CHAPTER V
DISCUSSIONS

In this present study, we extracted three varieties of mango seed kernel included Kaew, Mahachanok and Keaw morakot varieties and the Black queen grape seed using 95% ethanol, rice whisky (contain 40% ethanol), water and hot water to analyse for their antioxidant property focusing on their total phenolic content, reducing power and radical scavenging activity.

5.1 Total phenolic contents (TPC)

The total phenolic content was an analysis of antioxidant activity of any substance by measuring the amount of available phenolic compounds under the principle that the phenolic compounds had high antioxidant activity. Therefore the test substance contained high phenolic compounds, it will has high antioxidant activity as well. The use of Folin-Ciocalteu reagent for phenolic compounds measurement often found a linear relationship with antioxidant activity in electron transfer based assay. However, the mechanism of Folin-Ciocalteu reagent in the reaction with phenolic compounds that changed the yellow reagent to be blue green color was the electron transfer reaction of reduction mechanism (The Society for Free Radical Research-Thai, 2013). In previous studies found that the different phenolic compounds have different responses in Folin-Ciocalteu method (Kähkonen, 1999)

In this study, total phenolic contents in all mango and grape extracts were analyzed using the Folin-Ciocalteu method described Khummuang (2011) that applied from Singleton (1965). Total phenolic contents (TPC) were expressed as mg of gallic acid equivalent per gram extract weight. The results showed variation of total phenolic contents among mango seed kernels and grape seed extracted by 95% ethanol, rice whisky (contained 40% ethanol), water and hot water.

The total phenolic contents of mango seed kernel extracts from Thai varieties had been reported. The total phenolic contents from water extract of fresh mango seed kernel in Chok-a-nan variety was 399.8 mgGAE/g extract reported by Khummuang (2011) was less than the present work when compared with the highest values in 95% ethanol extract of Kaew variety. In contrast, the total phenolic contents from water extracts of all our three varieties were lower than the previous report. These differences might be due to extraction method, raw materials and the preparation of mango seed kernels extracts including effects from varieties, cultivars, ripeness, environmental factor such as soil type, sun exposure and rainfall (Manach, 2004).

Maisuthisakul (2009) reported that total phenolic content of mango seed kernel (Chokanan variety) from different extraction method which 95% ethanol was used to be an extraction solvent (shaking, refluxing and acid hydrolysis methods) was varied from 90.03 to 285.70 mg/g which expressed as tannic equivalents (TAE). Their report showed that the acid hydrolysis conditions had greater total phenolic content and antioxidant activity, however; the total phenolic contents in mango seed kernel from our work was mostly higher than those found in mango seed kernel extracts that extracted by 95% ethanol.

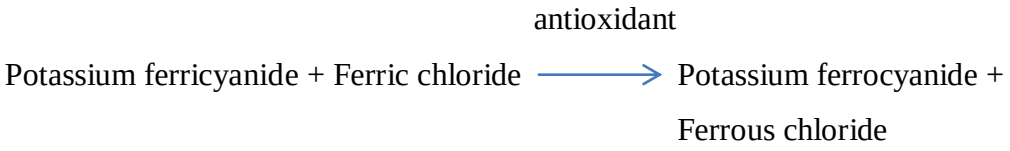
The highest total phenolic contents in rice whisky extract of Kaew variety was compared with Black queen grape seed extract in same extraction method. The phenolic content of grape seed extract was 1.6 times higher than mango seed kernel extract (683.22 mgGAE/g extract). The content of phenol compounds in Black queen grape seed extracts from this study was higher than those found in extract of White malaka grape seed extracted by 80% ethanol by Khunthacharoen (2010). Their study showed that the total phenolic compounds in grape seed extract was greater than in peel extract (67.43 and 10.43 mgGAE/g.dw respectively).

Phenolic compounds are important in plant constituents because their hydroxyl group provided the scavenging ability (Hatano, 1989 ; Jamuna, 2012). They role as antioxidant by act as reducing agent and scavenging free radicals (Dubost, 2007 ; Villano, 2007 ; Manach, 2004). These results indicated that mango seed kernels can be the source of antioxidant under the principle that phenolic compounds had high antioxidative function (The Society for Free Radical Research-Thai, 2013). In others words, mango seed kernel extracts contains phenolic compounds that have good

antioxidant potential and their effects on human nutrition and health are considerable (Hazra, 2008).

5.2 Reducing Power (RP)

The reducing power of three mango seed kernel varieties and grape seed extracts in this study were determined by according to the method of Ribeiro (2007) that base on the method of Oyaizu (1986). The reaction of extracts which had reduction potential, react the potassium ferricyanide (Fe^{3+}) and transform into the potassium ferrocyanide (Fe^{2+}) by electron transfer principle. Then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. (Manmohan, 2011)



The results show the ability of anti-oxidation of the extracts. These two forms had different absorption when quantified by absorbance measurement at 700 nm that the extracts will react Fe^{3+} to form Fe^{2+} (Inrod, 2008). Increased absorbance of the reaction mixture indicated the increased reducing power (Ajila, 2007).

