Bioactive compounds and volatile compounds of Thai bael fruit (*Aegle marmelos* (L.) Correa) as a valuable source for functional food ingredients

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Abstract: Bael fruit (*Aegle marmelos* (L.) Correa) is an attractive and characteristically sweet aroma fruit, native to Southeast Asia, known to be a good source of natural antioxidants and bioactive compounds. In this study, the results showed that fully ripe Thai bael fruit pulps had total, soluble, and insoluble dietary fiber contents of 19.84, 11.22, and 8.62 g/ 100 g dry weight (dw), respectively. Determination of antioxidant activities by 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays resulted in 6.21 μg dw/ μg DPPH and 102.74 μM trolox equivalent (TE)/ g dw, respectively. It was also found to have total phenolic, total flavonoid, total carotenoid, and ascorbic acid contents of 87.34 mg gallic acid equivalent (GAE)/ g dw, 15.20 mg catechin equivalent (CE)/ g dw, 32.98 μg/ g dw, and 26.17 mg/ 100 g dw, respectively. Volatile compounds in bael fruit pulp were analyzed using the solid-phase microextraction (SPME)/ gas chromatography (GC)/ mass spectrometry (MS) method. A total of 28 volatile compounds were identified, and the dominant components were monoterpenes and sesquiterpenes. Among these components, limonene was the major constituent producing the characteristic bael fruit flavor.

Keywords: Bael fruit, bioactive compounds, antioxidant activities, volatile compounds, functional food

INTRODUCTION

Bael fruit (*Aegle marmelos* (L.) Correa) is a tropical fruit native to Southeast Asia and belongs to the Rutaceae family. It is grown throughout India as well as in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand, and most of the Southeast Asian countries (Singh and Roy, 1984). In Thailand, it is commonly found growing in many regions, especially the lower north and central part consisting of Phichit, Prachin Buri, and Phitsanulok provinces. There are no standard names for bael fruit cultivars. The general cultivars known in Thailand are locally "Matoom Kai" (in Thai language "Matoom" means bael fruit and "Kai" refers to cultivar). It is usually used for household consumption and traditional medicine, but some are planted for trade in particular regions (Subhadrabandhu, 2001).

The peel of the fruit which is a very hard shell and green to brown in color depends on ripening stage. The appearance of yellow or orange edible pulp is like a boiled pumpkin, possesses a slightly sweet taste and a characteristic floral, terpene-like aroma, very fragrant and pleasantly flavored. Seeds are surrounded by slimy transparent mucilage. The bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants which may protect us against chronic diseases. Total dietary fiber found in this fruit can be divided into insoluble dietary and soluble dietary fiber (mucilage and pectin). In addition, it also contains many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, calcium, and phosphorus (Dikshit and Dutt, 1930; Parmar and Kaushal, 1982; Roy and Khurdiya, 1995). Therefore, bael fruit may indicate that it is one of the important plants used for indigenous traditional medicine. There are innumerable references of its uses in traditional medicine (Arseculeratne *et al.*, 1981; Karunanayake *et al.*, 1984; Singh, 1986; Nagaraju and Rao, 1990).

The uses of bael fruit in aspects of food have many forms in each country. For example, the ripe fruit is consumed fresh and also prepared as nectar, squash, sherbet, jam, marmalade, and cream in India (Morton, 1987). However, in Thailand these fruits are usually cut into pieces and dried, packed in bags or pulverized and packed as tea bags, and preserved in syrup as the bael fruit glacé which is normally used as dessert or an ingredient for cakes.

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Nowadays, the world market for functional foods and nutraceuticals is large and growing. According to the literature, the characteristic of Thai bael fruit in terms of bioactive compounds and characteristic flavor was considered to have a potential for use as functional food and value added processed products. There is considerable amount of literature that examines the bioactive compounds and volatile compounds in fruits such as guava, mango, and berries. However, there have been no data on bioactive and volatile compounds of Thai bael fruit. Therefore, the purpose of this study was to determine the bioactive compounds and volatile compounds in Thai bael fruit (Matoom Kai), a cultivar usually found and consumed in Thailand, and also to investigate the possibilities of utilizing Thai bael fruit as a valuable functional food ingredient or a resource for nutraceutical products in the future. Bael fruit is from a perennial plant which is present in Thai way of life from ancient times, but today this plant has some limits in its use and the risk of extinction. The results of this study can add to the scientific literature and useful application from bael fruit for producers and consumers to be aware of the importance and utilization of this useful resource.

