sweetness. In sharp contrast, the CHT-treated puree exhibited a significant increase in TSS to 13.76 ± 0.01 °Brix ($P < 0.05$), which may be attributed to heat-induced water evaporation and subsequent concentration of soluble components. This phenomenon is well-documented in thermal processing, where prolonged exposure to elevated temperatures results in moisture loss and increased solute density (Kumar et al., 2023). Moreover, heat can accelerate the breakdown of complex carbohydrates into smaller sugar units, further elevating the TSS (Su et al., 2019). However, such increases in °Brix might not always equate to improved quality, as thermally processed products are also more susceptible to flavor degradation, nutrient loss, and pigment deterioration factors not directly measured by °Brix but critical to consumer acceptance. The relatively stable physicochemical parameters

observed in HPP-treated samples, despite the intense pressure applied, support the notion that HPP is a gentle preservation technique that retains the compositional integrity of fruit-based products. Compared to heat treatment, which exerts substantial influence on pH, TA, and °Brix, HPP offers a method that minimizes undesirable changes, thereby preserving the fresh-like characteristics of the puree. These findings align with numerous studies advocating HPP as a superior alternative to conventional pasteurization for products where minimal processing and high nutritional and sensory retention are desired (Bi et al., 2020; Patras et al., 2009). In summary, the results clearly indicate that CHT significantly alters the physicochemical properties of "*Phulae*" pineapple puree, especially in terms of increased acidity and soluble solids. Conversely, HPP treatments at both 400 and 600 MPa demonstrated a preservation of key quality attributes such as pH, TSS, and TA, with changes that were minimal and statistically insignificant compared to the control. These outcomes emphasize the potential of HPP as a non-thermal food preservation technology capable of maintaining the original quality of tropical fruit purees, ensuring both safety and consumer appeal.

Table 4.1 Changes in pH; TSS; TA; and color parameters (L^*, a^*, b^*) of *"Phulae"* pineapple puree treated by CHT and HPP

Treatments	**pH**	**TSS (°Brix)**	**TA**	***L****	***a****	***b****
Untreated	3.96 ± 0.01[a]	13.54 ± 0.11[b]	0.44 ± 0.02[c]	49.41 ± 0.39[a]	-2.41 ± 0.23[a]	24.11 ± 1.10[a]
CHT	3.94 ± 0.01[b]	14.36 ± 0.23[a]	0.51 ± 0.03[a]	45.08 ± 0.78[d]	-2.34 ± 0.21[a]	19.69 ± 0.89[c]
HPP 400	3.92 ± 0.01[c]	13.51 ± 0.12[b]	0.48 ± 0.01[b]	46.63 ± 0.81[c]	-2.40 ± 0.21[a]	22.04 ± 0.87[b]
HPP 600	3.92 ± 0.01[c]	13.61 ± 0.14[b]	0.46 ± 0.00[b]	48.02 ± 0.83[b]	-2.71 ± 0.15[b]	21.27 ± 0.90[b]

Note Different letters in the same column indicate a significant difference ($p < 0.05$) among treatments. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min; TA, total acidity; TSS, total soluble solids; L^*, lightness; a^*, redness; b^*, yellowness.

4.2 Effect of HPP on Bioactive Compounds and Antioxidant Activity in *"Phulae"* Pineapple Puree

4.2.1 Vitamin C content

The vitamin C content of fresh "*Phulae*" pineapple puree was determined to be 216.9 ± 3.8 mg/kg, which aligns well with previously reported values for ripe "*Phulae*" pineapple pulp (Kongsuwan et al., 2009). Vitamin C, also known as ascorbic acid, is a water-soluble micronutrient that plays a vital role in human nutrition due to its antioxidant properties, contribution to immune function, and involvement in collagen biosynthesis. However, it is also one of the most labile bioactive compounds in fruits, being highly sensitive to oxidative degradation, enzymatic activity, pH, and, most notably, heat. As such, preserving vitamin C during processing is often considered an important indicator of the effectiveness of a food preservation technique (Lu et al., 2014). In this study, both HPP and CHT led to reductions in vitamin C levels compared to the untreated sample, although the magnitude of degradation varied significantly between the two methods. HPP-treated samples retained relatively high levels of vitamin C, with values ranging from 191.9 ± 2.5 mg/kg (400 MPa) to 191.2 ± 2.9 mg/kg (600 MPa), showing only a slight and statistically insignificant decline ($P > 0.05$). In contrast, the CHT-treated sample experienced a more substantial reduction, with vitamin C content dropping to 172.3 ± 3.1 mg/kg representing a statistically significant loss ($P < 0.05$) of nearly 20% compared to the untreated control (Figure 4.2). These results are in agreement with prior research, including studies by (Barba et al., 2017) and (Odriozola-Serrano et al., 2008), which demonstrated that HPP results in considerably lower degradation of ascorbic acid compared to conventional thermal treatments. The minimal loss of vitamin C in HPP-treated samples can be attributed to the non-thermal nature of the process. Although HPP involves intense pressure, the temperature rise is minimal and transient, which protects thermolabile compounds such as vitamin C from structural breakdown. Additionally, the absence of oxygen in sealed, vacuum-packaged samples during HPP further reduces the likelihood of oxidative degradation. In contrast, thermal treatment at 80°C for 10 min, as applied in the CHT sample, exposes vitamin C to both heat and potential oxidative conditions. Vitamin C

is known to degrade rapidly at elevated temperatures due to oxidation, hydrolysis, and ring-opening reactions, especially when catalyzed by trace metal ions or in the presence of oxygen. The significantly lower vitamin C content in the heat-treated sample reflects these mechanisms and underscores the vulnerability of this nutrient to thermal processing (Odriozola-Serrano et al., 2008; Zheng et al., 2012). The preservation of vitamin C in HPP-treated pineapple puree not only has nutritional implications but also contributes to the antioxidant capacity and shelf-life of the product. Ascorbic acid acts as a scavenger of free radicals and can also protect other sensitive phytochemicals, such as phenolics and carotenoids, by reducing the redox potential of the food matrix. Therefore, higher retention of vitamin C through HPP may help preserve the overall bioactive compound profile of the puree, making it more desirable from a health and functional food perspective (Barba et al., 2013). Moreover, this pattern of vitamin C retention observed in pineapple puree is consistent with findings in other fruit products, including strawberry, orange, mango, and passion fruit, where HPP-treated samples consistently showed higher vitamin C retention compared to those subjected to CHT. For instance, (Sánchez-Moreno et al., 2006) found that HPP preserved 90–95% of vitamin C in orange juice, compared to only 70–75% in heat treated samples. These cross-product comparisons further support the conclusion that HPP is a superior method for preserving temperature-sensitive nutrients. This study clearly demonstrates that while both HPP and CHT cause some loss of vitamin C in "*Phulae*" pineapple puree, the extent of degradation is significantly less under HPP conditions.

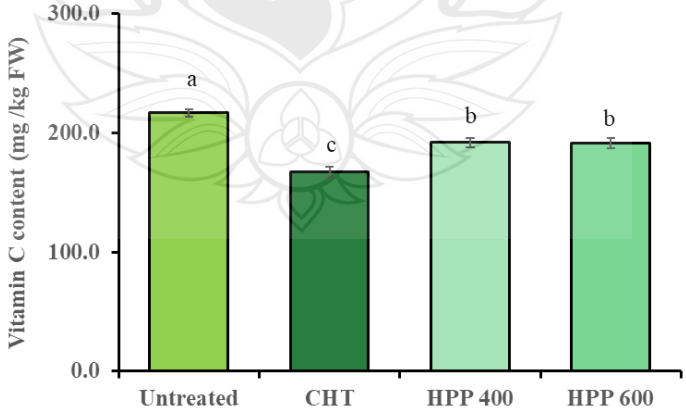


Figure 4.2 Effects of different processing on Vitamin C content (mg/kg) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; FW, fresh weight; HPP-400, high-pressure processing at 400 MPa for 10

min; HPP-600, high-pressure processing at 600 MPa for 10 min.

