

Full Length Research Paper

Physiochemical composition and antioxidant activities of underutilized *Mangifera pajang* fruit

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Underutilized plant species are defined by their unexploited economic potential, making them an appropriate focus for commercialization. The physiochemical composition and antioxidant activities of underutilized *Mangifera pajang* Kosterm fruit pulp and fruit juice powder were studied. The average kernel weight and length of *M. pajang* fruits was higher compared to *Mangifera india*. Chemical composition revealed that *M. pajang* juice powder (MPJP) was high in protein, carbohydrate, ascorbic acid, and ash whereas *M. pajang* pulp (MPP) was rich in fiber, gross energy, phenolic and β -carotene content. Additionally, MPJP extract exhibited the highest free radical scavenging activities by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. The antioxidant capacity of MPP and MPJP extracts were significantly correlated with the ascorbic acid and β -carotene but not with phenolic content. The high antioxidant activity with high ascorbic acid, proteins and carbohydrate content suggested that the MPJP can be used as a good source for preparation of health drink.

Key words: *Mangifera pajang*, antioxidant activity, physiochemical properties.

INTRODUCTION

Increased fruit and vegetable consumption has been promoted extensively because of health benefits of many non-nutrient phytochemicals associated with health maintenance and prevention of chronic diseases. As our understanding of the role of free radicals in human diseases has deepened, antioxidants have attracted broader interest because of their role in inhibiting free radical reactions and their help in protecting the human

body against damage by reactive oxygen species (Kaur and Kapoor, 2001). In Malaysia generally, fruits and vegetables with rich sources of antioxidant compounds are widely consumed along with some underutilized fruits, especially in rural communities (Ikram et al., 2009). Currently, research and development activities on underutilized fruits species have become priority areas in developing and developed countries. They are characterized by the fact that they are locally abundant but globally rare, and the scientific information and knowledge about them is limited (Gruere et al., 2009). Malaysia has a rich multiplicity of underutilized fruits and can be seen widely in the region of Peninsular Malaysia, Sabah and Sarawak. In addition, their economic potential is still unexplored. Many of these fruits are eaten locally to meet their nutritional requirements, having a broad range of flavor and color with potential health benefits (Ikram et

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Abbreviations: MPP, *Mangifera panjang* pulp; MPJP, *Mangifera panjang* juice powder; HPLC, high performance liquid chromatography; FRAP, ferric reducing antioxidant power; DPPH, 1, 1-diphenyl-2-picrylhydrazyl.

al., 2009).

Mangifera pajang Kosterm. (*M. pajang*) belongs to Anacardiaceae family, ovoid in shape, are one among the largest fruit to be known among mango species. The tree of *M. pajang* can grow up to 30 m tall and bear up to hundreds fruit and are commonly seen in East Kalimantan (Indonesia) and Borneo Island (Malaysia-Sabah, Sarawak and Brunei). The fruit pulp, which represents 50 - 67% of the total weight, is fibrous and juicy and can be eaten freshly, having a specific aromatic flavor and strong smell while peel is used for cooking curry. The fruit is commonly referred to as bambangan in Malay language and is considered highly seasonal and perishable with limited post-harvest shelf life (Aman, 1999). For this reason, large quantities of fruits are lost due to deficient post harvest handling. New economical strategies can be considered for *M. panjang* use, such as the production of *M. panjang* pulp (MPP) and *M. panjang* juice powder (MPJP) since, they can be easily stored, handled, transported and be used in formulation of diverse functional foods or as health drink.

Previously, Abu et al. (2009) have reported the antioxidant activity of bambangan fruits while Khoo and Ismail (2008) have determined their daidzein and genestein contents. However, there is no information on the physicochemical composition and antioxidant activities from MPP and MPJP. Hence, the objective of this study is to estimate the physical characteristics, chemical composition and antioxidant activities of MPP and MPJP and to compare the results with mango fruit widely described in literature.

MATERIALS AND METHODS

Fruits sampling and preparation

Fresh fruits of *M. pajang* Kosterm at their commercial ripening stage were collected from Bau, Sarawak, Malaysia. The fruits were then wrapped with papers, placed in boxes and transported via airmail to Nutrition Laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Malaysia. Upon arrival, the fruits were cleaned with tap water and left at room temperature for two hours. The fruits were manually peeled to obtain the peel, pulp and kernel fractions.