MATERIALS AND METHODS

Plant materials

Fresh Thai bael fruits *Aegle marmelos* (L.) Correa (Matoom Kai) were obtained from a bael fruit garden in Phichit, Thailand. Fully ripe bael fruits (Figure 1) were chosen for this study because this is what usually consumed fresh and used as a main ingredient in many food products. The pulps were removed from the fruits, and were analyzed for physicochemical properties, bioactive compounds, and volatile compounds. All chemicals and solvents used in this experiment were of analytical grade, purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA) and Sigma Aldrich Co., Ltd (Steinheim, Germany). All analyses was performed in triplicate.

Determination of physicochemical properties

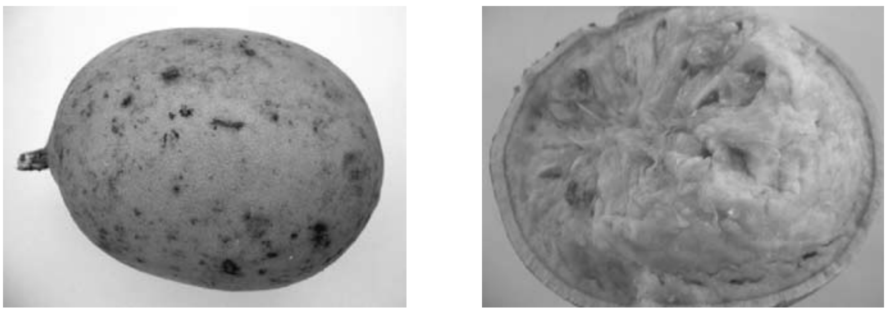
The pulps of bael fruit were analyzed for total soluble solids by using a hand-held refractometer (Atago 32-62°Brix, Japan), pH was measured using a digital pH meter (Eutech, Cyber Scan pH 1000 Bench, Singapore), reducing sugars were determined as glucose by using Nelson-Somogyi method (Nelson, 1944), total acidity and moisture content were analyzed according to standard AOAC methods (AOAC, 1995). The color of fresh bael fruit pulps was measured using a chromameter (Minolta, CR400) with reference to illuminant D_{65}, expressed with the value of Hunter. The L* value is a measurement of lightness and varies from 0 (black) to 100 (white); the a* value varies from –a* (green) to +a* (red); the b* value varies from –b* (blue) to +b* (yellow); C* (chroma or color saturation); and °h (hue angle).

Determination of dietary fiber

Total, soluble, and insoluble dietary fiber (TDF, SDF, and IDF) were analyzed according to standard AOAC methods (AOAC, 1995).

Antioxidant activity determination

Samples were prepared by the method modified from Velioglu *et al.* (1998). Fresh bael fruit pulps were ground and extracted with 95% ethanol in the dark at 25°C for 4.5 h. The free radical-scavenging activity of sample extracts was evaluated using the DPPH assay, according to the methods of Masuda *et al.* (1999) and Maisuthisakul *et al.* (2007). The percentage of DPPH radical-scavenging activity was calculated and plotted against the sample extract concentration (µg/ ml) to determine the amount of extract necessary to decrease DPPH radical



(a) (b)

Figure 1: Physical characteristics of "Matoom Kai": (a) outer hard shell skin and (b) internal pulp

concentration by 50% (called EC_{50}). The unit of EC_{50} was later converted to µg dry weight (dw)/ µg DPPH. FRAP assay was done according to Benzie and Strain (1996) with some modifications. Briefly, the fruit extracts (50 µl) were allowed to react with 950 µl of the FRAP solution for 4 min in dark conditions. The standard curve was linear between 82 and 625 µM trolox. Results were expressed in µM trolox equivalent (TE)/ g dw.

