UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF HIGH PRESSURE PROCESSING AND THERMAL PROCESSING ON PINEAPPLE-MANGO JUICE BLEND

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and I am forever grateful. This dissertation stands as a testament to your unconditional love and encouragement.

ABSTRACT

The activity of endogenous enzymes in fruit are believed to shorten the shelf life of the juices and may also cause changes in quality attributes such as color, flavor, and nutritional value. The methods used in preservation of fruit juice are thermal processing (60°C, 70°C and 80°C) and high pressure processing (400MPa and 600MPa). The result showed HPP treatment at pressure 600 MPa are the best method in order to reduce the activity of the enzyme also maintain the antioxidant activity and the total phenolic content compare to thermal treatment.

In order to increase the efficiency of HPP treatment on inactivation of endogenous enzymes, we suggest the combination of pressure and mild temperature need to be applied onto juice.The study of heat and pressure effect on pineapple-mango juice blend at different temperature and pressure range is very important to improve the shelf life of juice blend that is popular as convenient health drink.

ABSTRAK

Aktiviti enzim endogen dalam buah-buahan dipercayai memendekkan jangka hayat jus dan juga boleh menyebabkan perubahan dalam sifat kualiti seperti warna, rasa, dan nilai pemakanan. Kaedah yang digunakan dalam pemeliharaan jus buah adalah pemprosesan terma (60 ° C, 70 ° C dan 80 ° C) dan pemprosesan tekanan tinggi (400MPa dan 600MPa). Hasilnya menunjukkan rawatan HPP pada tekanan 600 MPa adalah kaedah terbaik untuk mengurangkan aktiviti enzim juga mengekalkan aktiviti antioksidan dan jumlah kandungan fenolik berbanding dengan rawatan haba.

Untuk meningkatkan kecekapan rawatan HPP terhadap ketidakaktifan enzim, kami mencadangkan gabungan tekanan dan suhu ringan untuk digunakan pada jus. Kajian mengenai rawatan haba dn tekanan pada jus campuran nenas dan mangga pada suhu yang berbeza dan pelbagai tekanan adalah sangat penting untuk meningkatkan jangka hayat campuran jus yang popular ini sebagai minuman kesihatan.

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LIST OF ABBREVIATION

cv. = cultivar

HPP = High Pressure Processing

POD = Peroxidase

PPO = Polyphenoloxidase

RA= Residual Activity

A=Activity

A_o=Activity of unprocessed enzyme

A_c = Absorbance of control

A_s=Absorbance of sample.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Fruits are an integral part of a healthy diet. They provide essential micronutrients such as vitamins, minerals, and also antioxidants, phytochemicals, sugars, dietary fiber, and so on. Preservation of fruits in the form of purees and juices is very popular as these products are convenient to consume and form an integral part of many formulations, beverages, and so on (Heckman et al.,2010). The main issue of the fruit processing industry is the limited stability of its products as they are susceptible to spoilage (Buzrul et al., 2008). Besides microbial spoilage, quality degradation due to endogenous enzyme activity is also a factor of concern. In whole fruits, the enzymes and the substrates are confined in separate compartments. But in squeezed purees and juices, this separation is obliterated which offers enzymes to come in contact with substrates resulting in the products with poor quality attributes (Hendrickx and others 1998). Oxidative enzymes like polyphenoloxidase (PPO), peroxidase (POD), β-glucosidase (BGL), and lipoxygenase (LOX) are responsible for the deterioration of color, flavor, and nutritional value (Liavoga et al., 2012). POD is involved in catalyzing the oxidation of cinnamic acids and tyrosine-containing cell wall proteins, which help in the oxidative cross-linking of cell wall polysaccharides (Van Dijk et al.,2009). Inactivation of these quality-degrading endogenous enzymes is needed for shelf-life extension.

Mixed fruit juice blends together can be produced from various fruits such as orange, pineapple and among others in order to combine all the basic nutrients present in these different fruits. This usually gives a better quality juice nutritionally and organoleptically. Studies showed that the practice of mixing different exotic fruits positively impact on the flavour and taste of the fruit and fruit products (Nwachukwu E *et al.,* 2007). Moreover, one could think of a new product development through the blends of different fruits in the form of a natural health drinks which may also serve as an appetizer.

High pressure processing (HPP) has already been established as alternative to thermal processing for the inactivation of food borne microorgnisms (Master et al., 2004) and some enzymes (Terefe et al.,2012) without an requirement of chemical preservatives. The HPP treatment can result in microbial destruction and product stabilization without affecting their sensory qualities. This technology therefore offers potential for processing of fruits and vegetables because their delicate sensory qualities are often adversely influenced by conventional heat treatments. Thermal food processing is known as the most reliable method for controlling enzymes activities in fruit. But, the use of heat can destroy nutrients such as heat labile vitamins and also components related to product flavor and taste (Deliza et al., 2005).

1.2 Problem Statement

Consumption of fruit and vegetable products containing numerous bioactive compounds can significantly reduce the risk of various degenerative diseases. Many researches have been carried out to determine the content of different phytochemicals and their positive

health effect. It has been demonstrated that fruit and vegetables are the source of polyphenols (phenolic acids, flavonols, catechin monomers, proanthocyanidins, flavones, flavanones and anthocyanins) (Suffredini et al., 2004). From the consumer's point of view anthocyanins play an important role because of their health promoting properties and its impact on a key quality parameter of juice, colour, and ultimately sensory acceptance of the product.

Enzymes are natural biocatalyst exist in fruits, it remains active after harvesting. Although this may be desired in cases where ripening takes place during postharvest storage, the activity of endogenous enzymes in fruit are believe to shorten the shelf life of the juices and it may also cause changes in quality attributes such as color, flavor, texture, and nutritional value (Toepfl et al., 2006). Peroxidase, POD important oxidoreductase enzyme, is also involved in the deterioration of color and flavor. Their residual activity is detrimental to the quality of processed products of fruits and vegetables resulting in effects such as browning, off flavor and loss of vitamins. Therefore, the inactivation of POD in the processing of fruits and vegetables is a major quality indicator of processed fruits and vegetables (Zhong et al., 2007)

Currently, thermal processing is used by the food industry to inactivate the enzyme activity in juices (Barba et al., 2012). The problem is, thermal processing may cause undesirable changes in product's sensory as well as nutritional attributes (Miller et al., 2012). Hence, high pressure processing is a great interest to researches and food companies as an alternative to thermal processing whereas it maintains the juice's nutritional attributes as well as the capability to inactivate endogenous enzyme activity (Master et al., 2004).

1.3 Objectives

The focus of this research is to compare the effectiveness of high pressure processing and thermal processing in inactivation of the fruits endogenous enzymes (POD) that is causing enzymatic browning in fruit products. The specific objectives of this research are:

1) To investigate the effects of high pressure processing and thermal treatment on the inactivation of endogenous enzyme in pineapple-mango juice blend.
2) To investigate the effects of high pressure processing and thermal treatment on the antioxidant activity and total phenolic content of pineapple-mango juice blend.

1.4 Scope of Study

This study will be focused on the method of thermal processing with temperature of 60°C, 70°C, 80°C and the high pressure processing with the pressure of 400MPa and 600MPa. The inactivation of enzyme, antioxidant activity and the total phenolic content also will be study on the effects of thermal processing and high pressure processing on pineapple-mango juice blend.

CHAPTER 2

LITERATURE REVIEW

2.1 Overall View

2.1.1 What is Juice Blend?

Juice is a beverage made from the extraction or pressing out of the natural liquid contained in fruit and vegetables. It can also refer to liquids that are flavored with these or other biological food sources such as meat and seafood (e.g., clam juice). Juice is commonly consumed as a beverage or used as an ingredient or flavoring in foods or other beverages, such as smoothies. Juice emerged as a popular beverage choice after the development of pasteurization methods allowed for its preservation without using fermentation (the approach used with wine production).

United States Department of Agriculture (USDA) estimated the total world production of citrus fruit juices to be 7.77 million tons in 2016-17. The largest fruit juice consumers are New Zealand (nearly a cup, or 8 ounces, each day) and Colombia (more than three quarters of a cup each day). Fruit juice consumption on average increased with country income level. To the American food industry, fruit juice is more profitable than only fruit.

Juices are often consumed for their perceived health benefits. For example, orange juice contains vitamin C, folic acid, potassium, and phytochemicals. However many fruit juices have a higher sugar (fructose) for example with grape juice having 50% more sugar than Coca-Cola. Evidence for fruit juice affecting the rate of cancer is unclear.

Some fruit juices have filtered out the dietary fiber present in the fruit. In other cases, other ingredients are added. High-fructose corn syrup, an ingredient in many juice cocktails, has been linked to the increased incidence of type II diabetes. High consumption of juice is also linked to weight gain in some studies, but not in others. Fruit juice can help meet the daily recommendations for fruit consumption.

2.1.2 View on Pineapple

Pineapple (*Ananas comosus*) is an economically important plant in the *Bromelanceae* family which encompasses about 50 genera and 2000 species mostly epiphytic. Deputy Agriculture and Agro-based Industry Minister Datuk Anthony Nogeh Gumbek said LPNM, which began operating in Sarawak in 2010, had provided assistance to about 600 pineapple growers in the state with 810ha of farmland. Pineapple and its juice is non-alcoholic drink and the demand continues to rise mainly due to increasing awareness of its health benefits (Nwachukwu et al., 2014). Its juice have an proximate composition of 81.2 – 86.2% moisture content, 13 – 19% total solid of which sucrose, glucose and fructose are the main compositions, 0.4% fiber and a rich source of vitamin C (Dull G.G, 2000). Pineapple also contains polyphenolic compounds and possesses antioxidant activity (Hossain, 2011). Its pulp is juicy and fleshy with the stem serving as a supporting fibrous core. It is an excellent source of antioxidant, vitamin C which is required for the collagen synthesis in the body. Pineapple juice is largely consumed around the world, mostly as canning industry by-products and in the blend composition to obtain new flavours in beverage and other products.

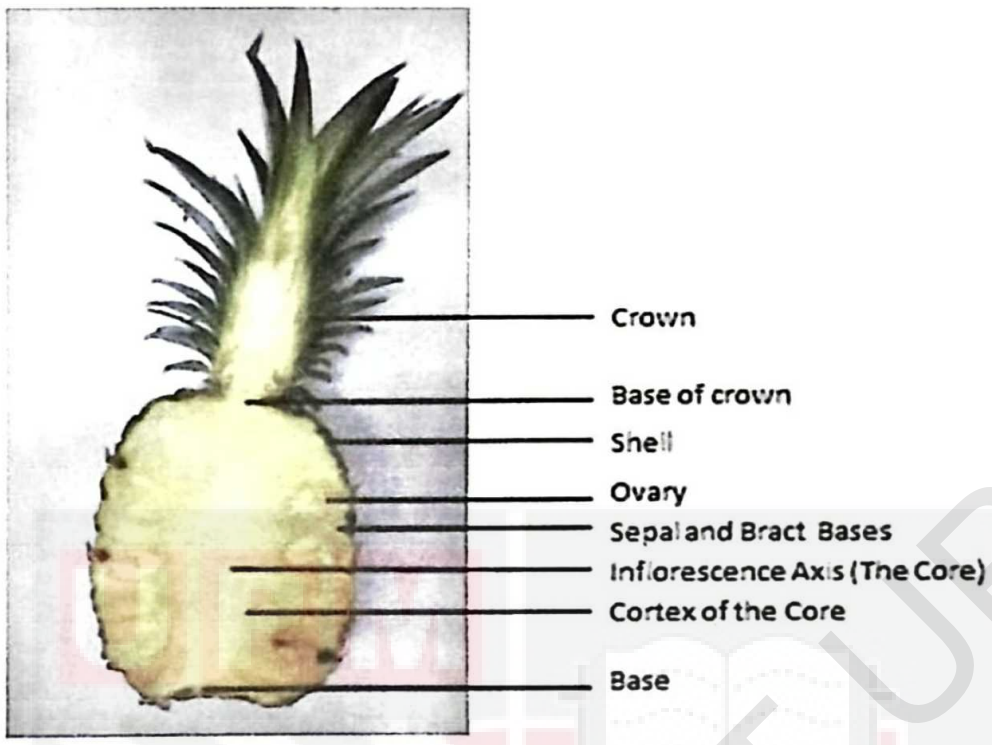

Figure 1: Morphological structure of pineapple

Table 1 : Nutritional value of fresh pineapple fruit (100 g)