4.2.2 TPC

The TPC of pineapple puree samples was significantly influenced by the processing method employed, as illustrated in Figure 4.3. The untreated "*Phulae*" pineapple puree exhibited a TPC value of approximately 64.9 ± 2.1 g GAE/kg, serving as the benchmark for the untreated sample. Phenolic compounds are a major class of bioactive phytochemicals known for their antioxidant properties, free radical scavenging activity, and their role in reducing oxidative stress in biological systems. As such, TPC is frequently used as a key indicator of the nutritional and functional quality of fruit-based products. As shown in the graph, CHT resulted in a significant reduction in TPC, with levels dropping to the lowest among all groups, approximately 56.2 ± 1.8 g GAE/kg. This reduction was statistically significant ($P < 0.05$) when compared to both the untreated sample and HPP-treated groups. The decrease in phenolic content following thermal processing is likely due to the thermal degradation, oxidation, or polymerization of phenolic compounds under high temperature conditions (Cao et al., 2012). Heat can cause the breakdown of phenolic structures or convert them into forms that are no longer detectable by standard Folin–Ciocalteu methods, which rely on the redox reactivity of phenols. In contrast, HPP at both 400 MPa and 600 MPa preserved significantly higher levels of TPC approximately 74.1 ± 2.4 g GAE/kg and 72.8 ± 2.3 g GAE/kg, respectively. These values were not only statistically similar to each other but also significantly higher than the CHT group and even higher than the untreatedsample. This unexpected enhancement of TPC in HPP-treated samples may be explained by pressure-induced cell wall disruption, which facilitates the release and improved extractability of bound or insoluble phenolic compounds that may otherwise remain embedded in the cell matrix in untreated fruits (Wu et al., 2021; Barba et al., 2012). The mechanical effect of high pressure may rupture plant tissue structures and free phenolics from vacuoles or cell walls, thereby increasing their availability for quantification. Moreover, the higher TPC in HPP-treated samples also corresponds with enhanced antioxidant capacity, as reflected in DPPH radical scavenging activity measurements. This correlation is consistent with existing studies that establish a strong positive relationship between TPC and antioxidant activities in fruit and vegetable matrices (Wu et al., 2021). Phenolic compounds, including flavonoids and phenolic

acids present in pineapple, act as hydrogen or electron donors, neutralizing free radicals and contributing significantly to the antioxidant profile of the puree. These findings further support the conclusion that HPP is a superior processing technique compared to CHT, particularly for preserving or even enhancing the antioxidant potential of pineapple puree. HPP-treated samples consistently retained higher levels of both TPC and DPPH activity, indicating a better preservation of the bioactive phytochemical matrix. These results align closely with previous studies in other fruits such as mango, strawberry, and blueberry, where HPP was shown to preserve or increase phenolic content and antioxidant activity relative to thermal processing (Cao et al., 2012; Barba et al., 2013). HPP at both 400 and 600 MPa not only prevents the degradation of phenolic compounds but may also enhance their extractability through pressure-induced tissue disruption. HPP at both 400 and 600 MPa not only prevents the degradation of phenolic compounds but may also enhance their extractability through pressure-induced disruption of cellular structures. In plant tissues, a substantial portion of phenolic compounds exists in bound form—either esterified or covalently linked to cell wall components such as lignin, cellulose, hemicellulose, or pectin matrices. These membrane-bound phenolics are less soluble and often inaccessible to conventional extraction processes. Under HPP, the semi-permeable nature of plant cell membranes is compromised, leading to the rupture of vacuoles and loosening of polysaccharide–phenolic linkages. This mechanical disruption facilitates the migration of previously bound phenolics into the soluble fraction, thus increasing their apparent TPC and antioxidant activity (Pérez-Lamela et al., 2021). Moreover, the absence of severe heat in HPP minimizes oxidative polymerization or enzymatic degradation (e.g., by polyphenol oxidase or peroxidase), which often occurs during CHT. Consequently, phenolic acids, flavonoids, and other bioactives not only remain stable but can become more bioaccessible. The increase in measurable TPC after HPP has been observed in various fruits such as strawberries, mangoes, and pineapples, and is attributed primarily to pressure-mediated cell wall disintegration rather than de novo synthesis (Ozkan et al., 2025).

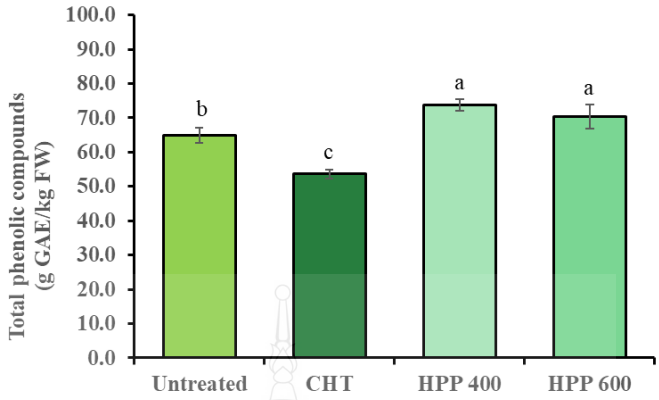


Figure 4.3 Effects of different processing on TPC (g GAE/kg) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; GAE, gallic acid equivalents; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP 600, high-pressure processing at 600 MPa for 10 min.

4.2.3 DPPH radical scavenging activity

In the present study, the untreated pineapple puree exhibited a DPPH scavenging activity of approximately 1.20 ± 0.04 mmol TE/kg, representing the level of antioxidant capacity inherent to "*Phulae*" pineapple (Figure 4.4). However, processing both thermal and non-thermal resulted in significant alterations in DPPH radical scavenging activity, though the extent of impact varied considerably depending on the treatment method. Interestingly, the CHT group demonstrated the highest DPPH radical scavenging activity among all treatments at 1.96 ± 0.03 mmol TE/kg, which was statistically higher ($P < 0.05$) than both the untreated and HPP-treated samples. This somewhat counterintuitive result may be explained by the thermal degradation of larger polyphenolic structures into smaller fragments, which may exhibit higher radical scavenging activity per unit weight (Cao et al., 2012). Additionally, heat-induced release of bound antioxidant compounds from cell walls and vacuoles may temporarily increase the accessibility of antioxidants to react with DPPH radical scavenging radicals. However, it should be noted that this enhanced DPPH activity following thermal treatment may not always correlate with actual nutritional superiority, as heat can also destroy key micronutrients and enzymatic antioxidants (e.g., vitamin C, catalase, superoxide dismutase) that contribute to long-term stability and health benefits (Poljsak et al., 2021). The HPP-treated samples presented moderate levels of DPPH

radical scavenging activity, with values of 1.77 ± 0.03 mmol TE/kg for HPP at 400 MPa and 1.22 ± 0.04 mmol TE/kg for HPP at 600 MPa. While both values were lower than that of the heat-treated sample, HPP at 400 MPa preserved a significantly higher antioxidant capacity compared to the control ($P < 0.05$), whereas HPP at 600 MPa was statistically like the untreated sample ($P > 0.05$) shown in Figure 4.4. These findings align with previous reports suggesting that moderate pressure levels (300–500 MPa) are optimal for releasing antioxidant compounds without causing structural breakdown, while excessively high pressure may begin to alter molecular structures or promote oxidative reactions that reduce overall activity (Wu et al., 2021). Despite the slightly higher DPPH activity in the CHT group, both TPC and vitamin C declined markedly, suggesting that the increase was due to heat-induced phenolic transformation rather than real antioxidant preservation. A similar trend was reported by Song et al. (2013), where heating increased DPPH activity in plant-based protein produce from cashew nut, walnut, soybean, black sesame, sesame, beet, onion, and gluten. In contrast, HPP maintained high TPC and vitamin C, reflecting true antioxidant retention. The DPPH activity in HPP 400, being significantly above that of the untreated sample, may reflect enhanced extractability of certain pressure-sensitive antioxidants, while the slightly lower activity at 600 MPa might be due to pressure-induced modifications in antioxidant molecular structure or reactivity. These patterns collectively reinforce the view that HPP is a viable and advantageous non-thermal alternative to heat-based processing for the preservation of antioxidant properties in fruit purees. Conversely, HPP retains not only DPPH scavenging activity but also structural and compositional quality, resulting in a product that is more representative of the fresh fruit in terms of functional and nutritional value. Furthermore, studies by Wu et al. (2021) and Barba et al. (2013) have demonstrated that HPP can maintain or even enhance the antioxidant capacity of tropical fruits by preserving sensitive bioactive compounds, including flavonoids, phenolic acids, and vitamin C. These compounds act synergistically to scavenge ROS, which are implicated in oxidative stress-related chronic diseases. As such, maintaining antioxidant capacity through HPP is not only a matter of nutritional labeling but also of potential health impact. While DPPH values varied across treatments, the broader interpretation of antioxidant preservation supports the use of HPP, particularly at 400 MPa, as a superior processing method that balances free radical

scavenging ability with the retention of key nutritional and phytochemical components. These findings provide additional evidence for HPP's value in the development of minimally processed, functional pineapple products with enhanced health appeal and shelf stability.