Preparation of MPP and MPJP

For the determination of physicochemical and antioxidant properties, the MPP obtained was divided into two portion. One portion was stored at -80°C and then freeze dried using a freeze dryer (35 XL, Virtis Co. Inc, NY, USA). Other portion was homogenized with distilled water (1:1, v/v). The mixture was filtered through 0.45 µm sieve, and the juice was collected and stored at -80°C and freeze dried. Later, the freeze dried samples were passed through 0.45 µm sieve to obtain their respective samples (MPP and MPJP) and stored in an airtight container and kept at -80°C until further analysis.

Sample extraction

MPP and MPJP extraction were carried out according to the method

described by Velioglu et al. (1998). Samples (200 mg) were extracted with 2 ml of 80% methanol and 1% hydrochloric acid for 2 h at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000 g for 15 min and the supernatant was collected in a 15 ml vial and used for determination of ascorbic acid, β-carotene, total phenolic content and antioxidant capacity (ferric reducing antioxidant power (FRAP) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH)) analyses.

Physical characteristics of *M. pajang* fruits

Physical characteristics of *M. pajang* fruits including average fruit, peel, pulp and kernel weight (g), fruits length (cm) and fruit perimeter (cm) were directly determined upon the arrival of the fruits at the Nutrition Laboratory 1, UPM. The fruits were weighed using a calibrated meter balance. Fruit length was measured with a vernier calliper. The determination was done in triplicate and the average mean was reported.

Chemical composition of MPP and MPJP

MPP and MPJP were analyzed in terms of their moisture, crude fat, crude protein, total ash and carbohydrate content using AOAC (1990) method. Dietary fiber analysis was carried out according to the method of Prosky et al. (1992), while gross energy was estimated according to FAO/WHO/UNU (1981) method.

Ascorbic acid and β-carotene estimation using high performance liquid chromatography (HPLC) method

Ascorbic acid estimation

The assessment of ascorbic acid content was carried out according to the method described by Thaipong et al. (2006) with some modification. Extraction of ascorbic acid was done by adding 15 ml of cold solution of 3% oxalic acid containing 8% glacial acetic acid to 5 ml of the samples and stirred for 5 min with a vortex until uniform consistency is obtained. The mixture was afterwards filtered and the total volume was adjusted to 25 ml with deionized water. The mixture was then centrifuged at 1000 g for 15 min and the supernatant was collected.

An aliquot of 2 ml of collected supernatant were filtered through 0.45 µm membrane (Milipore, USA) and 30 µl was immediately used for high performance liquid chromatography (HPLC) analysis as described by Ribeiro et al. (2007). The chromatographic conditions used were as follows: A Ultrasphere octadecylsilyl (ODS) Hypersil C₁₈ column (250 × 4.6 mm, 5 µm particle size, Thermo Scientific, Waltham, MA) was equipped with a HPLC separation module (1100 HPLC series, Agilent Technologies, USA). Meta-phosphoric acid and deionized water at pH 2.2 was used as mobile phase with a flow rate of 1.0 ml/min and detection was carried out at 238 nm.

β-carotene estimation

The extract (2.5 ml) of MPJ and MPJP were mixed with 40 ml methanol containing 1 g of potassium hydroxide and β-carotene was extracted using the method described by Tee and Lim (1991). The HPLC condition was followed according to the method described by Ribeiro et al. (2007) with slight modification. An aliquot of 2 ml of concentrated extracts were evaporated under running nitrogen, re-dissolved in 2 ml acetone, passed through a 0.45 µm Milipore membrane and 30 µl aliquots were injected into the HPLC system. The methanol, ethyl acetate and acetonitrile (70:20:10,

Table 1. Physical characteristics of *M. pajang* fruits compared to *M. indica* obtained from other studies.

Physical characteristics	Mangifera species		
	<i>M. panjang</i> ^a	<i>M. indica</i> ^A	<i>M. indica</i> ^B
Fruit weight (g)	599.44 ± 64.33	686.0	630
Pulp weight (g)	364.78 ± 44.55	544	453
Peel weight (g)	71.22 ± 29.61	75	119
Kernal weight (g)	163.44 ± 37.08	66.5	107
Fruit length (cm)	18.93 ± 2.79	16.9	-
Fruit perimeter (cm)	59.56 ± 6.48	34.7	-

*Values were the means ± standard deviations of three replicate analyses; ^A Pradeepkumar et al. (2006); ^BKansci et al. (2003).

v/v) was used as mobile phase at a flow rate of 2.0 ml/min and detection was carried out at the wavelength of 450 nm.