Determination of total phenolic and total flavonoid content

Samples were prepared in the same way as for the antioxidant activity determination. Total phenolic content was determined by the Folin-Ciocalteu assay (Waterhouse, 2005). Gallic acid (50-500 mg/l) was used for calibration of the standard curve. The results were expressed as mg gallic acid equivalent (GAE)/ g dw.

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al.*, 1999). Measurements were calibrated to a standard curve of prepared catechin solution (20-100 mg/l). The results were expressed as mg catechin equivalent (CE)/ g dw.

Analysis of total carotenoids and ascorbic acid

Total carotenoids were determined according to Gross (1991); Talcott and Howard (1999). The absorbance at 470 nm of extracts in acetone/ ethanol (1:1) with 200 mg/L butylated hydroxytoluene (BHT) was measured. The results were expressed in µg carotenoid/ g dw.

Ascorbic acid was determined based on the quantitative discoloration of 2,6-dichlorophenolindophenol (DCPIP), according to Pearson's (1976) method. The samples were extracted in 0.4% oxalic acid solution, and compared with standard ascorbic acid.

Volatile compounds analysis (modified from Chen et al., 2006)

Volatile compounds were isolated by using solid-phase microextraction (SPME) method. The volatiles were sampled by manual headspace solid phase microextraction at 60°C. The fibre (100 µm PDMS, Supelco) was pierced into the injection port of the GC/MS after 20 min of sampling, and then desorbed at 200°C for 5 min. Gas chromatography/ mass spectrometry condition: An Agilent 6890 GC equipped with an Agilent 5973 mass-selective detector (Agilent Technologies) was used, with the injector and detector maintained at 200 and 260°C, respectively. The column (HP-Innowax) dimensions were 0.25 mm i.d. × 30 m × 0.25 µm film thickness. The carrier gas (He) had a flow rate of 5.7 ml/ min. The temperature program was isothermal at 50°C for 10 min, increase to 240°C at 15°C/ min, then held for 10 min. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV; ion source and quadrupole maintained at 230 and 150°C, respectively; mass range m/z 10-350. Compounds were identified by matching mass spectra (quality match >80%) and retention indices with the Chemstation Wiley Spectral Library of standard compounds, as well as from NIST, Mass Finder, and the literature (Tressl *et al.*, 1982; Davies, 1990; Chung *et al.*, 1993; Píry *et al.*, 1995; Kocsis *et al.*, 2002; Choi, 2003; Högnadóttir and Russell, 2003; Mau *et al.*, 2003; Culleré *et al.*, 2004; Szafranek *et al.*, 2005).

RESULTS AND DISCUSSION

Physicochemical properties

Bael fruit pulps were analyzed for total soluble solids, pH, reducing sugar, total acidity, moisture content, and internal color. The results are presented in Table 1. Total soluble solids of the pulps were 39.50˚Brix, about twice as high as in most other fruits. The bael fruit pulp had pH, reducing sugar, total acidity, and moisture content of 5.37, 39.60 mg glucose/g fresh weight (fw), 0.94%, and 67.74%, respectively. For the physicochemical properties between Thai and Indian bael fruits for different cultivars, the results showed that pH, reducing sugar, and moisture content of Thai bael fruits were similar to Indian bael fruit. However, total soluble solids and total acidity of Thai bael fruit were higher than Indian bael fruit. The color of fruit in this study was orange and yellow corresponding to L*a*b* measurement, and similar to the internal color of Indian bael fruit pulp (various shades of yellow to orange and yellow) reported by Roy and Khurdiya (1995).

Bioactive compounds

Bioactive compounds found in bael fruit pulp are presented in Table 2. The results showed that bael fruit pulps had TDF, SDF, and IDF contents of 19.84, 11.22, and 8.62 g/ 100 g dw, respectively. The results indicate that the fruits are relatively rich in dietary fiber because they were in range of fruits which are defined as high dietary fiber fruits such as watermelon, orange, banana, mango, papaya, pineapple, and apple (TDF 9-24, SDF 4-8, and IDF 6-17 g/ 100 g dw) reported by Ramulu and Rao (2003).