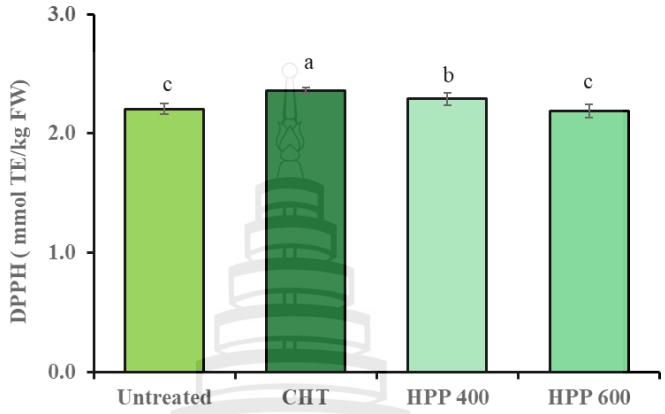


Figure 4.4 Effects of different processing on DPPH (mmol of TE/kg FW) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FW, fresh weight; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min; TE, Trolox equivalents.

4.2.4 FRAP assay

As shown in Figure 4.5, significant differences in FRAP values were observed across treatments. The untreated "*Phulae*" pineapple puree exhibited a ferric reducing power of approximately 418.4 ± 12.3 μmol ferrous sulfate/kg FW, serving as a reference for the unprocessed antioxidant capacity of the fruit. After thermal treatment via CHT at 80°C for 10 min, a substantial decline in FRAP value was recorded, dropping to 370.5 ± 10.7 μmol FE(II)/kg FW. This reduction was statistically significant ($P < 0.05$) when compared to all other treatments and highlights the detrimental impact of heat on the antioxidant capacity of the puree. Thermal processing is known to degrade heat-sensitive antioxidants such as vitamin C and phenolic compounds, which significantly contribute to the ferric reducing potential of fruits. Additionally, thermal exposure can promote oxidation of existing antioxidants or their conversion into polymeric, non-reactive forms, thereby decreasing their availability to participate in redox reactions (Cao et al., 2012). The result aligns with previous findings

suggesting that thermal treatments often impair both the quality and quantity of antioxidant compounds in tropical fruit products. In contrast, HPP at both 400 MPa and 600 MPa resulted in markedly higher FRAP values, measured at 474.1 ± 13.5 μmol FE(II)/kg FW and 468.9 ± 12.9 μmol FE(II)/kg FW, respectively. These values were significantly higher than both the untreated and heat-treated samples ($P < 0.05$), as indicated by the same superscript letter "a" in the graph, and suggest that HPP enhanced the ferric reducing capacity of the puree. This observation is consistent with the hypothesis that pressure-induced disruption of plant tissue structures facilitates the release of bound or cell wall-associated antioxidants, such as phenolic acids and flavonoid glycosides, which may not be readily extractable in their native cellular compartments (Wu et al., 2021; Barba et al., 2012). Interestingly, no statistically significant difference ($P > 0.05$) was observed between HPP at 400 MPa and 600 MPa, suggesting a saturation or plateau effect beyond a certain pressure threshold. This could indicate that maximum release or activation of antioxidant compounds occurs at or below 400 MPa and increasing the pressure further does not proportionally enhance the antioxidant potential. Similar findings have been reported in other tropical fruits, such as mango and guava, where moderate-pressure treatments yielded optimal antioxidant outcomes, while excessively high pressures either had no additional benefit or, in some cases, caused partial degradation of sensitive compounds (Barba et al., 2013; Cao et al., 2012). The enhanced FRAP values in HPP-treated samples also align with the increased TPC and relatively high DPPH activity observed in the same treatments. Since phenolic compounds are known contributors to ferric reducing power, their improved extractability or preservation under pressure likely explains the elevated FRAP results. Moreover, vitamin C is another key reducing agent which is better retained in HPP than in thermally treated samples, further supporting the higher FRAP activity in HPP groups. Together, these findings strongly indicate that HPP is a more effective method than thermal processing for preserving and enhancing the antioxidant potential of "*Phulae*" pineapple puree.

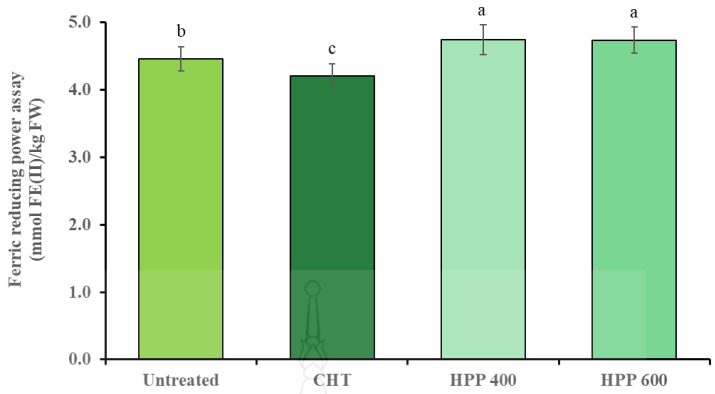


Figure 4.5 Effects of different processing on FRAP (mmol Fe (II)/kg FW) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; Fe (II), ferrous sulfate; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min.

The ability of HPP to retain and potentially increase FRAP activity supports its application in developing functional food products that aim to deliver antioxidant health benefits. Additionally, as consumers increasingly demand minimally processed, nutritionally dense products, HPP offers a promising strategy to maintain both quality and functional value without the detrimental effects commonly associated with heat treatment. FRAP assay results affirm the superior performance of HPP, particularly at 400 MPa, in maintaining and even boosting the ferric reducing antioxidant potential of pineapple puree. These outcomes further validate HPP's status as a non-thermal preservation technology capable of retaining crucial bioactive compounds while enhancing the health-promoting properties of fruit-based products. Future research could explore the long-term stability of these antioxidant attributes during storage and their bioavailability post-consumption.

4.2.5 ABTS radical scavenging activity

The results are presented in Figure 4.6, and they reveal noteworthy differences across the processing treatments. The HPP-treated sample at 600 MPa exhibited a significantly higher ABTS value of 4.55 ± 0.15 g TE/kg FW, which was statistically superior ($P < 0.05$) to all other treatments, including the untreated, CHT-treated, and HPP at 400 MPa. This marked increase suggests a pressure-dependent enhancement of

antioxidant capacity as measured by ABTS, with 600 MPa providing the optimal conditions for the liberation and preservation of antioxidant molecules in the pineapple puree matrix. The elevated ABTS activity under 600 MPa HPP may be attributed to several interrelated mechanisms. Firstly, high pressure disrupts cellular structures such as the tonoplast and cell wall, thereby facilitating the release of phenolic compounds and other antioxidants that may exist in bound or insoluble forms in the intact fruit tissue (Wu et al., 2021; Barba et al., 2013). These liberated compounds, including flavonoids, phenolic acids, and vitamin C, are known contributors to ABTS radical neutralization. Additionally, pressure-induced conformational changes in antioxidant molecules might improve their electron- or hydrogen-donating capacity, thereby increasing their reactivity in the ABTS assay (Guillamón et al., 2016). Interestingly, no significant difference ($P > 0.05$) was observed among the untreated sample (3.56 ± 0.12 g TE/ kg), CHT-treated sample (3.72 ± 0.13 g TE/ kg), and HPP at 400 MPa (3.41 ± 0.14 g TE/ kg) in Figure 4.6 This suggests that lower pressures (400 MPa) and CHT do not provide sufficient structural breakdown or chemical modification to enhance ABTS activity significantly. Moreover, thermal processing may degrade thermolabile antioxidants like ascorbic acid or polyphenolic constituents, thereby counteracting any potential benefit from heat-induced extractability (Cao et al., 2012). The similarity between the untreated and CHT-treated samples also implies that moderate thermal exposure (80°C for 10 min) does not drastically alter ABTS-related antioxidant activity although it may negatively impact other antioxidant mechanisms such as ferric reduction or DPPH radical scavenging. The result from HPP at 400 MPa, while slightly lower than the untreated sample, remains statistically comparable, suggesting that this pressure level may not be sufficient to significantly enhance ABTS radical scavenging capacity. This observation supports the concept of a threshold effect; wherein only higher pressures (such as 600 MPa) can unlock or activate enough antioxidant potential to result in a significant improvement in ABTS values. The findings are consistent with those of Guillamón et al. (2016), who reported similar trends in other fruit and vegetable systems, including tomato and mango pulp, where maximum antioxidant activity was achieved only at higher HPP levels. From a nutritional and functional product development perspective, the significant enhancement of ABTS antioxidant capacity at 600 MPa underscores the potential of HPP as a powerful non-thermal processing

technique to preserve and even improve the biofunctional properties of tropical fruits like pineapple. Given the increasing consumer demand for clean-label, minimally processed products with enhanced health benefits, these results provide compelling evidence that HPP at elevated pressures can yield superior antioxidant retention and activation without compromising quality. HPP at 600 MPa optimally enhances antioxidant potential in "*Phulae"* pineapple puree, while lower pressures and conventional heating do not significantly improve or may even reduce antioxidant performance. These findings, when interpreted alongside TPC, DPPH, and FRAP data, clearly position HPP as the most effective method for retaining the comprehensive antioxidant profile of pineapple puree. Further studies examining the bio accessibility and in vivo antioxidant effects of these compounds would provide valuable insights into the health impacts of HPP-treated tropical fruits.