Total phenolics content

The concentration of total phenolics content in extracts was measured by Folin-Ciocalteu method based on a colorimetric oxidation/reduction reaction (Velioglu et al., 1998). Sample extract (0.1 ml) was mixed with 0.75 ml of Folin and Ciocalteu's phenol reagent and allowed to stand at 22°C. 0.75 ml of sodium bicarbonates (60 g/L) solution was added to the mixture after 5 min, kept in the dark for 90 min and finally its absorbance was recorded using a spectrophotometer (UV 1601, Shimadzu, Koyoto, Japan) at 725 nm. Gallic acid was used as a standard (a calibration curve was constructed with different concentration of gallic acid between 0.01-0.06 mg/ml). The total phenolics content in the sample extract was expressed as gallic acid equivalents (GAE).

Antioxidant capacity determination of MPP and MPJP

FRAP assay

The determination of reducing power of MPP and MPJP extract was adopted and modified from Benzie and Strain (1996) using 2,4,6-tripyridyl-s-triazine (TPTZ) solution. 3 ml of FRAP reagent was added to cuvette, read at 593 nm and considered as blank reading. After that, total of 0.1 ml extracts and 0.3 ml of distilled water was added into the cuvette. After addition of the sample to the FRAP reagent, a second reading was performed after 4 min at 593 nm. The change in absorbance after 4 min from the initial blank reading was then compared with standard curve. Standard of known Fe (II) concentrations were run using several concentration ranging from 0.1 to 1.0 mM. A standard curve was then prepared by plotting the FRAP value of each standard versus its concentration. The final result was expressed as the concentration of antioxidant having a ferric reducing ability in 100 g of sample (mM/100 g).

Free radical scavenging activity assay by DPPH

The effect of methanolic extract of MPP and MPJP on DPPH radical was assessed by the method described by Brand-William et al., (1998) with some modification. The extract (0.2 ml) was mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature in the dark room for 20 min. The absorbance of the resulting mixture (A_s) was measured at 517 nm. The ability of extract to scavenge DPPH radical was calculated using the equation.

Scavenging effect (%) = $[(A_{(-ve)} - A_s) / (A_{(-ve)} - A_{(+ve)})] \times 100$. Where, A_s is the absorbance of the sample; $A_{(-ve)}$ is prepared by mixing distilled water with 1 ml DPPH; and $A_{(+ve)}$ is prepared by mixing 0.2 ml of butylated hydroxyanisole (BHA) with 1 ml of DPPH.

Statistical analysis

Each determination was done three times from the same extract in order to determine their reproducibility. Data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to test the mean difference between MPP and MPJP. Correlations among data obtained were calculated using Pearson's correlation coefficient (r) and $P < 0.05$ was considered significantly different.

RESULTS AND DISCUSSION

Physical characteristics of *M. pajang* fruits

The physical characteristics of *M. pajang* fruits are tabulated in Table 1 including fruit, pulp, kernel and peel weight. The perimeter and the length of the fruits were also determined. The fruits of *M. panjang* are big, semi-oval in shape and light brown in color. The average fruit weight of *M. pajang* in this study was 599.44 ± 64.33 g which is lesser when compared to the average fruit weight of *Mangifera indica* 'Chotta Jehangir' variety (Pradeepkumar et al., 2006) by 14.3 and 5% of 'Palmer variety' (Kansci et al., 2003) but, heavier than *M. indica* 'El-Kobbaneia' variety by 48% (Zaied et al., 2007).

It is clear from Table 1 that *M. pajang* fruits have a comparable pulp (60.7%,) and peel (11.8%) fractions and can be compared to *M. indica* (Kansci et al., 2003; Pradeepkumar et al., 2006) but have a higher kernel fraction by 27.2%. The results are in agreement with previously reported studies by Augustin and Ling (1987) that kernel of *M. pajang* is twice larger than kernel of *M. indica*. The values of *M. pajang* fruits length and perimeter were also higher as compared to *M. indica*.

Chemical composition of MPP and MPJP

Results for chemical composition of MPP and MPJP are

Table 2. Chemical composition of *M. pajang* pulp (MPP), *M. pajang* juice powder (MPJP) compared to *M. indica* pulp (MIP) and *M. indica* juice powder (MIJP) obtained from other studies.