The results of reducing power of the extracts were expressed as mg of ascorbic acid equivalent per gram extract weight. The analysis of reducing power is one mechanism for inhibit oxidation process, which analyse the ability of Fe^{3+} reduced to Fe^{2+} of extracts. In comparison of the reducing power of extraction solvents on 95% ethanol, rice whisky (contained 40% ethanol), water and hot water of the three mango seed kernel extract. It was found that the rice whisky extract of Kaew variety had highest reducing power than others. When compared with Black queen grape seed extract in same extraction method, the reducing power of grape seed extract was 1.4 times higher than those Kaew extract (392.95 mg AAE/g extract).

From the other recent studied of reducing power assay, it was found that the increase in extract concentration or amounts of extracts was increased the reducing power (Yildirim, 2000 ; Ribeiro, 2008). The reducing capacity of the extracts may serve as a significant indicator of its potential antioxidant activity (Hazra, 2008). However, the activities of antioxidants has been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging (Yildirim, 2000). This possible explanation from the present study is under the same condition and concentration of used extracts, the high reducing power value will show the high potential in antioxidant activity as shown in the highest reducing power of the variety of mango seed kernel in this study, Kaew variety, but still less than that found in Black queen grape seed variety. For the extraction solvent, the highest reducing power exhibited in rice whisky extracts while hot water extracts found the lowest reducing power. From the experiment, it was found that the interactions of mango seed kernels varieties and extraction solvents influence the reducing power value that means the reduction power value of mango seed kernel extracts was depended on the difference mango varieties and extraction solvents. The different antioxidant potentials from mango varieties and extraction solvents found in this study can be attributed, not only genotype or extraction solvent differences (Scalzo et al., 2005), but also agricultural practices (Wang, 2002). With the different source of mango, the differences in the antioxidant potential of mangoes may due to the different agricultural systems (Ribeiro, 2008). Also, the potential of reducing power of antioxidant components is much associated with their total phenolic contents that the extracts with higher levels of total phenolics also exhibit the greater reducing power (Sultana, 2007; Cheng, 2006; Siddhuraju, 2003).

5.3 Free radical scavenging activity

Free radical scavenging activity of all mango seed kernel and grape seed was determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay. DPPH radical is a stable radical has been used widely for determination of primary antioxidant activity (Shih, 2006). DPPH radical is an Organic nitrogen radical type with a maximum absorption at 517 nm. Then the DPPH showed the reduction activity

or received electron, the color of DPPH substance will be change from deep purple fade into yellow when measured by the spectrophotometer. These explained that the degree of discoloration of the solution indicates the scavenging efficiency of the substances added in to the solution (Xu, 2007). The ability of the extracts to scavenge DPPH free radicals was determined by the method described by Blois (1958).

In this study, the experimental results showed the relationship of mango seed kernel varieties and extraction solvents influenced the % DPPH scavenging activity which depended on the difference mango varieties and extraction solvents ($p < 0.05$). This related to the total phenolic contents and reducing power as discussed previously. The mango seed kernel extracts from the three mango varieties showed significantly different values of DPPH radical scavenging activities, ranged from 11.41% to 63.44%. The highest % DPPH scavenging activity was found in Kaew variety followed by Keawmorakot and Mahachanok respectively. In addition, the highest % DPPH scavenging activity was observed mainly in rice whisky extract of the three mango varieties following by 95% ethanol water and hot water respectively. To compare with grape seed extracts, it was found that % DPPH scavenging activity of grape seed extracts was lower than those found in mango seed kernel. In the previous study Babbar (2011) reported DPPH scavenging activity of 77% for grape seed extracted by 70% methanol that greater than our study which extracted by ethanol and water. While the study of Ribeiro (2008) at concentration 3000 - 4000 ppm of mango seed extracts (Uba variety of brazilian mango) showed the similar DPPH scavenging activity (%) compared with our Kaew variety at 100 ppm. It indicated that in low concentration of our extracts showed the great radical scavenging activity and may be used as antioxidant compound.

5.4 The possibility of applying the extraction methods that appropriate in the local industry

To study the possibility of applying the extraction methods that appropriate in the local industry. This study indicated that the rice whisky has highest possibility to be used as an appropriate extraction solvent for valuable substances extraction, in this case was antioxidant compounds. The rice whisky can easily find locally and effective as an extraction solvent. The villager can use the rice whisky to extract the useful substances in mango seed kernel and grape seed that came from agricultural wastes. Although, 95% ethanol had more effective than the rice whisky but the villager cannot acquired 95% ethanol which is the control chemicals and more expensive than rice whisky. About water and hot water as extraction solvents may not appropriate because the test results showed low antioxidant activity when compared with others. Likewise, Sultana (2009) reported that using aqueous ethanol (ethanol : water, 80:20 v/v) and aqueous methanol (methanol : water, 80:20 v/v) to extracted medicinal plant material were obtained higher phenolic contents and exhibited better antioxidant activity, as compared to the absolute ethanol and methanol. This supports the fact that phenolic compound are often extracted in higher amounts in more polar solvent such as aqueous ethanol as compare with absolute ethanol (Sultana, 2009; Anwar, 2006; Siddhuraju, 2003) which in our study used rice whisky (contain 40% ethanol) as aqueous ethanol.