PRINCIPLE	NUTRIENT VALUE	PERCENTAGE OF RDA
Energy	50 kcal	2.5%
Carbohydrate	13.52 g	10%
Protein	0.54 g	1%
Total Fat	0.12 g	<1%
Cholesterol	0 mg	0%
Dietary Fiber	1.40 g	4%
Folates	18 µg	4.5%
Niacin	0.500 mg	4%
Pyridoxine (vit B-6)	0.112mg	9%
Riboflavin	0.018 mg	1.5%

Thiamin	0.079 mg	6.5%
Vitamin A	58 IU	2%
Vitamin C	47.8 mg	80%
Vitamin E	0.02mg	<1%
Vitamin K	0.07 µg	0.5%
Sodium	1 mg	0%
Potassium	109 mg	2.5%
Calcium	13 mg	1.3%
Copper	0.110 mg	12%
Iron	0.29 mg	3.5%
Magnesium	12 mg	3%
Manganese	0.927 mg	40%
Zinc	0.12 mg	1%

Table 2 : Publish data on pH of pineapple

Fruit	Medium	pH	Reference
Pineapple		3.20 – 4.00	USFDA (2003)
Pineapple	Canned	3.35 – 4.10	USFDA (2003)
Pineapple	Juice, canned	3.30 – 3.60	USFDA (2003)
Pineapple	Canned	3.50	Food and Agricultural Products,

			Research and Technology Center (2005)
Pineapple	Fresh	3.30 – 3.50	Food and Agricultural Products, Research and Technology Center (2005)
Pineapple	Juice	3.50	Food and Agricultural Products, Research and Technology Center (2005)

2.1.3 View on Mango

The mango cv.Chokanan (Mangifera indica L.) is one of the most celebrated tropical fruits, which belongs to the family Anacardiaceae which is known for encompassing several highly poisonous plants. The Mangifera genus is comprised of 69 species, out of which the common mango tree cv.Chokanan (Mangifera indica L.) is the most popular. Out of these 69 species, approximately 27 species produce fleshy fruits that are edible with the common mango cv.Chokanan (Mangifera indica) being the most popular (Mukherjee & Litz, 2009).

In Malaysia, mango cv.Chokanan (Mangifera indica L.) is the one of the most popular cultivar grown for local and export market. According to (Spreer et al., 2009) there is a large stock of mango cv.Chokanan (Mangifera indica L.) every year as it has three harvest seasons in May, June and August. This is due to its ability to yield off-season flowering without applying chemicals for initiation. This characteristic allows the fruit to be processed into products including juice, nectars, puree, pickles, and canned slices that are globally accepted. The increasing demand for this cultivar is due to its vibrant colour, exotic flavors, distinctive taste and nutritional properties. Consumption of mango juice has been linked to the prevention of cardiovascular disease and cancer.

The mango fruit is known as a drupe or a stone fruit whereby an outer fleshy part (skin and flesh) surrounds a shell (the pit, stone, or pyrene) with a seed inside (Stern, 1997). It grows from the end of a long, string-like stem to form the fruit. Mango fruits may vary greatly in shape, size, weight and colour based on its cultivar. They are normally 2 to 9 inches long and are kidney shaped, ovate, elongated and nearly round. The fruit weight ranges from 50 gram to over 2 kilograms (Mukherjee & Litz, 2009). The outer most layer of the fruit (exocarp) is the leathery skin, which is normally waxy and smooth and turns pale green, yellow or yellow marked with red according to corresponding cultivars. The exocarp is inedible and contains saps that may cause irritation in some people. The mesocarp or the flesh of a mango is the edible part of the fruit, which is made up of shaped seed (endocarp). The flavour and taste of the mango flesh is pleasant and it is high in sugars and acid giving it a distinct flavour (Bally, 2006).

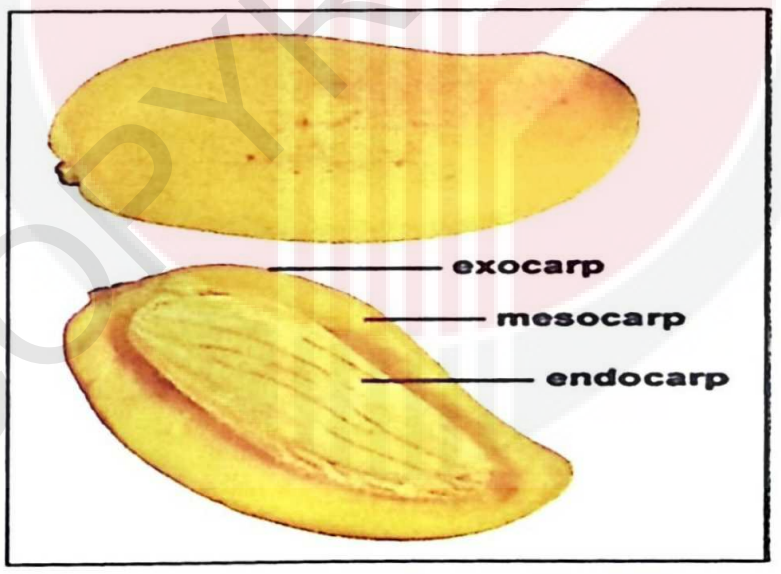

Figure 2 : Fruit structure of ripe mango

Table 3: Nutritional value of fresh mango fruit

PRINCIPLE	NUTRIENT VALUE	PERCENTAGE OF RDA
Energy	70 kcal	3.5%
Carbohydrate	17 kg	13%
Protein	0.5g	1%
Total Fat	0.27 g	1%
Cholesterol	0 mg	0%
Dietary Fiber	1.80 g	4.5%
Folates	14 µg	3.5%
Niacin	0.584 mg	3.5%
Pantothenic acid	0.160 mg	1%
Pyridoxine (vit B-6)	0.134 mg	10%
Riboflavin	0.057 mg	4%
Thiamin	0.058 mg	5%
Vitamin C	27.7 mg	46%
Vitamin A	765 IG	25.5%
Vitamin E	1.12 mg	7.5%
Vitamin K	4.2 µg	3.5%
Sodium	2 mg	0%
Potassium	156 mg	3%
Calcium	10 mg	1%
Copper	0.110 mg	12%
Iron	0.13 mg	1.5%

Magnesium	9 mg	2%
Manganese	0.027 mg	1%
Zinc	0.04 mg	0%

2.2 Fundamental of Enzyme

Enzymes are basic component that are required by living cells in order to make reactions to occur. Enzyme is a biological catalyst which is highly selective. The enzymes are involved in thousands of metabolic processes in all organisms (Bugg, 2012). Enzymes consist of polypeptide backbone of defined amino acid sequence, arranged in particular folding. Enzyme activity is defined by its three dimensional structures. The shape of natural folding is known as native state. Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules, known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life (Stryer, 2002).

Enzymes increase the reaction rate by lowering its activation energy. Some enzymes can make their conversion of substrate to product occur many millions of times faster. An extreme example is orotidine 5'-phosphate decarboxylase, which allows a reaction that would otherwise take millions of years to occur in milliseconds (Callahan, 2007). Chemically, enzymes are like any catalyst and are not consumed in chemical reactions, nor do they alter the equilibrium of a reaction. Enzymes differ from most other catalysts by being much more specific. Enzyme activity can be affected by other

molecules: inhibitors are molecules that decrease enzyme activity, and activators are molecules that increase activity. Many therapeutic drugs and poisons are enzyme inhibitors. An enzyme's activity decreases markedly outside its optimal temperature and pH.

2.2.1 Peroxidase (POD)

Peroxidase (POD) is found in almost all living organisms, its principal physiological function being to control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals that are harmful to all living organisms (Van Dijk and Tijskens, 2000). POD catalyzes single-electron oxidation of a wide variety of compounds in the presence of hydrogen peroxide (Tomas-Barberan and Espin, 2001). Plant POD consists of a complex spectrum of isoenzymes existing both in soluble and bound forms (Van Dijk and Tijskens, 2000). POD is believed to be involved in color and flavor degradation of horticultural products. POD catalyzes the oxidation of phenolic compounds in the presence of hydrogen peroxide leading to the formation of brown degradation products. However, whether this reaction takes place in plants is unclear since the low internal concentration in plants of hydrogen peroxide, which is essential in POD-catalyzed reactions, limits its activity. The possible role of PPO as a promoter of POD activity is suggested since hydrogen peroxide is generated during the PPO catalyzed oxidation of phenolic compounds (Tomas-Barberan and Espin, 2001). There are also studies that showed a high correlation between anthocyanin degradation and browning discoloration during postharvest storage with increased POD activity (Underhill and Critchley, 1995; Zhang et al., 2003; Zhang et al., 2005). Therefore, although the main agent responsible for enzymatic browning in fruits and vegetables is

PPO, a possible synergistic effect between PPO and POD cannot be excluded (Tomas-Barberan and Espin, 2001). On the positive side, POD catalyzes the formation of phenolic cross-linkings between neighboring cell wall polymers in adjacent cells improving the textural quality of some vegetables during thermal processing (Van Dijk and Tijskens, 2000). In general, due to the degradation of pectin and the resulting cell separation, significant tissue softening occurs during thermal processing of parenchyma-rich plant tissues.

2.3 Thermal Processing of Fruit Juice

The acid nature of most juices permits pasteurization, defined as the use of temperatures near 100°C to effect destruction of spoilage organisms (Ludikhuyze et al., 2002). Although spores conceivably can survive at a pH less than 4.6, outgrowth is unlikely. In contrast, at a pH greater than 4.6, heat resistance spore dictates a process temperature of greater than 115°C for an extended time. Hence pH reduction by acid addition to turn low acid or marginal pH juices into high acid products is widely practiced (Sanchez-Vega, 2009).

Thermal processing with high temperatures may be successful in the preservation of liquid food products such as milk and fruit juices, it is inappropriate for other food products such as fruits and vegetables. In the fresh produce industry, thermal processing are carried out at lower temperatures to minimize quality loss in fruits. The time and temperature variables for thermal processing of fruit depends on the fruit cultivar. Treatment conditions should be selected appropriately to avoid over processing.

The efficacy of thermal processing in inactivation microorganism is due to the adverse effects caused by heat on microbes which leads to damage of organic molecules (nucleic acids and proteins) required for the proper functioning of cells (Santhirasegaram et al., 2014). Relatively high temperatures are required for successful inactivation of spoilage causing microorganism which may in turn cause adverse effect on the quality of food products in terms of nutritional value and sensory attributes, such as flavour and colour (Rawson et al., 2011).

2.4 High Pressure Processing of Fruit Juice

The vast interest in the high pressure technology is due to its ability to preserve the freshness of foods and retention of bioactive compounds in addition to achieving required microbial reduction concerning food safety issue. High Pressure Processing (HPP) has already been established as an alternative to thermal processing for the inactivation of food borne microorganisms (Patterson, 2005) and some enzymes (Guerrero et al., 2005) without any requirement of chemical preservatives and/or additives at comparatively lower temperature than thermal processes. HPP has successfully been used for various specialty foods and numerous reports are available on its application to food (Ramaswamy, 2003). HPP processing retains the sensory qualities of the food better as compared with thermal processing (Deliza et al., 2005). HPP has been successfully applied to various fruit products, giving better quality retention of colour, flavor and vitamins (Toepfl et al., 2006) than thermal processing. A few fruit products treated with HPP such as juices and jams have been commercialized in some developed countries. However, the success of the process depends on the inactivation of enzymes and destruction of microorganism to a safe level.