Chemical composition (%)	MPP [*]	MPJP [*]	MIP ^A	MIJP ^B
Moisture	86.84 ±0.09	10.01 ±0.16	79.06	0.8
Protein	1.13 ±0.05	3.78 ±1.17	0.98	1.3
Fat	1.98 ±0.18	1.75 ±0.07	0.32	0.1
Carbohydrate	21.02 ±0.23	76.09 ±1.62	15.56	95.8
Ash	0.43 ±0.03	3.30 ±0.05	0.50	0.7
Total fiber	5.26 ±0.56	0.80 ±0.08	3.70	1.4
Insoluble fiber	4.84 ±0.08	0.12 ±0.06	1.0 ^C	ND
Soluble fiber	0.42 ±0.03	0.68 ±0.03	1.0 ^C	ND
Gross energy (kcal/100 g)	428.68 ± 3.7	335.23 ±16.1	ND	389

*Values were the means ± standard deviations of three replicate analyses of dried samples; ^AMamiro et al. (2007); ^BPrasad et al. (2000); ^CRamalu and Rao (2003); ND, Not determined.

presented in Table 2. The protein, carbohydrate, soluble fiber and ash contents in MPJP were higher, while moisture, gross energy, total and insoluble fiber contents were higher in MPP, whereas no difference in fat content were observed. For further understanding, the chemical composition of MPP and juice powder was also compared with *M. indica* pulp and *M. indica* juice powder obtained from others studies (Prasad et al., 2000; Ramulu and Rao, 2003). The chemical composition of pineapple, grape and mango juice powders were lower in term of moisture, protein, fat and ash content compared with MPJP (Prasad et al., 2000).

Hymavathi and Khader (2005) reported moisture content of 3.85 % for *M. indica* powder whereas Prasad et al. (2000) reported 0.8 %. Hence, MPJP has 2.5 - 12.5 times higher moisture content when compared with *M. indica*. The differences observed in the moisture content may be due to different methods used, ingredients, and equipment used in the preparation of the juice powder. In our study, the juice powder was prepared without adding any ingredient, while Hymavathi and Khader (2005) used concentrated milk and wheat flour, while Prasad et al. (2000) used sucrose in the preparation of mango juice powder. The drying techniques also play a vital role to have a good end product with better moisture content. Generally, freeze-drying is adapted commercially because it can retain pigment stability during processing.

Protein and ash content of *M. pajang* juice powder was three and five times higher, respectively, than *M. indica* juice powder (Prasad et al., 2000). This indicate that, intake of MPJP will provide a good source of protein and more minerals since ash represent the total mineral content in food. Total dietary fibre for MPP was higher than *M. indica* by 29% (Peter et al., 2007) and 62% (Ramulu and Rao, 2003). MPP is four times higher with insoluble fiber content, and less in soluble fiber as compared to common mango (Ramulu and Rao, 2003). From the results observed in this study, MPJP are rich in soluble dietary fiber (0.68%) compared to insoluble fraction (0.12%) which has been associated with a number

of health benefits. Dietary fiber consumption regardless of soluble as well as insoluble fibers was reported to have positive effects in lowering the risk of cardiovascular disease, gastrointestinal disease, colon cancer and obesity (Rosamond, 2002).

Antioxidant properties of MPP and MPJP

Antioxidant constituents

The content of ascorbic acid, β -carotene, total phenolics and antioxidant activity (FRAP and DPPH) of MPP and MPJP are given in Table 3. The determination of total phenolics content was based on the production of complex molybdenum-tungsten blue, which can be detected spectrophotometrically at 725 nm (Velioglu, et al., 1998). Total phenolics content among MPJP was lower compared to MPP. Our data showed that MPP had low phenolic content (by 48 - 87%) as compared to *M. indica* reported by Ribeiro et al. (2007). As reported earlier, the total phenolics contents for *M. pajang* are more pronounce in peel and kernel fraction (Abu et al., 2009).

Ascorbic acid content for MPJP was three times higher when compared to MPP (Table 3) and *M. indica* pulp (18 - 65 mg/100 g) and *M. indica* pulp powder (63.25 mg/100 g) (Hymavathi and Khader, 2005; Peter et al., 2007; Ribeiro et al., 2007). Ascorbic acid content in the present study was similar to the previously reported for *M. indica* pulp by FAO (1981). Many factors may have an influence on the ascorbic acid content in fruits including cultivar and tissues, climatic condition, maturity stage and post-harvest factor. Ascorbic acid is an important and essential diet component for human health and functions as anti-oxidant and therefore provides some protection against oxidative stress-related diseases such as cardiovascular disease, and in respiratory infection (Khaw and Woodhouse, 1995).

The β -carotene content of MPP and MPJP was relatively high when compared to studies in *M. indica*. Ribeiro et al.