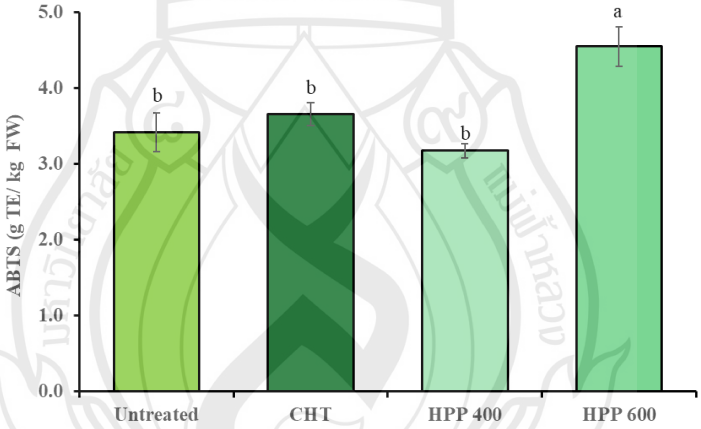


Figure 4.6 Effects of different processing on ABTS (g TE/ kg FW) in "*Phulae*" pineapple puree. ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min; TE, Trolox equivalents.

4.2.6 ORAC assay

The ORAC assay was employed to assess the ability of antioxidants present in "*Phulae*" pineapple puree to neutralize peroxyl radicals, which are one of the most prevalent ROS in biological systems. The ORAC method is considered one of the most biologically relevant antioxidant assays because it evaluates antioxidant performance

over time using a fluorescein probe and AAPH as a free radical generator, simulating oxidative stress under physiological conditions. As illustrated in Figure 4.7, ORAC values for all treatments including untreated, CHT, HPP at 400 MPa, and HPP at 600 MPa ranged from 25.5 to 36.6 mmol TE/kg FW. Although there was variation in the mean values, statistical analysis showed no significant differences among the treatment groups ($P > 0.05$). This lack of statistically significant change suggests that neither thermal processing nor HPP induced substantial alterations in the overall oxygen radical scavenging capacity of the pineapple puree. The untreated sample exhibited an ORAC value of approximately 36.6 mmol TE/kg, which serves as a baseline for comparison. The CHT-treated sample showed a slightly lower mean (around 30.2 mmol TE/kg), while HPP at 400 MPa and 600 MPa yielded values of approximately 28.7 and 35.5 mmol TE/kg, respectively. Despite minor numerical differences, the overlapping error bars and statistical insignificance indicate that the antioxidant molecules responsible for ORAC activity in pineapple puree are relatively stable under both heat and pressure-based treatments. This stability can be attributed to the robust nature of certain antioxidants present in pineapple, such as ascorbic acid, simple phenolic acids (e.g., gallic acid, caffeic acid), and bromelain-associated peptides, which may withstand moderate thermal and pressure stress. These compounds are often less susceptible to oxidation or structural degradation than more complex polyphenols or lipid-soluble antioxidants. Moreover, some processing-induced structural disruptions may enhance the bio accessibility or release of bound antioxidants, potentially compensating for degradation losses. Supporting literature echoes this observation. For instance, Pérez-Lamela et al. (2021) and Siddiq et al. (2013) reported that pineapple and similar tropical fruits maintain a relatively consistent ORAC value following standard pasteurization or HPP. Similarly, (Barba et al., 2017) demonstrated that while antioxidant profiles may fluctuate depending on the specific assay (e.g., DPPH or ABTS), ORAC values tend to remain stable due to the cumulative and time-integrated nature of this method, which reflects the additive effects of multiple antioxidant classes. It is also worth noting that the lack of significant differences in ORAC contrasts with trends observed in other antioxidant assays such as ABTS, DPPH, and FRAP, where HPP at higher pressures (particularly 600 MPa) often led to enhanced antioxidant readings. This divergence highlights the complementary nature of antioxidant assays. Each method targets

different mechanisms and antioxidant species. For example, ABTS and DPPH focus on electron or hydrogen atom donation, while ORAC assesses the ability to quench peroxyl radicals over a sustained period. Thus, the ORAC assay offers a unique perspective, especially when used alongside other analytical approaches. The ORAC results suggest a high degree of antioxidant resilience in "*Phulae*" pineapple puree, regardless of whether it undergoes conventional heating or high-pressure processing. This is encouraging from a food processing standpoint, as it implies that functional antioxidant properties can be retained across various preservation methods. Such findings reinforce the suitability of both CHT and HPP for producing shelf-stable pineapple products without compromising antioxidant quality. Further exploration into the synergistic or antagonistic behavior of individual antioxidant compounds under processing conditions could provide more nuanced insights into their role in ORAC stability.

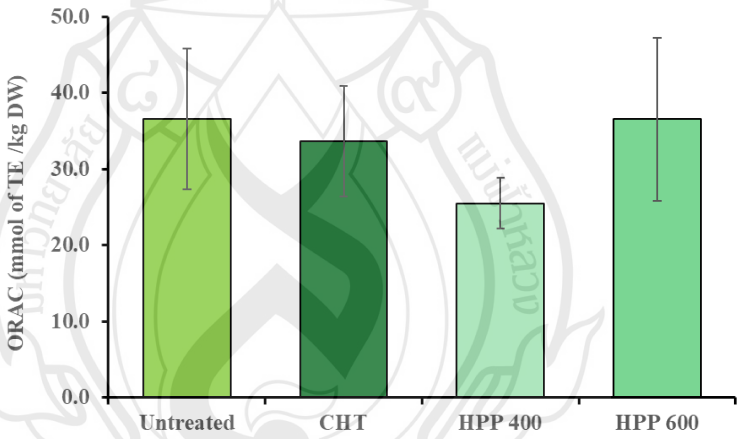


Figure 4.7 Effects of different processing on ORAC (mmol of TE/kg FW) in "*Phulae*" pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents.

4.3 Effect of HPP on Anti-inflammatory Properties in *"Phulae"* Pineapple Puree

4.3.1 Bromelain enzyme activity

In the current study, the enzymatic activity of bromelain in the untreated *"Phulae"* pineapple puree was recorded at 3,733.3 ± 45.1 CDU/L, serving as the benchmark for untreated, biologically active puree. Following HPP, bromelain activity remained well-preserved, with values of 3,441.7 ± 38.7 CDU/L and 3,488.3 ± 41.2 CDU/L for the 400 MPa and 600 MPa treatments, respectively (Figure 4.8). These values were not statistically different from the control ($P > 0.05$), indicating that HPP did not significantly compromise the structure or functionality of the enzyme. The retention of over 92% of bromelain activity under both HPP conditions underscores the ability of this non-thermal technique to maintain the biofunctional quality of pineapple puree. Pressure-induced structural changes in proteins typically occur at much higher-pressure thresholds or under conditions of elevated temperature, neither of which were present in this study, thereby preserving enzyme integrity (Ha et al., 2013; Barba et al., 2012). Conversely, a dramatic reduction in Bromelain activity was observed following CHT at 80°C for 10 min, with enzyme activity plummeting to 2,025.0 ± 32.5 CDU/L. This represents a loss of nearly 46% compared to the untreated sample and a significantly lower level than both HPP-treated counterparts ($P < 0.05$).

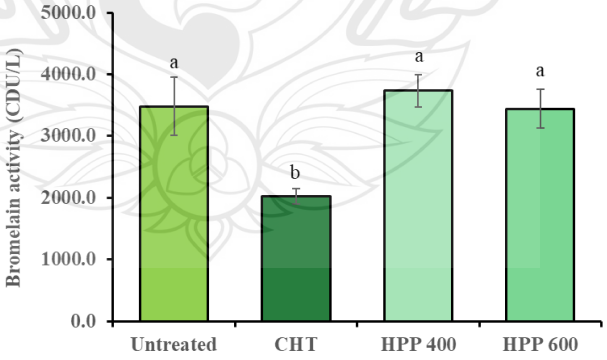


Figure 4.8 Effects of different processing on bromelain (CDU/L) in *"Phulae"* Pineapple puree. CDU, casein digestion units; CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min.