Table 4 : Summary of representative studies on the effect of high pressure from different sources

Source	Medium	Conditions Investigated	Effect	Reference
Apple	Cloudy apple (golden delicious) juice	200-500 MPa, 15-65°C, 0.5-10.5 min	Activity increase up to 40°C at all conditions; some inactivation at higher temperature 32% and 39% inactivation at 100 MPa and 650 MPa	Baron et al. (2006)
	Purified in citrate-phosphate buffer	150-650 MPa, 25°C, 10 min		
Banana	Purified in Tris-HCl buffer	10°C, 600-700 MPa	Inactivation of the labile fraction (92%) at $P^a \geq 600$ MPa at 10°C; inactivation at	Ly-Nguyen et al. (2002a)
		30-76°C, 0.1-900 MPa	$P \geq 800$ MPa at room temperature; no inactivation of the stable fraction up to 900 MPa; antagonistic effects at $P \leq$ 300-400 MPa, $T^b \geq 64$°C	Ly-Nguyen et al. (2002b)
Strawberry	Purified in Tris-HCl buffer (pH 7.0)	850-1000 MPa, 10°C, kinetic	Inactivation of the labile fraction (90%) at $P \geq 850$ at 10°C; no inactivation of stable fraction up to 1000 MPa	Ly-Nguyen et al. (2002c)

Grapefruit	Grapefruit juice	500-900 MPa, T=20-50°C not controlled, temperature effect not accounted for 10-62°C, 0.1-800 MPa, kinetic	50% to a maximum of 85% inactivation (only the labile isoform) after 1 second at 600-900 MPa	Goodner et al. (1998)
Guava	30% Guava juice (pH 4.7, 3° Brix)	600 MPa, ambient temperature	600 MPa, 10 min; Partial inactivation (45%)	Lin and Yen (1995)
Orange	Orange juice (pH 3.45)	500-900 MPa, 20-50°C not controlled, temperature effect not accounted for 400-600 MPa, 25-50°C, kinetic 500 MPa MPa, 600 MPa, 800 MPa, 25-50°C	10-93% inactivation, respectively, after 1 sec at 600-900 MPa (only the labile isoenzyme); slower inactivation at 500	Goodner et al. (1998)

2.5 Effect on Enzyme Activity after High Pressure Processing and Thermal Processing

High pressure may cause inactivation or activation of enzymes depending on the applied pressure and the type of enzyme. Application of pressure less than 100 MPa has been observed to activate some enzymes, especially monomeric enzymes such as chymotrypsin and PPO (Buckow et al., 2009). The presence of a substrate may have protective effects but can also lead to a strong destabilization of a protein (Fernandez-Garcia et al., 2002). The changes of water properties with increasing pressure, temperature, and the presence or absence of solutes reflect changes in the arrangement of water molecules that might explain the baro-protective effects of solutes on proteins under denaturing conditions. Moderate high pressure can be used to enhance beneficial enzyme reactions in plant tissue as it allows increased enzymesubstrate contact due to tissue disruption. It is also not unusual that the thermostability of enzymes is increased under specific pressures, allowing enzyme-catalyzed reactions to take place at moderately high temperature and faster rate (Ludikhuyze et al., 2003; Knorr et al., 2006). Under the high pressure environment, the mechanism of enzyme inactivation can be hypothesized similar to protein denaturation (Ludikhuyze et al., 2001). The application of pressure may induce reversible or irreversible and partial or complete unfolding of the native structure of the enzyme. This eventually leads to a change in enzyme activity as its specificity is related to the structure of its active site.

Table 5 : Summary of representative studies on the effect of high pressure on PPOs from different sources

Source	Medium	Conditions investigated	Effect	Reference
Apple(cv. Amasaya)	Cloudy apple juice (pH 3.5)	250-450 MPa, 25-50°C, 0-60 min	~50% activity increase at 450 MPa, 25◦C, 15 min; 90% inactivation at 450 MPa, 50◦C, 60 min	Bayindirli et al. (2006)
Mango	Puree (pH 4.5)	379-586 MPa, 0.03-20 min, 25°C	25% inactivation at all conditions after 20 min	Guerrero-Beltran et al. (2005a)
Raspberry	Whole fruit	600–800 MPa/5–15 min	54% and 42% activity increase after 5 min and 10 min at 600 MPa	Garcia-Palazon et al. (2004)
Strawberry	Whole fruit	600–800 MPa/5–15 min	100% inactivation (800 MPa/10 min), ~78% inactivation (600–800 MPa/5 min)	Garcia-Palazon et al. (2004)
Banana	Banana Puree (pH 3.4)	517 MPa and 689 MPa, 21°C, 10 min	Increased activity after 10 min at 517 MPa, 21% inactivation at 689 MPa	Palou et al. (1999)
Guava	30% Guava juice (pH 4.7, 3°	600 MPa, 10 min, 25°C	5% inactivation	Lin and Yen

	Brix)			
	30% Guava juice (pH 3.9, 3°Brix)			
	30% Guava juice (pH 3.9,12°Brix)			
Pear (cv. La France)	Purified latent PPO in phosphate buffer (pH 7.0)	300–700 MPa	Activation at P > 300 MPa that increased with treatment time, maximum activation at 600 MPa	Asaka et al. (1994); Asaka and Hayashi (1991)
			70% inactivation at 600 MPa/10 min	(1995)
			50% inactivation	

Table 6 : Summary of representative studies on the effect of high pressure on PODs from different sources

Source	Medium	Treatment condition	Effect	Reference
Strawberry (cv.Festival)	Strawberry halves puree	100–600 MPa, 20–60◦C, 2–10 min	Significant inactivation at all condition, maximum of 58% inactivation at 600 MPa, 60◦C, 10 min.	Terefe et al. (2009a, 2010)
Blueberry (cv.C97-390)	McIlvaine buffer (pH 3.6)	100–690 MPa, 24–90◦C, 10 min	Complete inactivation at 90◦C regardless of the pressure, slight inactivation at 100 MPa and 690 MPa and 30–70◦C, maximum 35% inactivation at 690 MPa and 70◦C, 40% increase at 400 MPa and 50◦C	Terefe et al. (2009c)
Strawberry	Whole fruit	400–800 MPa/15 min, ambient temperature	13% activity increase at 400 MPa and 5 min, 35% inactivation (600–800 MPa/15 min)	Garcia-Palazon et al. (2004)
Apple	Apple pieces	600–1000 MPa, 20◦C, 15 and 30 min	Two-fold increase in activity at 600 MPa, ~40% inactivation at 1000 MPa, no effect of treatment time	Prestamo et al. (2001)
Banana	Extraction buffer (pH 7.0)	70–139 MPa, 50–70◦C, 5–25 min	98.6% inactivation at 110 MPa and 70◦C, 5 min	MacDonald and Schaschke (2000)

Guava	30% Guava juice (pH 3.9, 12◦ Brix)	100–500 MPa, 25◦C, 5–60 min	No effect up to 300 MPa, 10.4% inactivation at 500 MPa, ≥5 min	Lin and Yen (1998)
Orange	Orange juice	50–400 MPa, 20–60◦C, 15 min	Inactivation at 50–400 MPa and 25–32◦C, maximum of 50% inactivation at 400 MPa, 32◦C, 15 min, decreased inactivation or activation at higher temperature.	Cano et al. (1997)

2.6 Antioxidant Activity

Antioxidant are compound capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals.

Antioxidant are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals.

Endogenous antioxidant are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non-enzymatic compound, such as uric acid, bilirubin, metallothioneins. When endogenous factors cannot ensure a rigorous control and complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound.

Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxanisole, butyhydroxytoluene, gallates, etc. (Aklima, 2014).

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as deterioration of fats and other constituents of foodstuffs (Alonso, 2002).

2.7 Total Phenolic Compound

Phenolic compounds are secondary plant metabolites, which are produced in response to different pressures, such as infection, injury, ultraviolet (UV) radiation, ozone, pollution, etc. Flavonoids are the most abundant and biologically active phytonutrients, which can reduce inflammation, inhibit tumor growth, have an action proapoptotic and anti-angiogenic, antimicrobial properties, anti-virus, and anti-aging, modulate the immune system, enhance capillary resistance, protect the cardiovascular and nervous system, limiting weight gain, promote wound healing and other polyphenolics used in various food and cosmetic industry as a natural material (natural dyes, conservative agent, a natural antioxidant, nutritional supplements) (Munin & Edwards-Levy, 2011).

CHAPTER 3

METHODOLOGY

3.1 Preparation of Pineapple-Mango Juice

Pineapple cv. Josapine and Mango cv. Chokanan fruits at commercial maturity were purchased from Pasar Borong Selangor. After the fruits were washed, the skins were remove using meat slicer. Then, the flesh of the fruit was cut into smaller pieces using food slicer. The juice was then produced using fruit cold press. It was followed by filtering each fruits using stainless steel screen. The juices were prepared using 100M, 100P, 70P:30M, 30P:70M and 50P:50M ratio which means100% mango, 100% pineapple, 70% of pineapple juice is mix with 30%, 30% of pineapple juice is mix with 70% and 50% of pineapple juice is mix with 50% of mango juice to form a pineapple-mango juice.

Pineapple-mango juice was placed in glass bottles (Duran Bottle) temporarily before packing process. Next, 30 grams of pineapple-mango juice were vacuum packed in 100 mm x 150 mm pouches. This bag was 1 mm thick with low oxygen transmission rate and could withstand temperature up to 130°C and very suitable for thermal and high pressure processing. The packed samples were stored at -20°C and thawed in laboratory refrigerator for a night before treatment.

Table 7 : Formulation of Pineapple-Mango Juice Blend

Sample	Pineapple Juice (%)	Mango Juice (%)
A	100	0
B	0	100
C	70	30
D	30	70
E	50	50

3.2 Thermal Treatment of Pineapple-Mango Juice

For thermal pasteurization treatment, the packed samples were placed in thermostatic water bath (Sul Supplies (M) Sdn. Bhd. Malaysia). According to (Azam Ali, 2008) fruit juices are pasteurized at temperature between 60°C to 80°C for 5-30 min. In this experiment, the thermal treatment was performed at 60°C, 70°C and 80°C for time interval (5 min to 30 min). The samples were immediately cooled in an ice-water bath before enzyme extraction.

3.3 High Pressure Processing of Pineapple-Mango Juice

Packed juice samples were treated using Avure 2L-700 HPP Laboratory Food Processing System. The HPP consists of computerized control and treatment chambers. Distilled water was used as pressure medium in treatment chamber. The HPP chamber composed with thermocouple to register the temperature during HPP cycle. The packed samples were processed at 400MPa and 600MPa for time interval (5min to 30min).

The processed samples are stored in lab refrigerator before the enzyme extraction. Based on previous results, which indicated effectiveness of PPO inactivation at HPP

>600 MPa (Buckow et al.,2009; Garcia-Palazon et al., 2004; Sulaiman and Silva, 2013; Weemaes et al., 1998a).The pressure-temperature-time processing conditions refer to the constant pressure phase of the HPP cycle. The total pressure increase took less than 2 min.

3.4 Enzyme Activity Measurement

3.4.1 Peroxidase (POD) Extraction and Assay

Extraction of POD from Pineapple-Mango juice was performed using the method suggested by Terefe et al. (2010). Fruit juice was mixed using commercial blender for 5 min with 0.2 M Sorensen's phosphate (SSP) buffer (pH 6.5) which containing 4% polyvinylpolypyrrolidone (PPVP), and addition of 1% (*v/v*) Triton X-100, and 1 M sodium chloride (R&M Chemicals, Malaysia). The homogenates were then centrifuged in 3 mL centrifuge tubes at 14,000 x *g* at 4°C for 30 min. The supernatant containing POD was taken out and POD activity was assessed spectrophotometrically. The reaction mixture was made of 70 µL of crude enzyme extract in 1.5mL 0.05 M SSP buffer, with addition of 200 microL 1.5% (*v/v*) hydrogen peroxide solution and 200 µL 5% (*w/v*) pyrogallol(Sigma Aldrich, Germany) in 0.05 M SSP buffer. In the blank, SSP buffer at pH 6.5 was used. Absorbance data for POD assay was taken at 420 nm for 5 min with interval of 1 min UV-visible spectrophotometer. POD activity was calculated from the slope of linear portion from the curve drawn between reaction time (min) against absorbance (mAbs) and was expressed as mAbs/min.

3.5 Residual Enzyme Activity

Enzyme activity was determined by taking the gradient of the plot of absorbance against time. From that data, percentage of residual enzyme activity was calculated by taking the ratio of processed and unprocessed juice (Chakraborty et al., 2014).

$$\text{Residual activity (\%)} = \frac{A}{Ao} x\ 100 \qquad \text{Equation 1}$$

Where A is the activity of processed juice and A0 is the activity of unprocessed juice.