Table 3. Ascorbic acid, β -carotene, total phenolics and antioxidant activity (FRAP and DPPH methods) of *M. pajang* pulp (MPP) and *M. pajang* juice powder (MPJP).

Antioxidant parameters	MPP*	MPJP*
Ascorbic acid (mg/100 g)	46.31 \pm 5.84	132.14 \pm 3.99
β -carotene (mg/100 g)	42.21 \pm 1.80	35.59 \pm 9.87
Total phenolics (mg GAE/100 g)	26.09 \pm 3.20	19.30 \pm 5.84
FRAP assay (mM/100 g)	26.50 \pm 3.81	39.58 \pm 2.73
DPPH radical scavenging activity (%)	43.25 \pm 1.95	52.61 \pm 1.3

*Values were the means \pm standard deviations of three replicate analyses of dried samples.

(2007) reported that β -carotene in *M. indica* pulp were between 0.5 - 25 mg/100 g which is lower than MPP (42.21 mg/100 g). On the contrary, report from Peter et al. (2007) indicated high amount of β -carotene of *M. indica* pulp. The β -carotene content for MPJP was ten times higher as compared to *M. indica* pulp powder (Hymavathi and Khader, 2005). The MPP showed β -carotene content lower than MPJP by 17% which might be due to the fast degradation during processing stage. Previous report showed β -carotene are relatively unstable and often undergo degradation reactions during processing and storage (Hymavathi and Khader, 2005). Among all the carotenoids types, β -carotene provides the highest vitamin A activity (Ribeiro et al., 2007) which contributes to protection against free radicals related diseases. Studies showed that β -carotene is a very potent antioxidant in inhibiting the progression of atherosclerosis and cancer (Krinsky and Johnson, 2005).

Antioxidant capacity

Two types of assays were selected to assess the antioxidant capacity of MPP and MPJP namely FRAP and scavenging activity on DPPH. FRAP assay measures the reduction of a ferrion analog, the Fe^{3+} complex of tripyridyltriazine $\text{Fe}(\text{TPTZ})^{3+}$ to the intensely blue coloured Fe^{2+} complex $\text{Fe}(\text{TPTZ})^{2+}$ by antioxidants in acidic medium.

The MPJP extract exhibited higher reducing capacity as compared to MPP (Table 3). Our data give a higher FRAP value for MPP as compared to that previously reported by Abu et al. (2009) by 43%. The reducing ability of the MPP and MPJP extracts were strongly correlated with ascorbic acid ($r = 0.99$, $p < 0.01$) and β -carotene (0.98 , $p < 0.01$) but not with total phenolics content. The result contradicts with the previously reported that total phenolics content of *M. pajang* fresh pulp strongly correlated with FRAP values (Abu et al., 2009); the difference might be due to the variability in the total phenolics content. The differences in term of nutritional composition like phenolics content can be contributed by the location where the fruits were sampled. The present study *M. pajang* fruits were sampled from Sarawak, Malaysia whereas the previous work (Abu et al., 2009) was done using *M. pajang* fruits from Sabah, Malaysia.

Other mechanism to assessed antioxidant activity is to determine the scavenging effect on proton radicals through the DPPH assay system. The present data showed the MPJP had a significantly higher percentage of scavenging activity as compared to MPP by 17%. Abu et al. (2009) reported a similar finding in their study. The antioxidants constituent, which might be responsible for the scavenging activity in the present study, are the ascorbic acid and β -carotene. Pearson correlation analysis revealed that the DPPH values for the studied MPP and MPJP extracts were strongly correlated with ascorbic acid ($r = 0.97$, $p < 0.01$) and moderately with β -carotene content ($r = 0.83$, $p < 0.01$). However, no correlation was observed between total phenolics content and the scavenging activity of the extracts. Shivashankara et al. (2004) and Ribeiro et al. (2008) also reported a strong positive correlation between antioxidant activity of mango extract with ascorbic acid.

Conclusion

In conclusion, our data suggest that both MPP and MPJP are rich sources of carbohydrates, proteins and fibers with high antioxidant activity contributed by ascorbic acid and β -carotene. The MPP and MPJP evaluated in this study are from edible material and are already being used by rural communities for food purposes and have potential for development into sources of various marketable foodstuffs. Hence, the use of MPP and MPJP may not only be attractive, modification of these into value added products as demonstrated in this study, is likely to be economically attractive, especially to small and medium scale industries in the regions where these fruits are found. Further investigations on health promoting aspects of MPJP in animal and human models are in progress.

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