The decline in enzymatic activity is most likely due to irreversible thermal denaturation, which unfolds the enzyme's tertiary structure, resulting in aggregation or inactivation of the active site (Peterson et al., 2007). Bromelain, like most proteolytic enzymes, is known to be highly heat-sensitive, with reported inactivation temperatures as low as 60°C depending on matrix conditions such as pH and ionic strength. Additionally, heat may promote the formation of disulfide bridges or cause peptide bond cleavage, both of which can severely impair enzymatic functionality. These findings are consistent with previous studies that have shown thermal processing to significantly impair bromelain activity in pineapple products (Huang et al., 2021). For instance, Ha et al. (2013) reported that bromelain retained less than 30% of its original activity after thermal treatment at 70°C for 15 min. In contrast, HPP-treated samples retained over 85% activity even under pressures up to 600 MPa, further confirming that HPP is a superior alternative for maintaining enzymatic function in heat-sensitive fruit enzymes. This is particularly relevant in the context of fruit puree production, where preserving native enzymatic profiles not only supports nutritional claims but also enhances product functionality, such as digestion aid or anti-inflammatory action. Furthermore, bromelain is considered a major contributor to pineapple's overall bioactivity. It has been shown to influence gastrointestinal health, reduce joint pain, and support immune modulation (Kansakar et al., 2014). Of relevance to this study, bromelain also exhibits hyaluronidase inhibitory activity, which has implications for anti-inflammatory responses and skin-related applications. When interpreted in conjunction with the HA inhibition results, a strong correlation becomes evident between the retention of bromelain activity and the ability of the processed puree to inhibit HA activity. The significantly higher HA inhibition observed in HPP-treated samples compared to the heat-treated sample suggests that preservation of bromelain under pressure not only maintains enzymatic quantity but also functional efficacy. These findings highlight the importance of selecting processing methods that preserve the biofunctional components of fruit-based products. In the case of "*Phulae*" pineapple puree, HPP at both 400 and 600 MPa proved highly effective in maintaining the proteolytic activity of bromelain, while CHT significantly compromised its integrity. This has practical implications for the food industry, particularly in the formulation of functional beverages, supplements, or therapeutic foods aimed at delivering enzymatic

health benefits. In addition, the data align with consumer trends favoring minimally processed foods that retain fresh-like qualities and natural bioactive compounds without reliance on synthetic additives. The use of HPP as a superior preservation method for bromelain in pineapple puree. While conventional heat treatment significantly reduces enzymatic activity due to thermal denaturation, HPP allows for the retention of both enzymatic structure and function (Sandate-Flores et al., 2017). This preservation not only ensures the nutritional and sensory quality of the puree but also reinforces its potential application in health-promoting products. Future studies could explore the kinetics of bromelain activity during extended storage under HPP and CHT conditions to further validate its long-term functional stability in commercial products.

4.3.2 Total protein content

Protein quantification in the "*Phulae*" pineapple puree samples was carried out using a colorimetric protein assay kit (Dojindo Molecular Technologies, Inc.), employing the Bradford method based on Coomassie Brilliant Blue G-250 dye. This widely accepted assay detects proteins through non-covalent binding of the dye, which interacts primarily with basic and aromatic amino acid residues, notably arginine, tryptophan, tyrosine, and phenylalanine (Bradford, 1976). Upon binding, the dye undergoes a spectral shift from red to blue, which can be quantified spectrophotometrically at 595 nm. This method is sensitive, rapid, and suitable for estimating total protein content in fruit-based matrices where protein concentrations are relatively low but still biologically and nutritionally significant. The analysis revealed a total protein content of 297.50 ± 1.34 mg in the untreated pineapple puree (Figure 4.9), which reflects the native protein composition of the fruit, including key enzymes such as bromelain which is a proteolytic enzyme known for its anti-inflammatory, digestive, and pharmacological properties (Suthiluk et al., 2023). However, a marked and statistically significant reduction ($P < 0.05$) in protein content was observed following CHT at 80°C for 10 minutes, with protein levels dropping to 105.83 ± 2.17 mg. This considerable loss representing nearly a 65% decrease can be attributed primarily to heat-induced protein denaturation, aggregation, and precipitation. Thermal denaturation often alters the three-dimensional structure of proteins, leading to reduced solubility and a consequent decline in dye reactivity. Aggregated or denatured proteins may also form insoluble complexes that are removed during centrifugation steps prior

to analysis, thereby reducing the apparent protein concentration in the assay. In contrast, the HPP treatments were far more effective in preserving protein content. Samples treated at 400 MPa and 600 MPa retained protein concentrations of 275.36 ± 1.55 mg and 280.17 ± 1.62 mg, respectively, values that were not significantly different ($P > 0.05$) from the untreated sample. This indicates that HPP has a minimal impact on protein solubility and structural integrity. Unlike thermal processing, HPP is a non-thermal technique that exerts isostatic pressure uniformly throughout the sample, which can inactivate microorganisms and certain enzymes while maintaining the native conformation of most proteins. The ability of HPP to preserve protein integrity has been documented in various fruit and vegetable systems, where bioactive enzymes, structural proteins, and other functional peptides remain largely intact post-treatment (Niu et al., 2022; Bi et al., 2020). Furthermore, the preservation of protein in HPP-treated pineapple puree is particularly important from both a nutritional and functional standpoint. Pineapple proteins include bromelain and other endogenous enzymes, which contribute to the fruit's biological activity, digestive properties, and potential health benefits. Bromelain is highly sensitive to heat and loses activity rapidly when exposed to temperatures above 60 °C. Therefore, the reduced protein content observed after CHT is likely accompanied by a substantial loss of bromelain enzymatic activity. HPP, by avoiding high temperatures, likely helps retain not only the quantity but also the functionality of these valuable proteins, enhancing the puree's nutritional profile and potential applications in functional food formulations. The findings of this study are consistent with previous research demonstrating the detrimental effect of thermal treatments on protein retention in fruit-based products. For instance, Suthiluk et al. (2023) reported a significant decline in soluble protein and enzymatic activity in heat-treated pineapple puree. On the other hand, HPP was shown to maintain high levels of soluble protein and preserve enzymatic activities critical for flavor development, texture, and nutritional value. In passion fruit puree, Niu et al. (2022) also observed similar protein retention trends, reinforcing the idea that HPP is an effective preservation method for minimally processed fruit products. The protein quantification results clearly highlight the superiority of HPP over CHT in maintaining the protein quality of pineapple puree. While heat treatment significantly compromised protein solubility due to denaturation, aggregation, and degradation. HPP treatments preserved

protein levels close to that of the untreated control. These results underline the potential of HPP as a gentle, non-thermal processing technology for producing high-quality, protein-rich fruit purees that retain their nutritional and functional attributes.

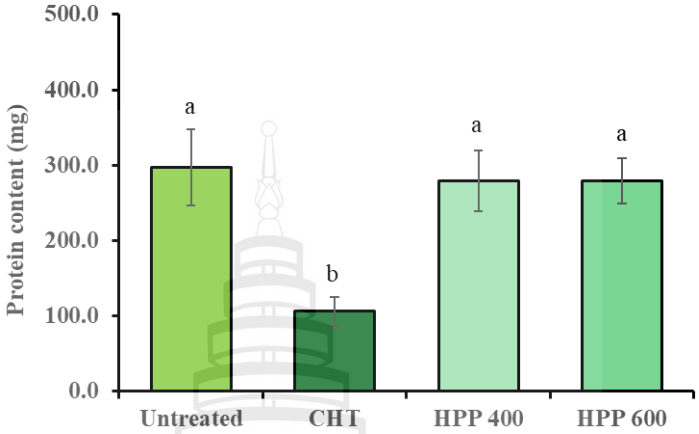


Figure 4.9 Effects of different processing on protein content (mg) in "*Phulae*" pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; HPP- 400, high- pressure processing at 400 MPa for 10 min; HPP -600, high-pressure processing at 600 MPa for 10 min.