3.6 DPPH Radical Scavenging Activity Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of antioxidant solution was determined according to the method of Yang et al. (2008). Briefly, 1 mL of sample solution was added to 3 mL of 0.1 mM DPPH solution. The mixed solution was kept for 30 min at ambient temperature (29°C) in dark and the absorbance of the solution was measured at 517 nm. The percentage inhibition was calculated according to the equation:

$$\text{Inhibition (\%)} = \frac{Ac - As}{Ac} \times 100 \qquad \text{Equation 2}$$

Where A_c is the absorbance of control (containing DPPH solution), A_s is the absorbance of sample.

3.7 Determination of Total Phenolics

The total polyphenol content was determined as slightly modified by Skerget el al. (2005). To 0.5 mL of diluted antioxidant extract, 2.5 mL of 10%(v/v) Folin-Ciocalteu reagent was added, followed by the addition of 2 mL of 7.5%(w/v) Na2CO3, then mixed well on a vortex vibrator for 5 min and incubated in the dark at

ambient temperature (29°C) for 1 hour prior to measuring the absorbance at 765 nm.. A standard curve of gallic acid (y=0.0024x – 0.0515) was prepared and results were reported as milligrams of gallic acid equivalent (GAE) per 100 ml juice extract.

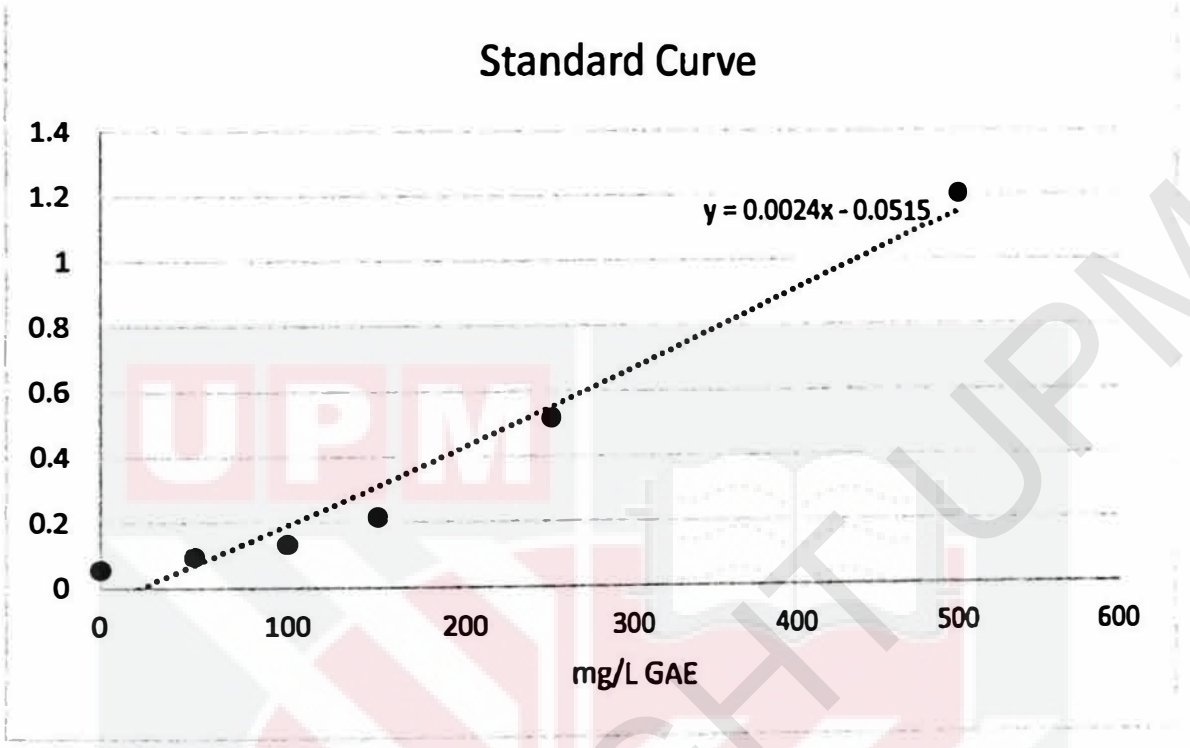

Figure 3: Standard curve for total phenolic content

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of Thermal Processing on POD

Figure 1 until Figure 3 demonstrate the POD residual activities in pineapple cv. Josapine and mango cv. Chokanan juice blend during the thermal processing at temperature ranging from 60°C to 80°C for 30 min respectively.

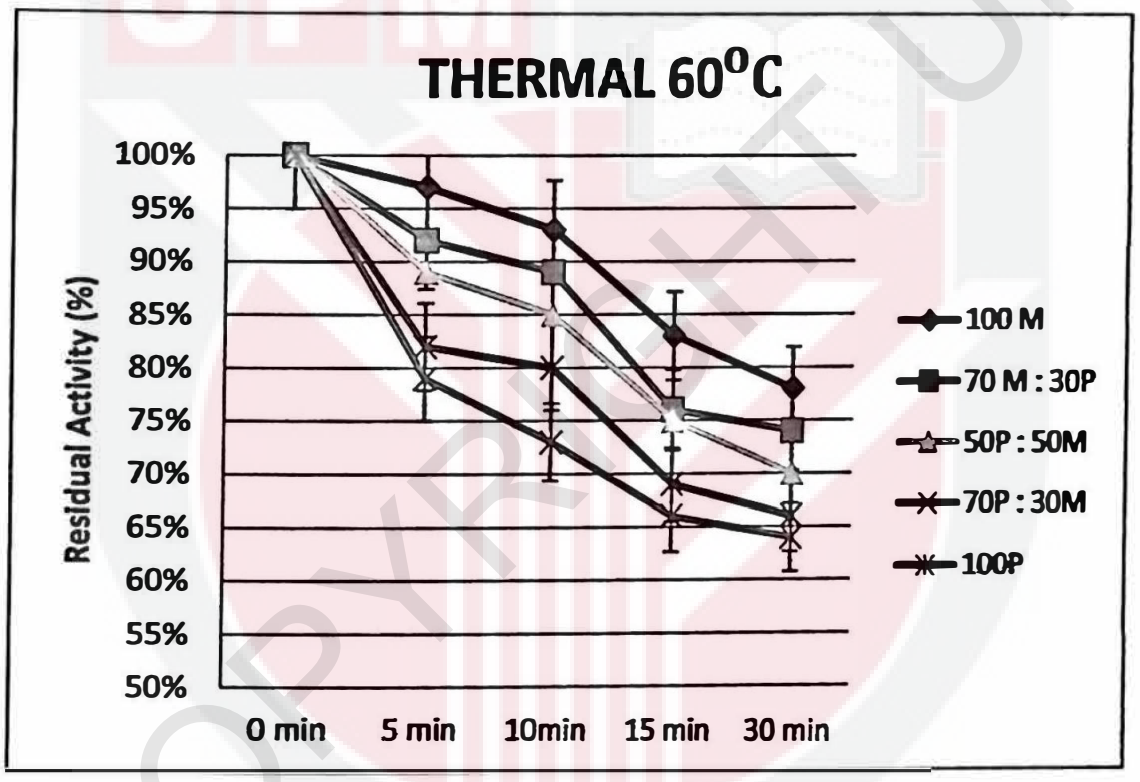

Figure 1 : Percentage POD residual activity after thermal processing at 60°C

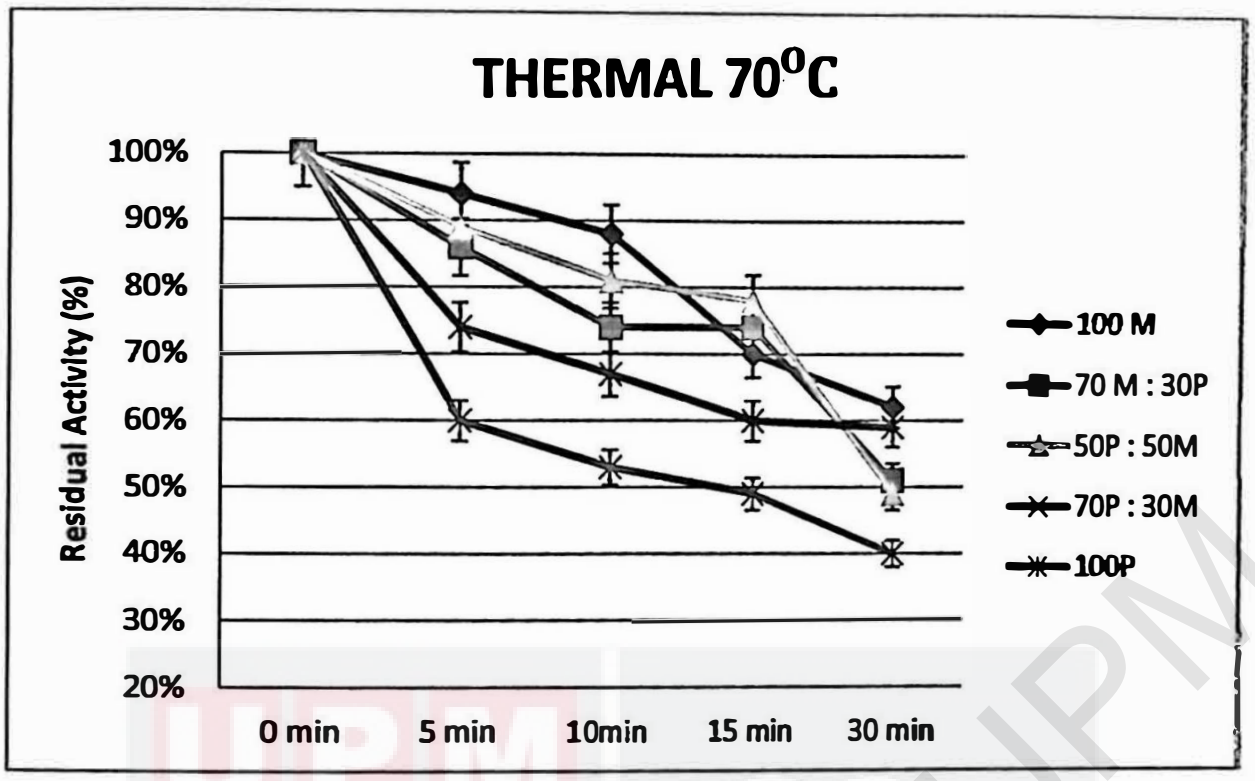

Figure 2 : Percentage POD residual activity after thermal processing at 70°C

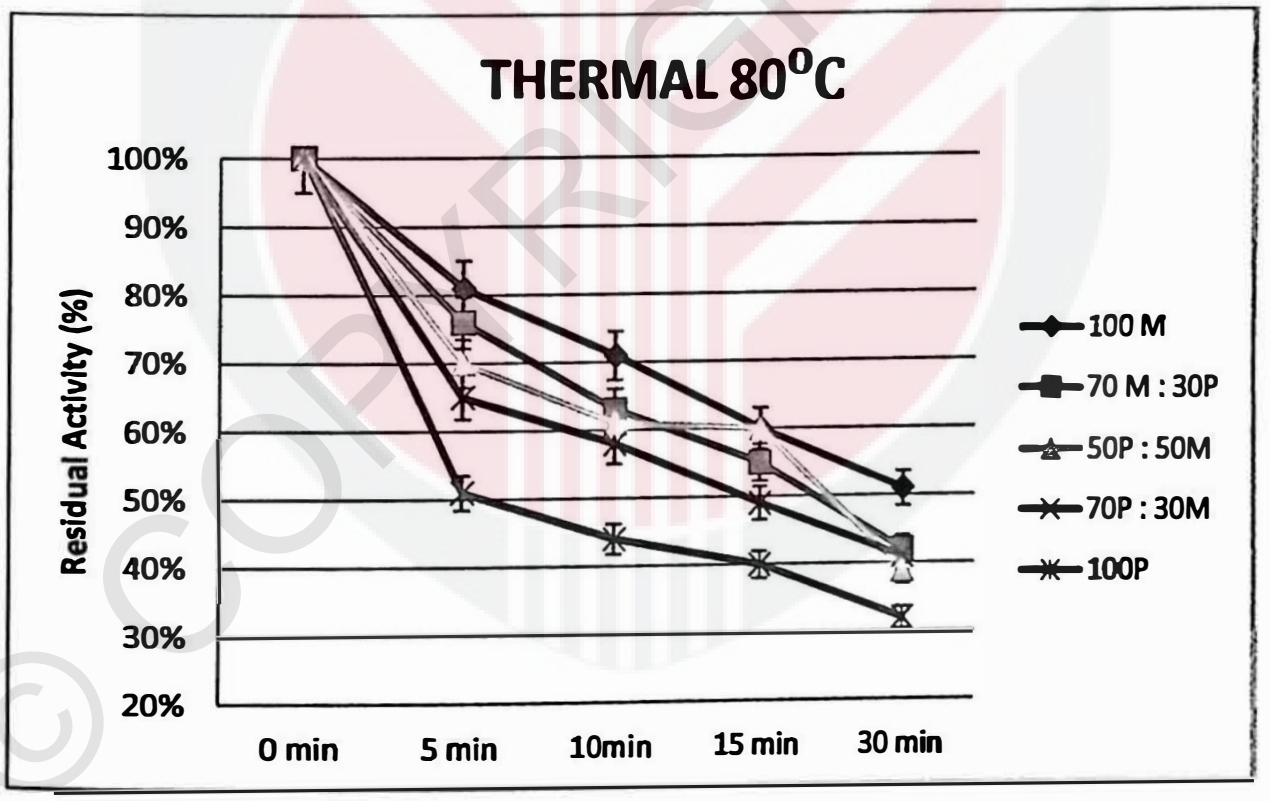

Figure 3 : Percentage POD residual activity after thermal processing at 80°C

Based on this three graph, a process of 5 min at 60°C partially inactivate 100% pineapple juice POD (RA= residual activity = 79%) and the lower RA was obtained when same temperature was applied for 15 min (64%). (RA=58%) partially

inactivate watermelon juice at temperature of 25°C was reported by (Liu et al., 2013). The lower RA obtained in 80°C is when 100% pineapple juice reach 30 min of process (32%).Thermal treatment generally in the range of 80°C to 95°C is commercially applied for the inactivation of spoilage enzymes in fruit pulp & juices. (Barba et al., 2012). However, processing at lower temperature which is less than 80°C showed higher percentage of residual activity for fruit juice. (Teixeira et al., 2006), considered POD completely inactivated after pasteurization at 80 °C and affirmed the treatment was efficient in keeping product quality during storage. Another study with POD showed its complete inactivation commercial fruit juice at temperatures above 62°C (Hirsch et al. 2008) and Anthon et al. POD was inactivated at temperatures above 76°C for periods longer than 30 s (Clemente, 2002).

4.2 Effect of Thermal Processing on Antioxidant Activity

Figure 4 until Figure 6 demonstrate the antioxidant activities in pineapple cv. Josapine and mango cv. Chokanan juice blend during the thermal processing at temperature ranging from 60°C to 80°C for 30 min respectively.

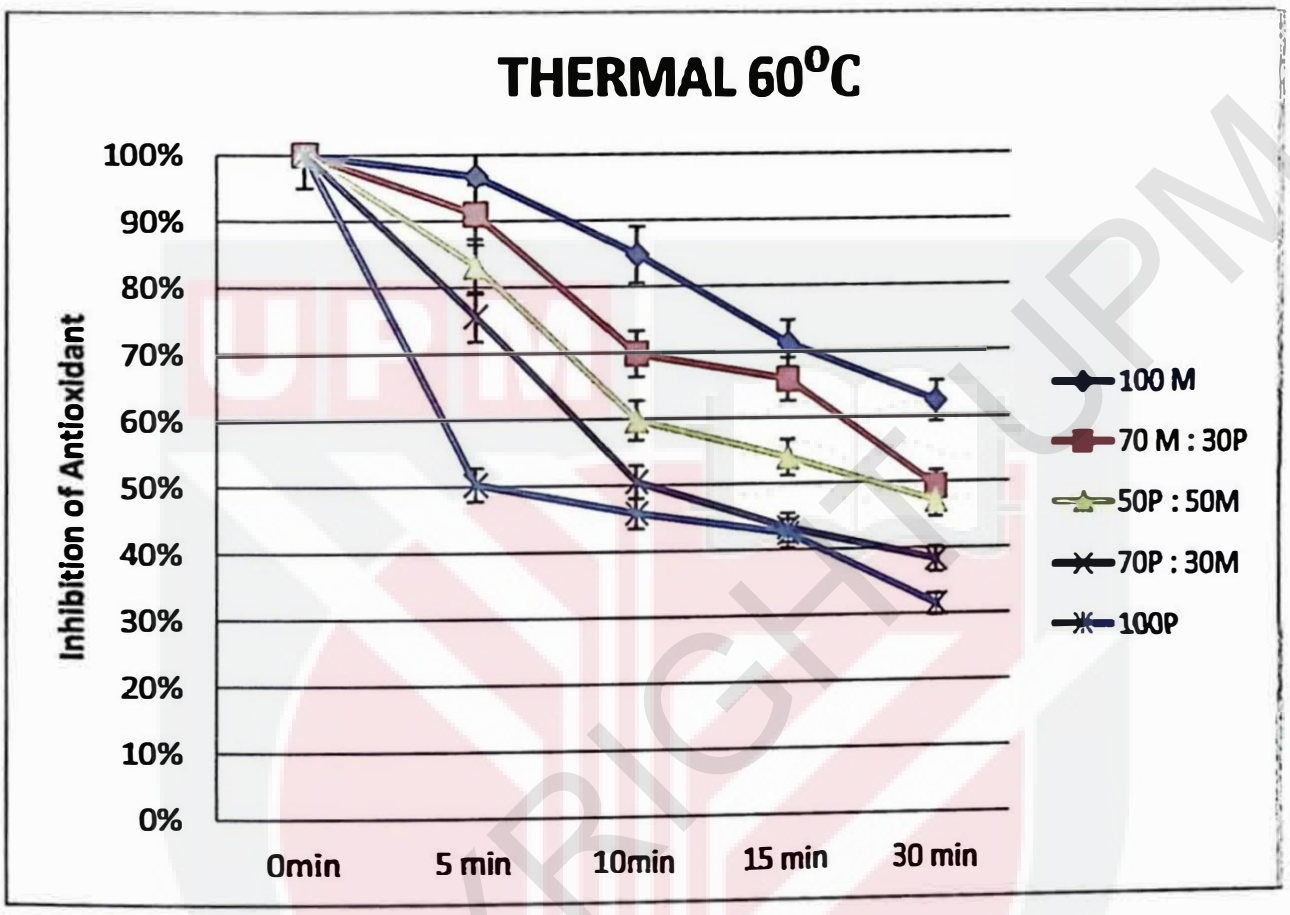

Figure 4 : Percentage inhibition of antioxidant after thermal processing at 60°C

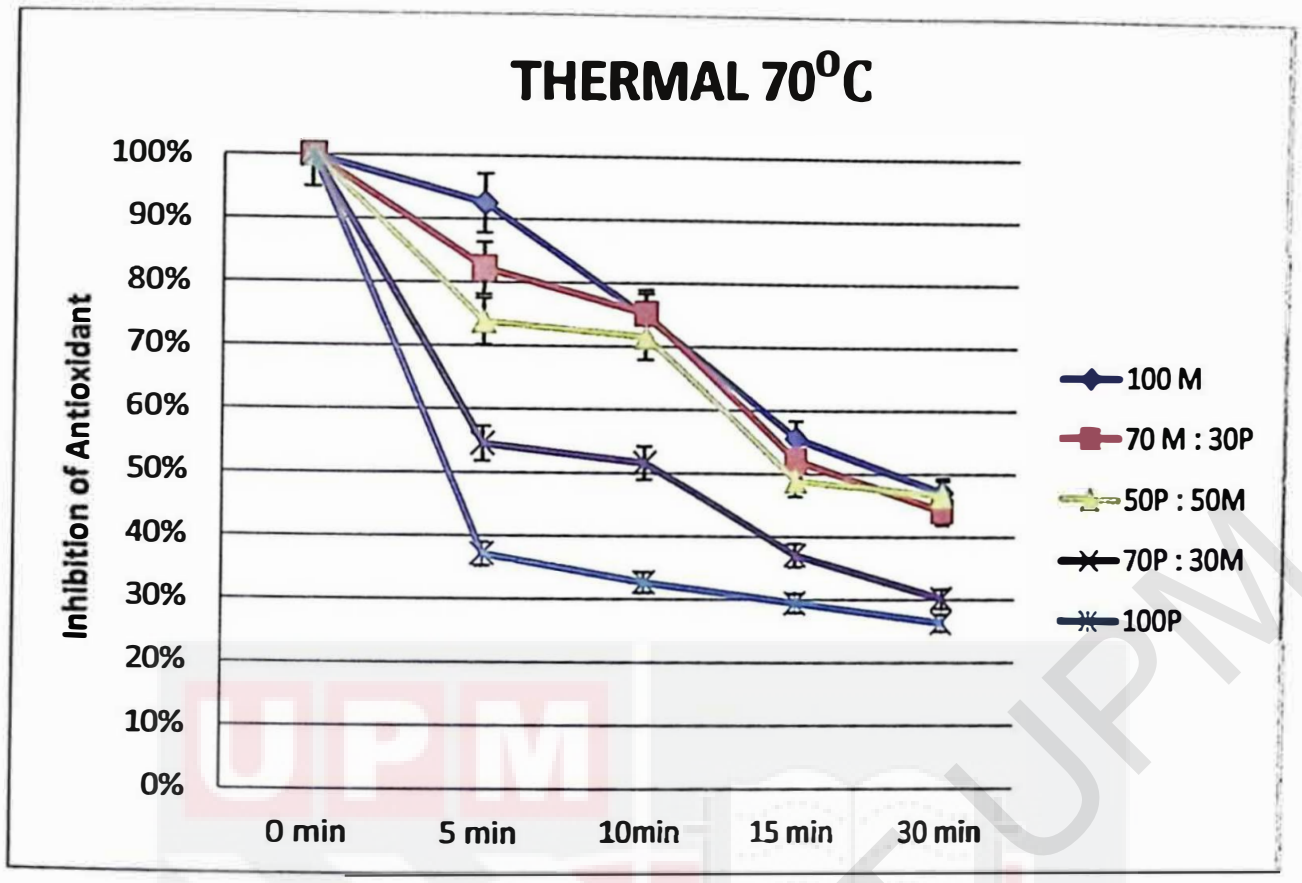

Figure 5 : Percentage inhibition of antioxidant after thermal processing at 70°C

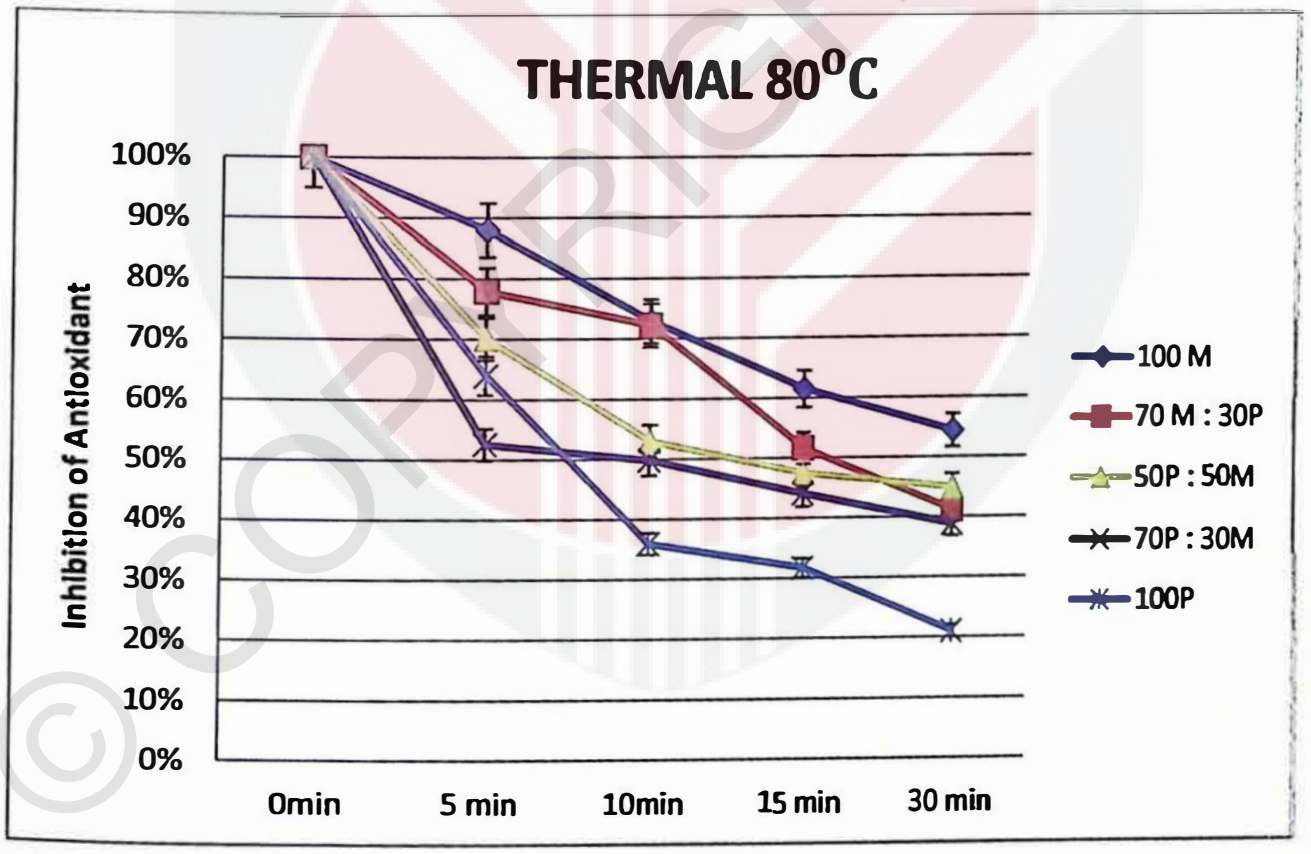

Figure 6 : Percentage inhibition of antioxidant after thermal processing at 80°C

Antioxidant activity of the pineapple-mango juice was determined by DPPH assay. The DPPH radical scavenging assay measures the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, which forms diphenylpicrylhydrazine.

(Shon et al., 2003). For 100% of pineapple juice, the DPPH inhibition at 60°C is (45.75%) for process time of 10 min. With the same process time, DPPH inhibition at 80°C decrease to (35.97%). However, the antioxidant activity depends on the chosen method, concentrations and physicochemical properties of the studied antioxidant (Kulisic *et al.*, 2004).