4.3.3 NO radical scavenging activity

NO radical scavenging assay was conducted to assess the capacity of pineapple puree to neutralize reactive nitrogen species (RNS), specifically the NO radical, which plays a critical role in oxidative stress, inflammation, and immune modulation. Unlike assays such as DPPH and FRAP that focus on electron transfer or metal reduction, the NO assay evaluates the sample's ability to inhibit nitrite formation resulting from the reaction between nitric oxide and oxygen under physiological pH, typically using sodium nitroprusside as a donor. As shown in Figure 4.10, the untreated pineapple puree exhibited a NO scavenging capacity of approximately 47.0 ± 1.6 mmol TE/kg FW, establishing the baseline antioxidant response of unprocessed "*Phulae*" pineapple. Notably, both HPP-treated samples (400 MPa and 600 MPa) retained similar levels of NO scavenging activity, 46.8 ± 1.5 and 47.2 ± 1.7 mmol TE/kg FW, respectively. These values were not statistically different ($P > 0.05$) from the untreated sample indicating that HPP had minimal to no impact on NO radical scavenging potential. This stability under pressure reinforces the characterization of HPP as a non-thermal preservation

technique that avoids the degradation of thermolabile bioactive compounds. The preservation of NO scavenging activity under HPP may be due to the structural integrity of compounds with anti-inflammatory and nitrogen-reactive potential, such as certain phenolic acids, vitamin C, flavonoids, and sulfur-containing compounds. These molecules appear to remain functionally intact under pressure processing, contributing to consistent NO inhibitory effects across both HPP levels tested. The CHT-treated sample demonstrated a significantly reduced NO scavenging activity of 27.4 ± 1.4 mmol TE/kg FW, which was statistically lower ($P < 0.05$) than all other groups. The decline of nearly 40% from the untreated value highlights the negative impact of thermal processing on pineapple's ability to mitigate NO radicals. Heat exposure can degrade or denature essential antioxidant molecules, especially those sensitive to high temperatures and prolonged heating durations. For example, vitamin C and some polyphenols both implicated in NO scavenging are known to degrade rapidly under thermal stress. Moreover, heat can also lead to the formation of inert polymerized phenolic structures, which have reduced bioactivity and antioxidant function. These results are consistent with findings by Gharezi et al. (2012), who reported a marked reduction in NO scavenging capacity in several thermally processed fruits and vegetables, depending on the stability of their inherent bioactive compounds.

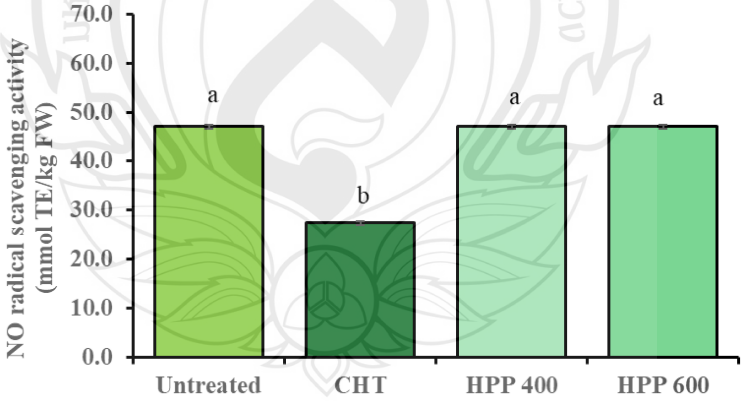


Figure 4.10 Effects of different processing on NO radical scavenging activity (mmolTE/kg FW) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min; NO, nitric oxide; TE, Trolox equivalents.

Their study suggested that processing methods that minimize thermal exposure are more favorable for maintaining NO-inhibitory effects, which is echoed in the preservation observed under HPP in this study. The selectivity of NO scavenging compounds to different processing treatments further emphasizes the need to evaluate antioxidant activity using multiple assays, as different mechanisms may be sensitive to pressure or heat. NO scavenging activity cannot be overstated, particularly given nitric oxide's role in chronic inflammation, cardiovascular dysfunction, and neurodegenerative conditions. Thus, maintaining the capacity of pineapple puree to scavenge NO radicals is critical for preserving its anti-inflammatory and health-promoting potential. The superior performance of HPP in preserving this activity makes it a promising processing method for functional foods designed to deliver bioactive health benefits beyond basic nutrition. HPP can preserve a natural status of NO. The preservation of activity itself is an important finding, especially when contrasted with the degradation seen in heat-treated puree. These data support the notion that HPP at 400–600 MPa effectively maintains NO radical scavenging functionality, further solidifying its utility in producing minimally processed, health-oriented pineapple products. Combined with data from DPPH, FRAP, ABTS, and ORAC assays, these results present a comprehensive picture of HPP's superior performance in preserving a broad spectrum of antioxidant responses in pineapple puree.

4.3.4 HA inhibitory activity

HA inhibition assay was utilized to evaluate the potential of "*Phulae*" pineapple puree to inhibit the activity of the hyaluronidase enzyme, which plays a significant role in the degradation of hyaluronic acid in connective tissues. Elevated hyaluronidase activity is associated with inflammatory processes, tissue degradation, tumor metastasis, and allergic reactions, making the inhibition of this enzyme an important marker for anti-inflammatory and anti-metastatic properties in functional foods (Chobotova et al., 2010). As shown in Figure 4.11, the HA inhibition capacity of the untreated pineapple puree was measured at 77.1 ± 2.8%, serving as a reference for the natural biofunctional potential of the unprocessed sample. Upon CHT at 80°C for 10 min, HA inhibition dropped markedly to 58.0 ± 2.2%, indicating a statistically significant decrease ($P < 0.05$) in bioactivity. This substantial decline suggests that heat treatment causes denaturation or degradation of heat-sensitive bioactive compounds,

particularly proteolytic enzymes and polyphenols responsible for inhibiting hyaluronidase. In contrast, both HPP treatments at 400 MPa and 600 MPa retained high levels of HA inhibition 78.8 ± 2.4% and 79.9 ± 2.1%, respectively which were not significantly different ($P > 0.05$) from the untreated sample. This clearly demonstrates that HPP effectively preserves or even slightly enhances HA-inhibitory activity compared to both untreated and heat-treated samples. The ability of HPP to maintain these functional properties is attributed to its non-thermal nature, which minimizes molecular degradation, protein unfolding, and enzymatic inactivation, all of which are common during thermal processing. A major contributor to pineapple's HA-inhibitory activity is bromelain, a complex of cysteine proteases found in the stem and fruit of pineapple. Bromelain is well-documented for its anti-inflammatory, immunomodulatory, and anti-tumor properties, with a proposed mechanism that includes direct inhibition of hyaluronidase activity, thereby reducing tissue permeability and inflammatory cell migration (Harrach et al., 1995). However, bromelain is a heat-labile enzyme, and studies have shown that even moderate thermal processing can irreversibly denature its protein structure, significantly reducing its biofunctional effects (Chobotova et al., 2010). The graph supports this mechanism: the CHT sample's reduced HA inhibition aligns with a corresponding drop-in activity, as observed in previous sections. This confirms that thermal degradation of bromelain and potentially other synergistic bioactive compounds compromises the functional efficacy of heat-treated pineapple puree. In contrast, HPP preserves enzyme structure and function by applying isostatic pressure uniformly, avoiding localized overheating. Studies by (Ha et al., 2013) and (Rastogi et al., 2007) have demonstrated that HPP at moderate to high pressures can maintain protease activity and functional protein conformations, including those of bromelain. The minimal structural damage to enzymes under HPP conditions allows for retention of anti-inflammatory properties, making it a favorable method for the preparation of functional fruit-based products with therapeutic potential. Moreover, the preservation of HA inhibition by HPP correlates well with the retention of TPC, vitamin C, and antioxidant capacity observed in the same samples, further reinforcing the hypothesis that HPP safeguards a range of bioactive compounds contributing to health-promoting effects. Since phenolic compounds can also inhibit hyaluronidase through competitive or non-competitive

binding mechanisms, the maintenance of their integrity under HPP could also contribute to the observed HA inhibition. From a functional food development perspective, the HA inhibition activity serves as a biomarker for the anti-inflammatory potential of pineapple puree. The superior performance of HPP-treated samples in maintaining this activity demonstrates that HPP is not only suitable for microbial and shelf-life control but also excels at preserving health-related functionalities, particularly those associated with enzyme stability and inflammation modulation. HPP in preserving biofunctional compounds, especially bromelain and phenolics, that contribute to hyaluronidase inhibition. The significant reduction in activity following heat treatment highlights the vulnerability of these bioactive to thermal degradation, whereas HPP emerges as a superior, non-thermal alternative for maintaining or enhancing the health-promoting properties of pineapple puree. These findings reinforce the potential of HPP in the formulation of nutraceuticals, functional beverages, and therapeutic food products with anti-inflammatory applications.