4.3 Effect of Thermal Processing on Total Phenolic Compound

Figure 7 until Figure 9 demonstrate the total phenolic compound in pineapple cv. Josapine and mango cv. Chokanan juice blend during the thermal processing at temperature ranging from 60°C to 80°C for 30 min respectively.

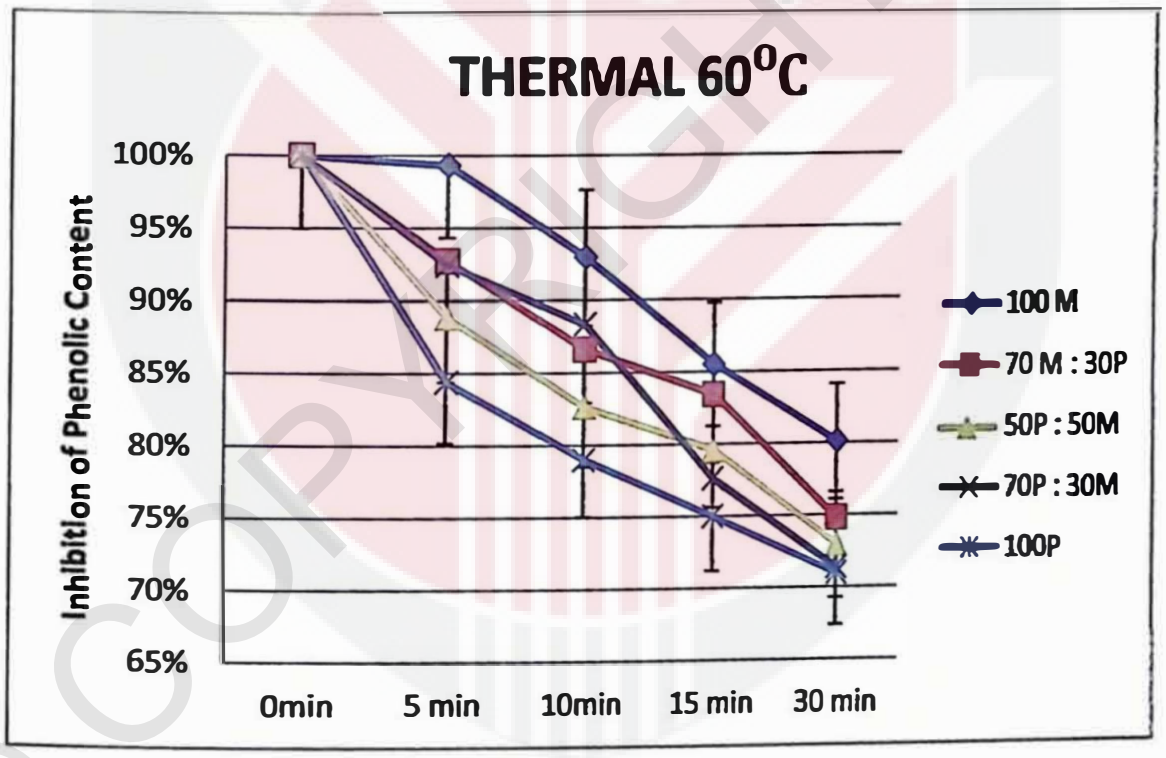

Figure 7 : Total phenolic content after thermal processing at 60°C

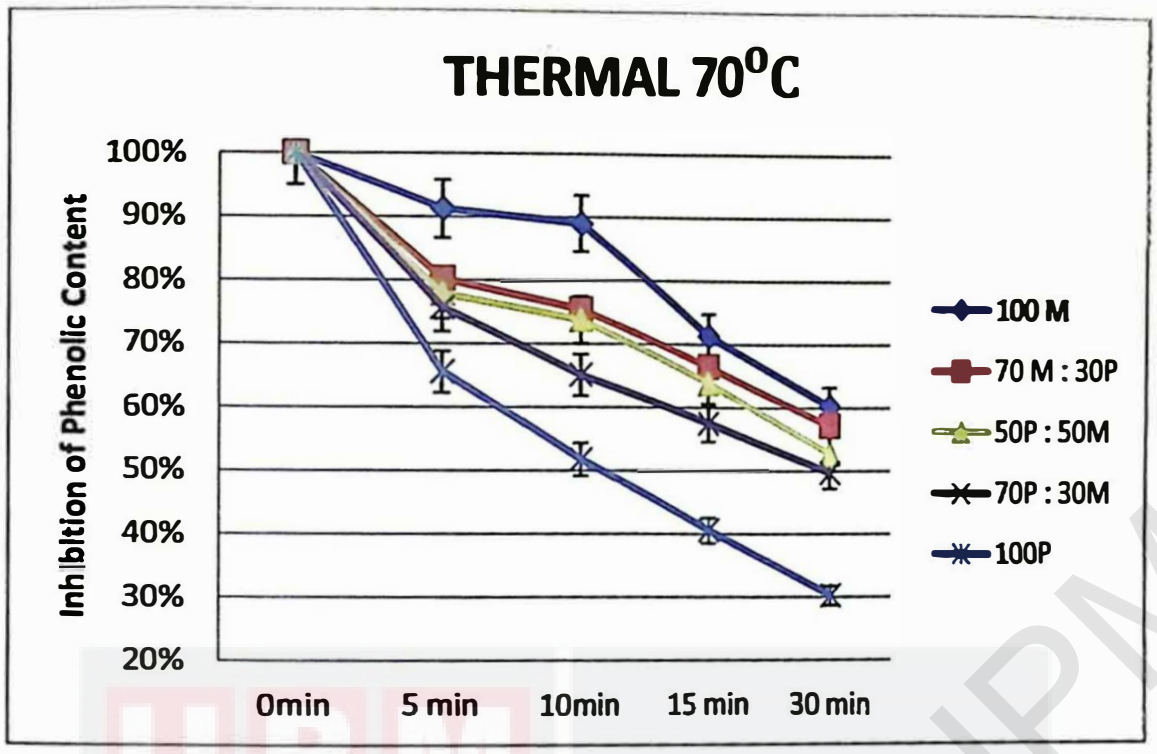

Figure 8 : Total phenolic content after thermal processing at 70°C

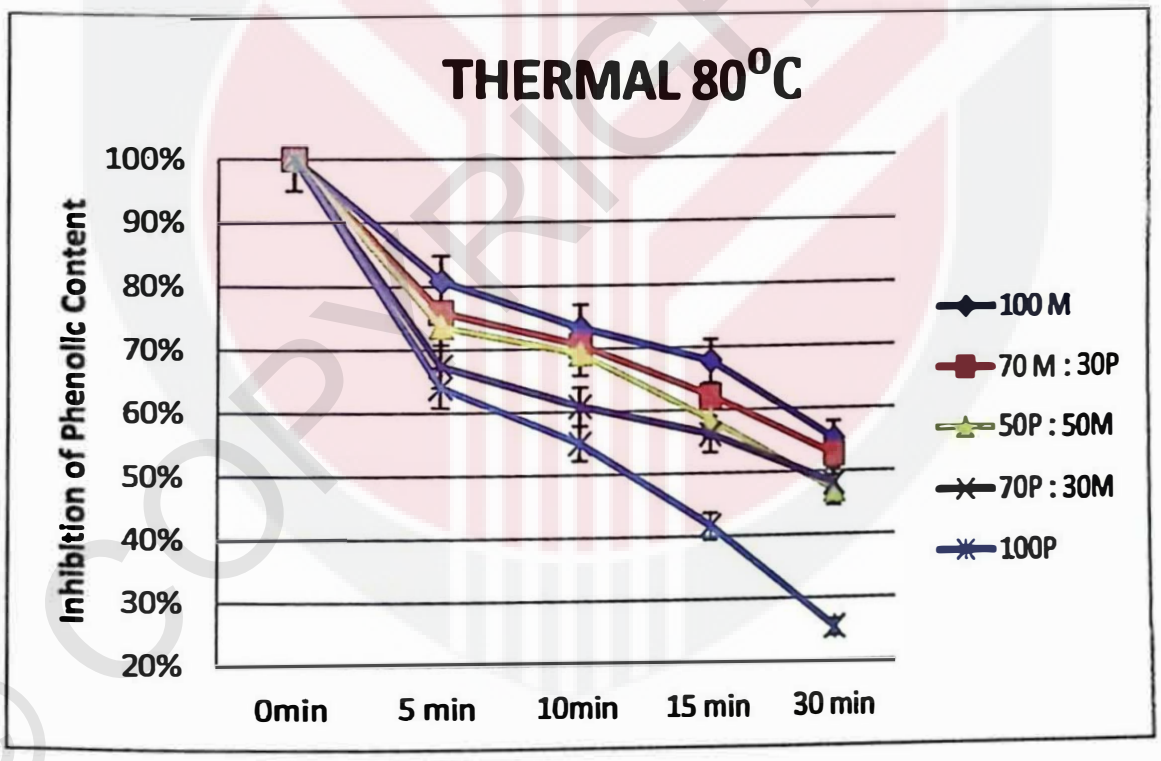

Figure 9 : Total phenolic content after thermal processing at 80°C

Total phenolics of pineapple-mango juice was determined by Follin-Ciocalteu assay. The Follin-Ciocalteu method is based on the detection of phenolic compounds by reduction of reagent, which contains tungsten and molybdenum oxides (John Wilet et

al., 2002). Based on the figure 10,11 and 12, it was found that losses of the total phenolic contents were found to be 29.03%, 69.74% and 74.6% at 60°C, 70°C and 80°C, respectively after 30 min heating of 100% pineapple juice. It was demonstrated that total phenolics significantly decreased affected by various temperatures and incubation times. Similar result was reported concerning thermal stability of anthocyanins in three pomegranate juices (Fischer et al., 2013). However, the variation among the total phenolics of the commercial juices may be due to various factors such as different varieties of the fruit samples and/or percentage of pure juice of their final products (Mahdavi *et al.,* 2010). The Folin-Ciocalteu method was used because many individual phenolic compounds that provide antioxidant activity in fruits cannot be identified or measured by high-performance liquid chromatography (HPLC) methods (Isfahlan AJ et al., 2010).

4.4 Effect of High Pressure Processing on POD

Figure 10 and Figure 11 show the POD residual activities in pineapple cv. Josapine and mango cv. Chokanan juice blend during the high pressure processing a pressure of 400MPa and 600MPa for 30 min respectively. The temperature at all processing pressures was maintained below 60°C.

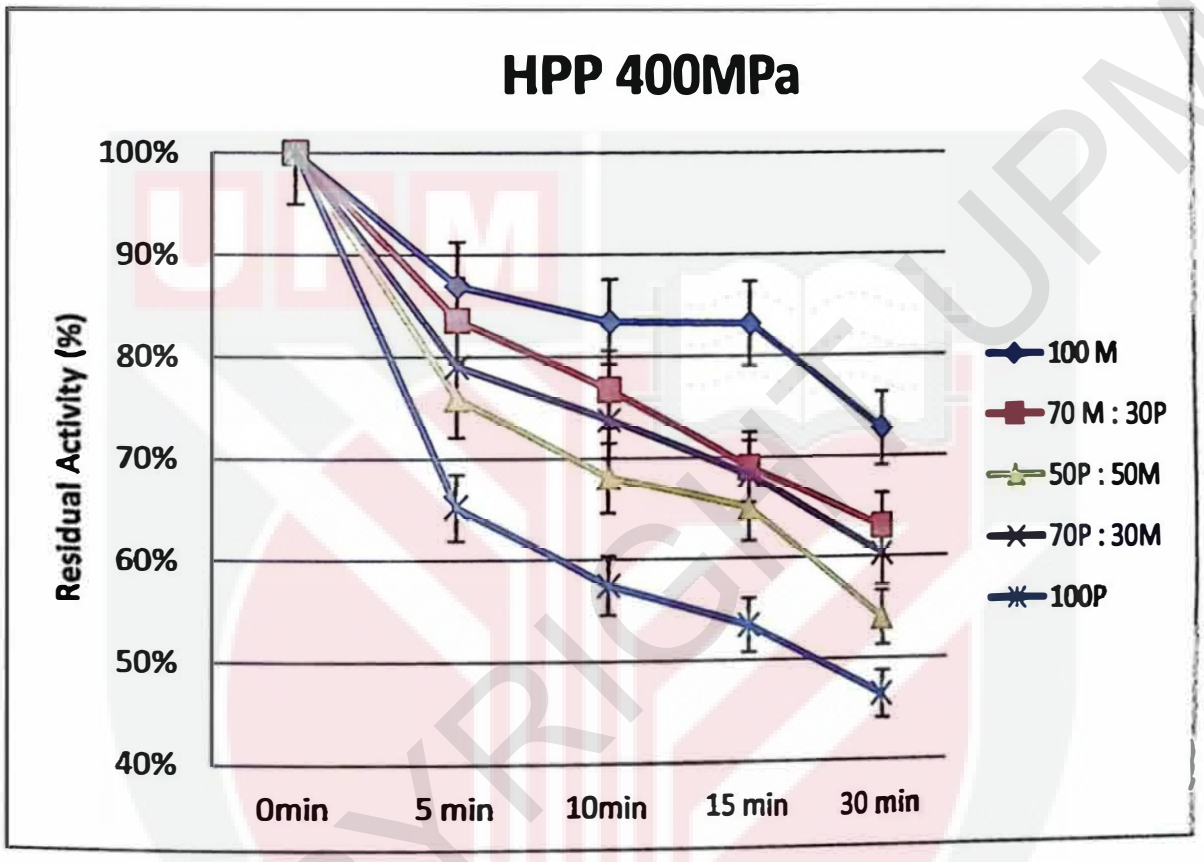

Figure 10 : Percentage POD residual activity after HPP at 400MPa

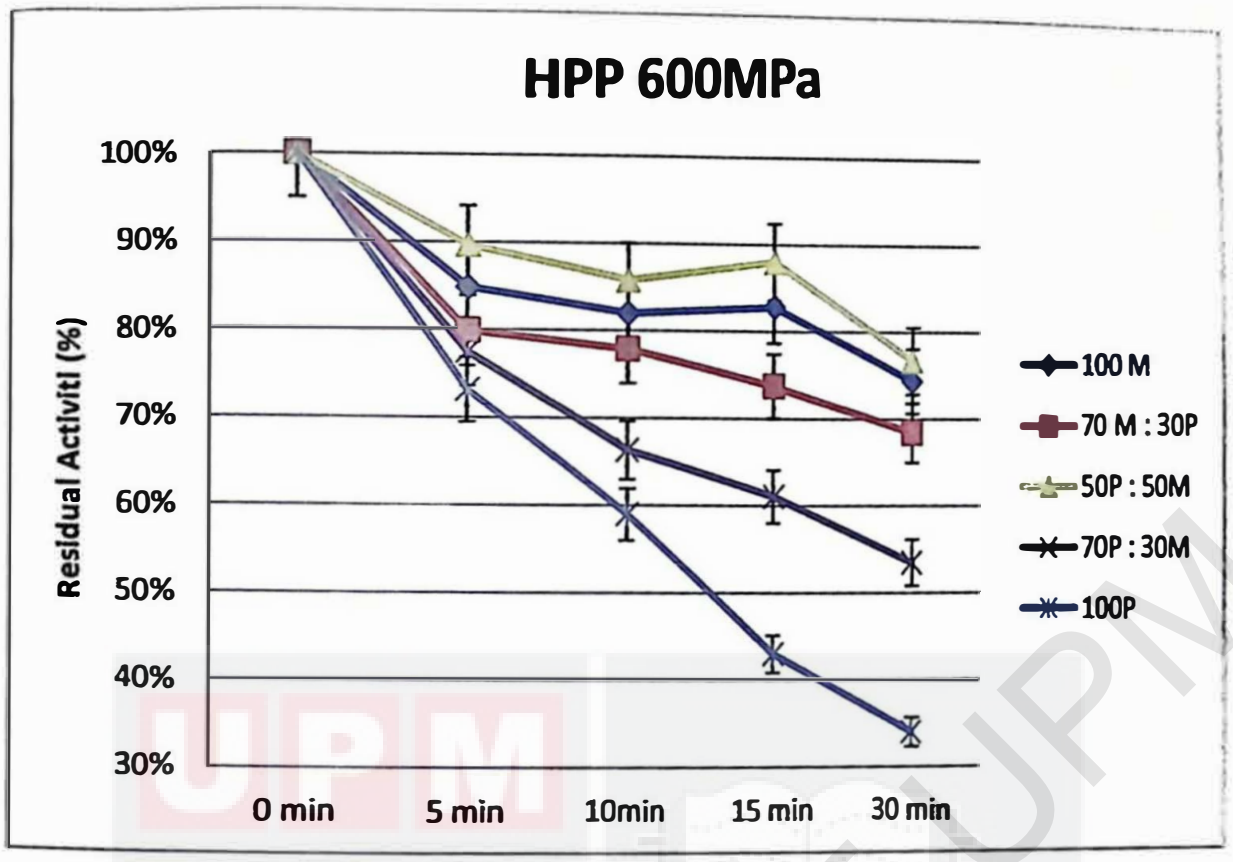

Figure 11 : Percentage POD residual activity after HPP at 600MPa

Based on the figure above, we can see clearly that the percentage of POD residual activity slightly decrease when pressure increase from 400MPa to 600MPa. For example, residual activity of 100% mango decreases from 46.47% to 34% after 30 min of processing. (RA=70%) partially inactivate kiwifruit juice at pressure of 600MPa after 30 min of processing was reported by (Fang et al., 2008). With the same pressure, (Liu et al., 2003) was reported that (RA= 58%) in watermelon juice after 5 min processing time. HPP has been applied at lower temperatures (below 40 °C). HPP at room temperature (>30 °C) can reduce the microbial load to a desired level but the inactivation of spoilage enzymes need the assistance of moderate temperature (Chakraborty et al., 2014). Several literatures have described the effect of HPP combined with/ without temperature on nutritional and physico-chemical properties of fruit juices and purees (Dede et al., 2007).

4.5 Effect of High Pressure Processing on Antioxidant Activity

Figure 12 and Figure 13 show the inhibition of antioxidant in pineapple cv. Josapine and mango cv. Chokanan juice blend during the high pressure processing a pressure of 400MPa and 600MPa for 30 min respectively. The temperature at all processing pressures was maintained below 60°C.