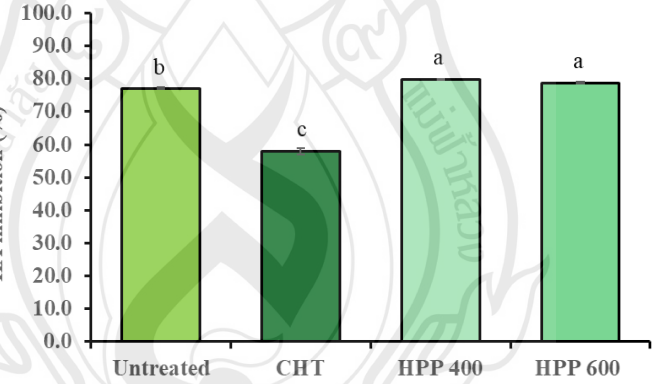


Figure 4.11 Effects of different processing on HA inhibition (%) in *"Phulae"* pineapple puree. CHT, conventional heat treatment at 80 °C for 10 min; HA, hyaluronidase; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min.

4.4 Correlations Between Different Antioxidant and Anti-inflammatory Activities of *"Phulae"* Pineapple Puree

Table 4.2 shows the correlation coefficients between different antioxidant parameters (TPC, DPPH, FRAP, ABTS, ORAC) and anti-inflammatory related

activities (NO, HA, and Bromelain) of *"Phulae"* pineapple puree. The results reveal several significant relationships, indicating that the antioxidant properties of the puree are closely associated with its bioactive enzyme activities. A strong positive correlation was observed between TPC and FRAP ($r = 0.91$, $p < 0.01$), suggesting that phenolic compounds play a major role in the ferric-reducing antioxidant capacity of the puree. Similarly, TPC was also significantly correlated with NO scavenging activity ($r = 0.86$, $p < 0.01$), HA ($r = 0.87$, $p < 0.01$), and bromelain activity ($r = 0.79$, $p < 0.01$). These strong associations imply that phenolic compounds may synergistically enhance both antioxidant and anti-inflammatory properties, likely due to their ability to donate electrons or hydrogen atoms to neutralize reactive species (Barros et al., 2012). Negative correlations were found between DPPH and most other parameters, particularly with TPC ($r = –0.43$, $p < 0.05$), FRAP ($r = –0.47$, $p < 0.05$), and NO ($r = –0.75$, $p < 0.01$). This may be attributed to the differences in reaction mechanisms between DPPH and other antioxidant assays. DPPH radical scavenging is more specific to hydrophobic antioxidants, while FRAP and ORAC are sensitive to both hydrophilic and hydrophobic compounds (Lu et al., 2014). Thus, the low correlation suggests that *"Phulae"* pineapple puree may contain more hydrophilic antioxidants that are better represented by FRAP and TPC rather than DPPH. Interestingly, FRAP exhibited high positive correlations with HA ($r = 0.88$, $p < 0.01$) and bromelain ($r = 0.83$, $p < 0.01$). This suggests that higher reducing power is associated with greater enzyme activity and HA-stimulating potential, which may be beneficial for anti-inflammatory and wound-healing properties (Utpal et al., 2025). NO scavenging also showed a strong positive correlation with both HA ($r = 0.96$, $p < 0.01$) and bromelain ($r = 0.92$, $p < 0.01$), indicating that the enzyme activity and bioactive compounds in the puree contribute to its potential anti-inflammatory effects by modulating nitric oxide levels. The strong interrelationships among TPC, FRAP, NO, HA, and bromelain activity emphasize the multifunctional bioactivity of *"Phulae"* pineapple puree. The results support that phenolic compounds and bromelain enzyme synergistically contribute to both antioxidant and anti-inflammatory mechanisms. These findings are consistent with previous studies reporting that phenolics and bromelain from pineapple exhibit cooperative roles in oxidative stress regulation and inflammatory response modulation (Huang et al., 2021).

Table 4.2 Correlations between different antioxidant and anti-inflammatory activities of *"Phulae"* pineapple puree

	TPC	DPPH	FRAP	ABTS	ORAC	NO	HA	Bromelain
TPC	1	-0.43*	0.91**	0.20	-0.26	0.86**	0.87**	0.79**
DPPH	-0.43*	1	-0.47*	-0.42*	-0.47	-0.75**	-0.66**	-0.33
FRAP	0.91**	-0.47*	1	0.34	-0.21	0.87**	0.88**	0.83**
ABTS	0.20	-0.42*	0.34	1	0.56	0.25	0.05	0.09
ORAC	-0.26	-0.47	-0.21	0.56	1	-0.12	-0.9	-0.19
NO	0.86**	-0.75**	0.87**	0.25	-0.12	1	0.96**	0.92**
HA	0.87**	-0.66**	0.88**	0.05	-0.09	0.96**	1	0.77**
Bromelain	0.79	-0.33	0.83**	0.09	-0.19	0.92**	0.77**	1

Note *. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.5 Effect of HPP on Volatile Compounds in *"Phulae"* Pineapple Puree

The volatile compound profiles of "*Phulae*" pineapple puree subjected to different processing treatments including CHT, HPP at 400 MPa and 600 MPa were comprehensively analyzed using SPME coupled with GC-MS. To better understand the impact of these treatments on the aroma profile, PCA was employed. The PCA scores plot and biplot (Figure 4.12 – 4.13) revealed that the first principal component (PC1) explained 60.4% of the total variance, while the second principal component (PC2) accounted for an additional 25.2%, indicating that together, they effectively summarize the major differences in volatile composition among the samples. The clustering of samples based on factor scores clearly demonstrated significant distinctions in volatile profiles according to treatment method, underlining how processing alters pineapple puree aroma (Table 4.3). In total, 18 volatile compounds were identified, predominantly esters and terpenes, which are key contributors to pineapple's characteristic fruity and

tropical aroma. This result is consistent with earlier literature such as (Zheng et al., 2012), who noted esters as the primary volatile constituents in pineapples. Among these, esters like methyl propionate, butanoic acid 2-methyl methyl ester, and hexanoic acid methyl ester play crucial roles in imparting sweet, fruity, and pineapple-like notes (Thu et al., 2017). Untreated pineapple puree (green circles in the PCA) exhibited the highest proportion of methyl propionate at 35.2%, a compound recognized for its sweet, rum-like, and fruity aroma that strongly aligns with consumer expectations for fresh pineapple flavor (Chakraborty, 2016). Other notable esters in untreated puree include butanoic acid 2-methyl-, methyl ester (37.0%), contributing ethereal and tutti-frutti notes, and octanoic acid methyl ester (6.3%) with fruity and orange-like nuances. The application of CHT markedly altered the volatile profile. Methyl propionate was drastically reduced to 4.5%, while butanoic acid 2-methyl-, methyl ester significantly increased to 59.0%. This shift suggests that heat treatment promotes the formation or release of certain esters at the expense of others, thereby modulating the aromatic balance. The diminished level of methyl propionate may lead to a loss of fresh, sweet notes, whereas the surge in methyl 2-methyl butanoate imparts a more ethereal and pineapple-like aroma, potentially changing the overall sensory perception. Additionally, compounds such as 3-carene and beta-ocimene monoterpenes associated with sweet, pungent, floral, and green aromas were elevated after heat treatment, which could partially compensate for the loss of other volatiles. HPP demonstrated a pressure-dependent influence on volatile composition. At 400 MPa, methyl propionate was retained at 19.1%, while methyl 2-methylbutanoate was present at 45.4%, indicating partial preservation of the fresh aroma alongside enhancement of some fruity esters. This intermediate profile suggests that moderate pressure preserves key volatiles better than heat treatment, maintaining a more balanced and complex aroma. Increasing the pressure to 600 MPa resulted in methyl propionate levels rising back to 36.4%, close to untreated puree, but methyl 2-methylbutanoate dropped to 23.9%. This indicates that higher pressure processing might selectively preserve or enhance some compounds while reducing others, leading to a distinctive aroma profile. Moreover, HPP treatments uniquely induced the release of monoterpenes such as β-myrcene and limonene (compounds 6 and 8), which were absent or minimal in untreated and heat-treated samples but present in HPP-treated puree. These compounds contribute to citrusy,

piney, and evergreen notes, enriching the aroma complexity. The presence of these monoterpenes aligns with previous studies (Muratore, 2016) highlighting how HPP can enhance or release volatile compounds not detected in untreated samples, possibly through structural modifications of the fruit matrix. Other volatile components like isovaleric acid (sweaty, cheesy odor) decreased progressively with treatment severity, from 3.3% in untreated puree to near undetectable in HPP 600 MPa (0.1%), indicating a reduction of potentially off flavors. The aldehyde nonanal (rose-orange scent) appeared only in HPP samples, adding subtle floral and citrus nuances. The terpene fraction, including woody and musky notes from compounds such as copaene and cis-muurola-3,5-diene, showed variation as well. Cis-muurola-3,5-diene increased from 0.03% in untreated puree to 3.06% in HPP 600 MPa, suggesting that higher pressure could release or generate more complex terpenoid aromas. CHT can diminish certain fresh-like esters and alter the aroma profile, HPP, especially at 400 MPa, preserves the original fruity and sweet notes of pineapple puree, with the added benefit of releasing unique monoterpenes that contribute to a richer and more diverse aroma. However, higher pressure (600 MPa) creates a different aroma balance that may enhance some fruity compounds but reduce others, highlighting the need to optimize processing parameters depending on the desired sensory outcome. These findings have important implications for the food industry, suggesting that HPP can be a superior method for preserving or even enhancing pineapple puree aroma, potentially improving consumer acceptance. Future sensory evaluations correlating these volatile changes with human perception will be essential to validate these chemical findings and tailor processing methods to meet market preferences while ensuring product quality and shelf-life.

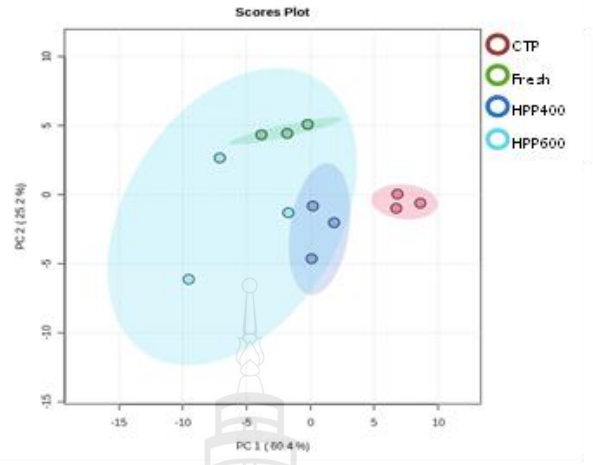


Figure 4.12 The score plot obtained from principal component analysis. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP-600, high-pressure processing at 600 MPa for 10 min.

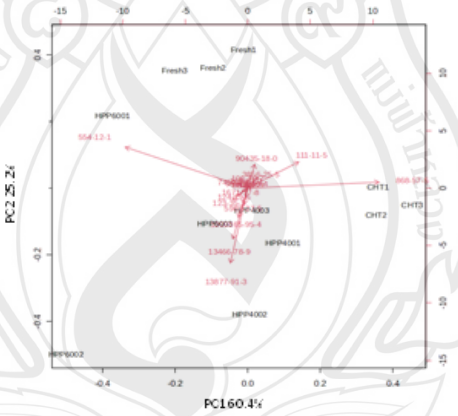


Figure 4.13 The biplot obtained from principal component analysis (PCA), with 18 metabolites labeled 1–18. See Table 2 for the identify of each metabolite. CHT, conventional heat treatment at 80 °C for 10 min; HPP-400, high-pressure processing at 400 MPa for 10 min; HPP -600, high-pressure processing at 600 MPa for 10 min.

Table 4.3 Identification of volatile compounds in *"Phulae"* pineapple puree

No.	Compounds	Odor Description	Classification	CAS No.	RI	Peak Area (%)			
						Untreated	CHT	HPP 400 MPa	HPP 600 MPa
1	Methyl propionate	Sweet, rum-like, fruity	Ester	554-12-1	611	35.24	4.54	19.06	36.40
2	Isovaleric acid	Sweaty, Cheesy, Rancid	Carboxylic acid	90435-18-0	651	3.31	1.58	0.85	0.10
3	Butanoic acid, 2-methyl-, methyl ester	Ethereal, fruity, tutti-frutti, pineapple-like	Ester	868-57-5	689	36.97	59.02	45.43	23.88
4	Butanoic acid, 2-methyl-, ethyl ester	Fruity, juicy, tropical	Ester	7452-79-1	856	1.92	0.03	2.08	0.80
5	Hexanoic acid, methyl ester	Fruity, pineapple-like	Ester	106-70-7	931	0.43	-	-	-
6	Beta.-Myrcene	Earthy, spicy, citrusy, piney, evergreen	Monoterpene	123-35-3	998	0.47	-	0.75	0.75
7	1-Pentanone, 1-(4-methylphenyl)	Raspberry-like	Ketone	1671-77-8	1033	0.04	-	-	-
8	D-Limonene	Citrusy	Monoterpene	5989-27-5	1036	0.53	0.66	0.93	0.85
9	3-Carene	Sweet, pungent	Monoterpene	13466-78-9	1055	4.48	6.84	8.66	9.23
10	Beta.-Ocimene	Floral, green, herbal	Monoterpene	13877-91-3	1056	8.78	14.35	19.29	18.80
11	Alpha.-Phellandrene	Pepper, mint, and fruity	Monoterpene	99-83-2	1067	0.03	-	-	-
12	Nonanal	Rose-orange	Aldehyde	124-19-6	1112	-	-	0.56	0.63
13	Octanoic acid, methyl ester	Fruity, orange-, pineapple like	Ester	111-11-5	1123	6.30	9.93	-	-
14	Octanoic acid, ethyl ester	Fruity, orange-, pineapple- like	Ester	106-32-1	1132	0.13	-	-	-
15	Isoledene	Woody, musky	Terpene	95910-36-4	1224	0.12	-	-	-
16	Decanoic acid, methyl ester	Fruity, orange-like	Ester	110-42-9	1233	0.04	-	-	-
17	Copaene	Woody, spicy	Terpene	3856-25-5	1289	1.13	1.19	-	-
18	Cis-muurola-3,5-diene	Woody, earthy, herbal	Terpene	1000365-95-4	1297	0.03	1.85	1.64	3.06

Note CAS No., Chemical Abstracts Service Number; CHT, conventional heat treatment at 80 °C for 10 min; HPP, high pressure processing; RI, retention index.

CHAPTER 5

CONCLUSIONS

In conclusion, HPP offers significant benefits over CHT in preserving the overall quality and biofunctionality of *"Phulae"* pineapple puree. As a non-thermal technology, HPP better maintained key physicochemical properties such as pH, TSS, and color. It also showed superior retention of sensitive nutritional and functional components. HPP had higher level of vitamin C content by approximately 22-25% and bromelain activity by 80-95% than CHT, highlighting its effectiveness in preserving thermolabile nutrients and enzymes. HPP also enhanced bioactive functions. NO scavenging activity increased by approximately 54%, and HA inhibition improved by around 30% compared to heat-treated samples, suggesting a stronger anti-inflammatory potential. Furthermore, antioxidant capacity assessed through DPPH, FRAP, and ABTS assays was significantly improved in HPP-treated samples, especially at higher pressure levels. The correlation analysis revealed strong positive relationships between TPC and antioxidant activities (DPPH, FRAP, and ABTS), confirming phenolics as key contributors to the puree's antioxidant capacity. Bromelain activity also showed positive correlations with anti-inflammatory markers (HA inhibition and NO scavenging), highlighting its complementary biofunctional role. Moreover, inverse correlations between color parameters and phenolic content suggest that HPP helps preserve both pigment integrity and bioactivity compared to heat treatment. HPP helped preserve volatile aroma compounds, particularly esters and terpenes, which are essential for the fresh and fruity aroma profile of pineapple. Further studies are recommended to investigate the effects of HPP on product stability, shelf life, and long-term retention of bioactivities during storage.

CHAPTER 6

SUGGESTIONS

1. HPP should be further explored and optimized for large-scale processing of fruit puree, particularly *"Phulae"* pineapple.

2. Investigate the shelf-life stability of HPP-treated puree under both refrigerated and ambient conditions to understand long-term effects on product quality, including microbial safety, enzymatic activity, and nutrient degradation.

3. Explore the combined use of HPP with other preservation techniques, such as mild heat, pulsed electric fields (PEF), or natural preservatives, to enhance microbial inactivation while maintaining sensory and nutritional properties.

4. Conduct sensory evaluations using trained panels and consumer groups to assess whether the physicochemical and aromatic improvements in HPP-treated puree led to higher consumer acceptance and preference in real-market conditions.

5. Study consumer perception to guide the formulation, labeling, and packaging of HPP-treated *"Phulae"* pineapple products suited for specific market segments.

6. Investigate the potential functional and clinical applications of HPP-treated puree, particularly its effects on inflammatory biomarkers or oxidative stress in human trials, to support its positioning as a functional food or nutraceutical.

7. Include an assessment of the economic feasibility, energy consumption, and environmental impact of HPP compared with CHT in future research, especially in regions like northern Thailand where *"Phulae"* pineapple is an important local commodity.

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