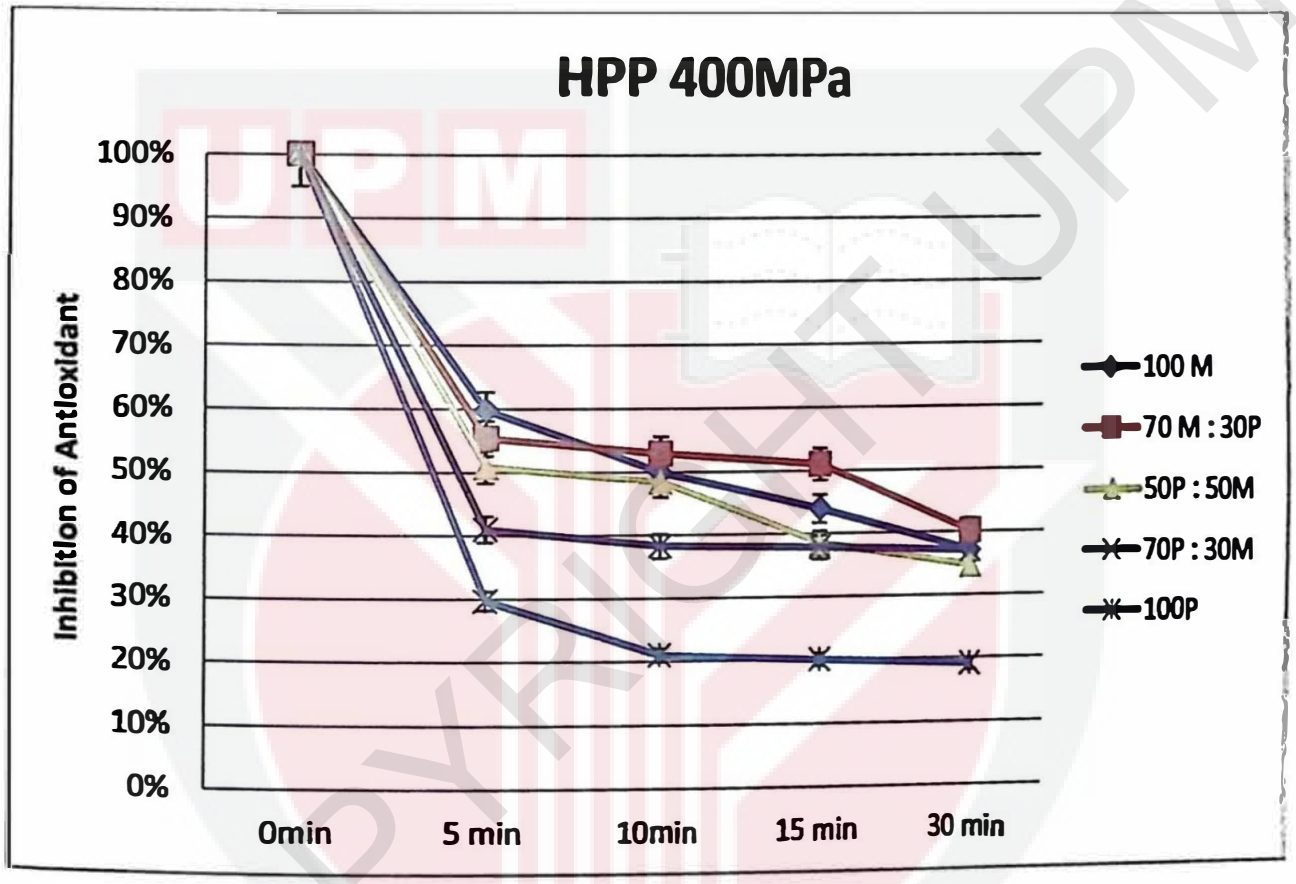

Figure 12 : Inhibition of Antioxidant after HPP at 400MPa

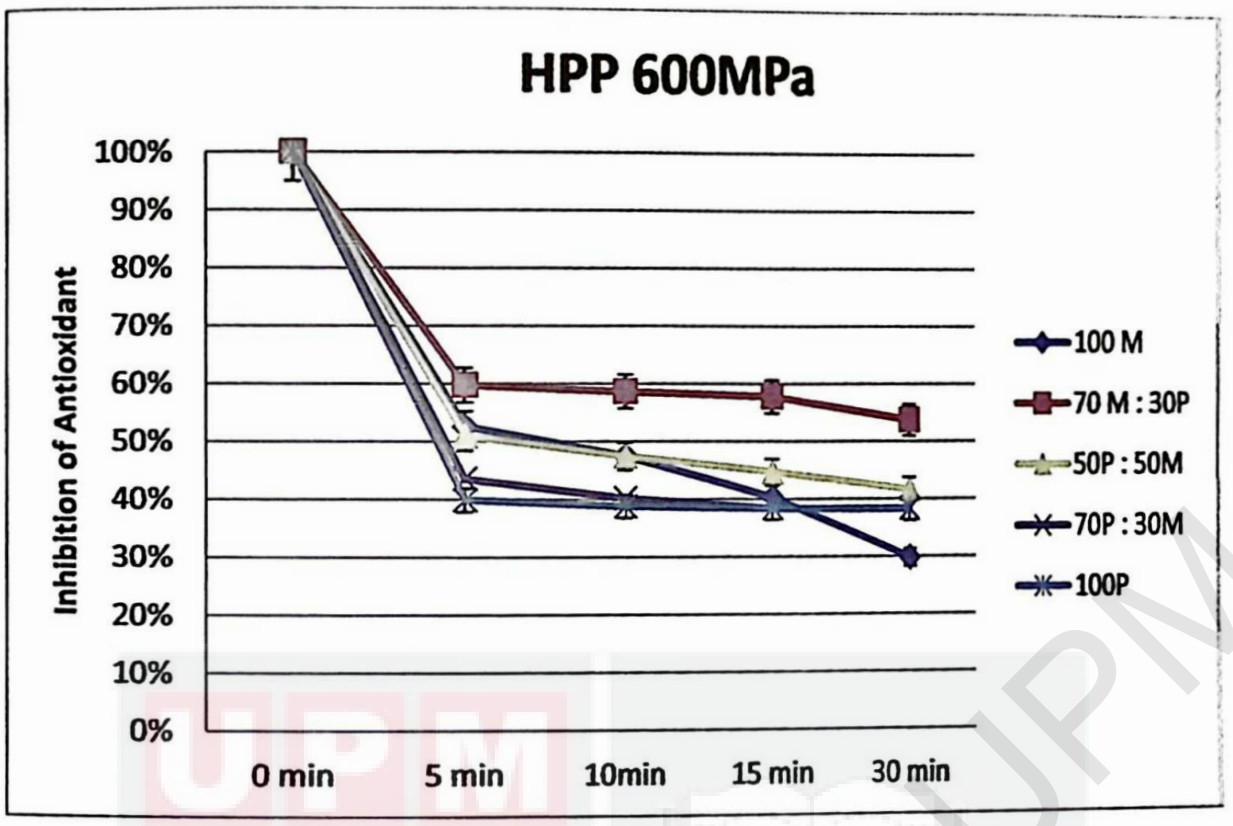

Figure 13 : Inhibition of Antioxidant after HPP at 600MPa

Antioxidant activity of the pineapple-mango juice was determined by DPPH assay. The DPPH radical scavenging assay measures the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, which forms diphenylpicrylhydrazine. (Shon et al., 2003). For 100% of pineapple juice, the DPPH inhibition at 400MPa is (21.03%) for process time of 10 min. With the same process time, DPPH inhibition at 600MPa increase to (38.76%). Free radical-induced oxidative damage is strongly implicated in the development of a number of common chronic disease states (Gutteridge et al., 2000) and many of the health benefits associated with consumption of vegetables and fruits have been linked to their potent antioxidant properties (Halliwell, Rafter, & Jenner, 2005).

4.6 Effect of High Pressure Processing on Total Phenolic Compound

Figure 14 and Figure 15 show the total phenolic compound in pineapple cv. Josapine and mango cv. Chokanan juice blend during the high pressure processing a pressure of 400MPa and 600MPa for 30 min respectively. The temperature at all processing pressures was maintained below 60°C.

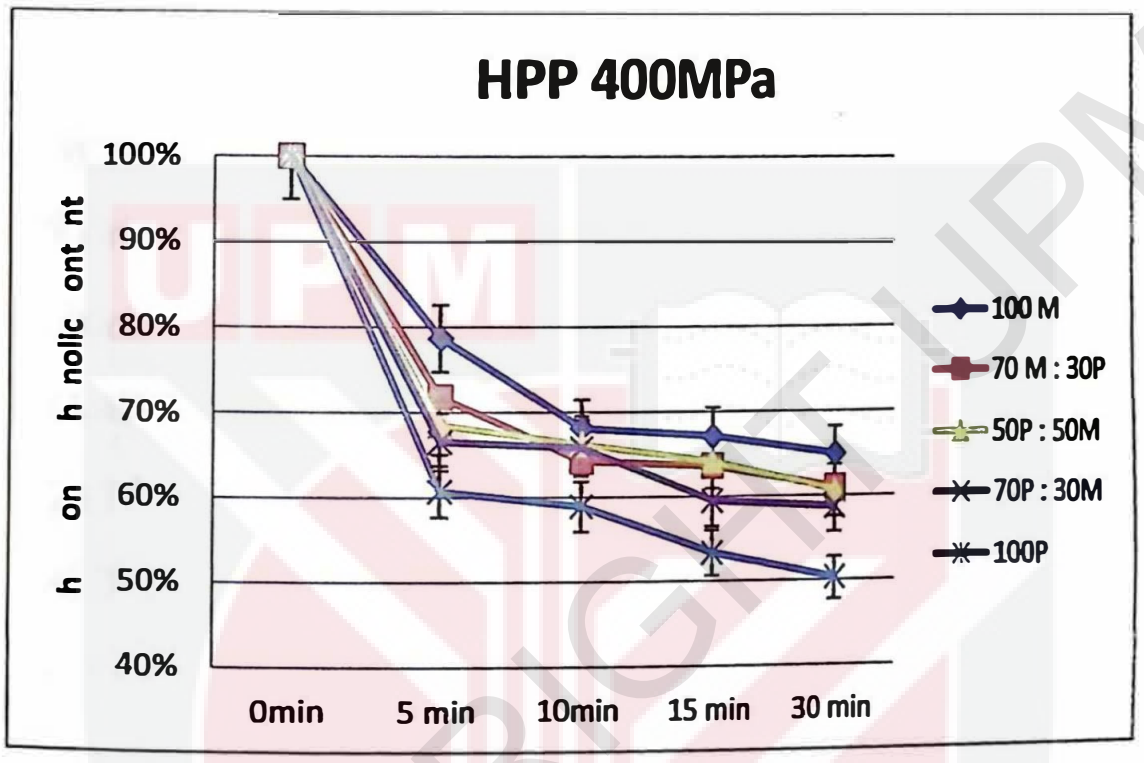

Figure 14 : Total phenolic content after HPP at 400MPa

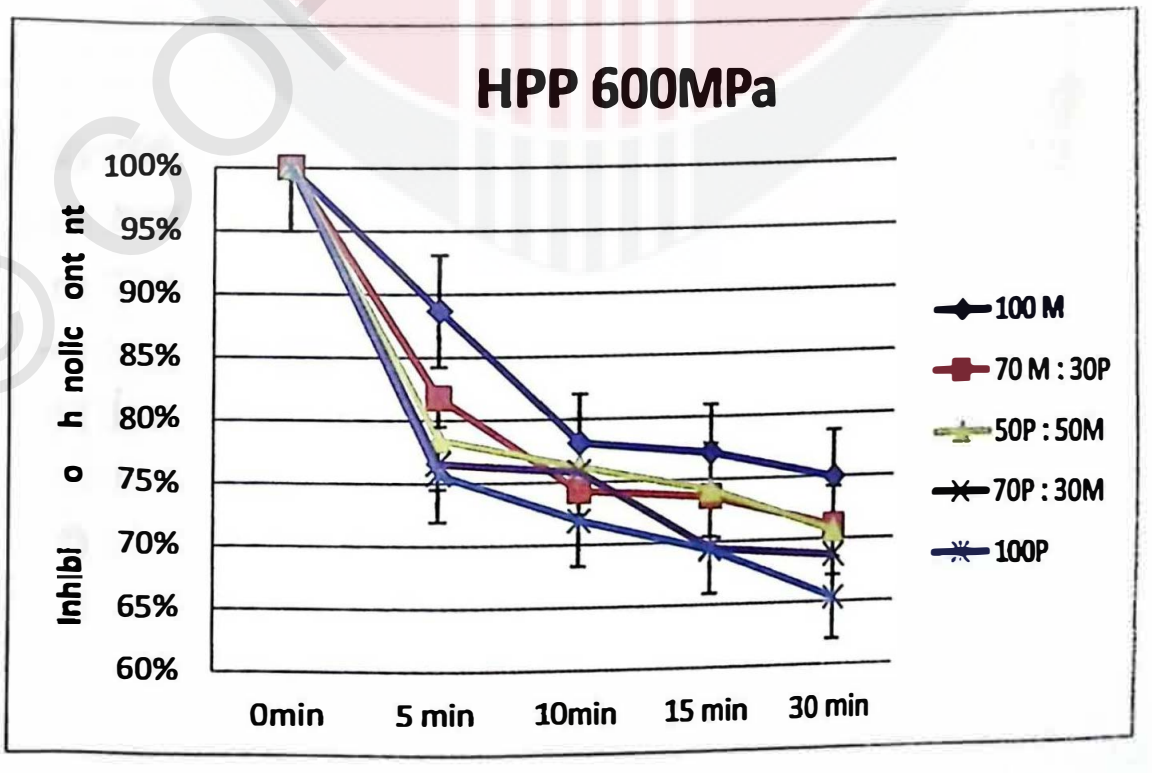

Figure 15 : Total phenolic content after HPP at 600MPa

Total phenolics of pineapple-mango juice was determined by Follin-Ciocalteu assay. The Follin-Ciocalteu method is based on the detection of phenolic compounds by reduction of reagent, which contains tungsten and molybdenum oxides (John Wilet et al., 2002). Based on the figure 17 and 18, it was found that losses of the total phenolic contents were found to be 49.68% and 34.68% at 400MPa and 600MPa, respectively after 30 min heating of 100% pineapple juice. The increase in TPC might be attributed to the higher extractability of some phenolics by the application of instantaneous pressure (Van Eylen et al., 2009; Varela-Santos et al., 2012).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

This study was aimed to employ high pressure processing to pasteurize liquid foods as an alternative to thermal treatment. Focus was given on the tropical fresh fruit juice. In general, the effects of thermal treatment and HPP on endogenous food enzymes were investigated. Residual activity of POD enzymes were calculated and it was proven that HPP treatment at pressure at 600 MPa and temperature below 60°C can reduce the activity of the enzyme also maintain the antioxidant activity and phenolic content. Hence, HPP definitely provides promising alternatives for juice treatment in order to preserve its attributes (color, flavor, and odour).While in thermal processing, temperature at 80^{o}C can reduce the enzyme activity but it causes undesirable changes in nutritional and quality of pineapple-mango juice which reduce the antioxidant activity and phenolic content. As conclusion, high pressure processing poses a potential treatment for shelf life extension and nutrition preserved in juice. This study can provide a useful data on HPP for commercial production of pineapple and mango juice.

5.2 Recommendation for Future Work

In order to increase the efficiency of HPP treatment on inactivation of endogenous enzymes, the combination of pressure and mild temperature is suggested. A shorter exposure time at high temperature can be tested in order to increase the enzyme inactivation and efficiency. For more accurate and precise data, all of the sample should come from the same plantation area. For ripeness, the sample should be

collected from the same batches, in order to avoid big differences in absorbance data which may cause variation in the final residual activity. Samples need to be stored in cool temperature (-4 °C) because there is a possibility of enzyme activity occurring when temperature is above suggested temperature. In centrifugation step, it is preferred to use a centrifuge that can run at temperature below 4 °C

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