Antioxidant activities were measured in ethanol extract obtained using DPPH and FRAP assays. The DPPH assay was selected to evaluate the

Table 1: Physicochemical properties of "Matoom Kai"

Physicochemical characteristics	Mean±SD
Total soluble solids (°Brix)	39.50±0.87
pH	5.37±0.03
Reducing sugar (mg glucose/ g fw[a])	39.60±0.89
Total acidity (%)	0.94±0.04
Moisture (%)	67.74±0.25
Internal color	
L*	40.11±0.27
a*	7.82±0.20
b*	35.84±0.24
C*	36.68±0.23
°h	77.70±0.33

[a] fw = fresh weight basis, All values were performed in triplicate.

Table 2: Bioactive compounds of "Matoom Kai"

Bioactive compounds	Mean±SD
Total dietary fiber (TDF) (g/ 100 g dw[a])	19.84±0.01
Soluble dietary fiber (SDF)	11.22±0.06
Insoluble dietary fiber (IDF)	8.62±0.04
Antioxidant activities	
DPPH assay (EC_{50}, µg dw/ µg DPPH)	6.21±0.34
FRAP assay (µM TE[b]/ g dw)	102.74±3.01
Total phenolics (mg GAE[c]/ g dw)	87.34±4.44
Total flavonoids (mg CE[d]/ g dw)	15.20±0.51
Total carotenoids (µg/ g dw)	32.98±0.51
Ascorbic acid (mg/ 100 g dw)	26.17±0.85

[a] dw = dry weight basis, [b] TE = trolox equivalent
[c] GAE = gallic acid equivalent, [d] CE = catechin equivalent
All values were performed in triplicate.

antioxidant activities of bael fruit extracts in terms of EC_{50}, the antioxidant activities obtained from this assay (6.21 µg dw/ µg DPPH) were comparable to that of fruits, vegetables, herbs, and chewing plants such as *Diospyros kaki* L., *Garcinia mangostana* L., *Spondias pinnata* Kurz, *Leucaena glauca* Benth, and *Piper betel* Linn. (0.3-7 µg dw/ µg DPPH) which contain high antioxidant activity reported by Maisuthisakul *et al.* (2007). The antioxidant activities as determined by FRAP assay of bael fruit (102.74 µM TE/g dw) were comparable to herbs and vegetables such as roselle, Asian pennywort, Thai basil, beet, and sweet potato (86-150 µM TE/g dw) reported by Ou *et al.* (2002) and Wong *et al.* (2006). Therefore, bael fruit is another fruit that has high antioxidant activities.

It can be indicated that the major antioxidants in bael fruit are phenolics, flavonoids, carotenoids, and vitamin C (Morton, 1987; Roy and Khurdiya, 1995). The results showed that bael fruit pulps had total phenolic content of 87.34 mg GAE/ g dw. Total phenolics in bael fruits were in the range of traditional Chinese medicinal plants associated with anticancer (2.2-503 mg GAE/g dw), as well as higher than for common fruits and vegetables including kiwifruit, orange, pear, garlic, carrot, and spinach (1.2-10.8 mg GAE/ g dw) reported by Cai *et al.* (2004). The results for total flavonoid content showed that bael fruit pulps had relatively high total flavonoids (15.20 mg CE/ g dw) when compared with medicinal plants such as *Alcea kurdica*, *Stachys lavandulifolium*, *Valeriana officinalis*, *Lavandula* *officinalis*, and *Melissa officinalis* (0.22-10 mg CE/ g dw) reported by Bouayed *et al.* (2007). In addition, total flavonoids in bael fruit were also higher than for fruits and vegetables including apple, raspberry, peach, broccoli, celery, and tomato (2.5-190.3 mg CE/ 100 g fw) reported by Marinova *et al.* (2005). In the case of total carotenoids, bael fruit pulps had total carotenoid content of 32.98 µg/ g dw which is in the range for fruits and vegetables such as papaya, mango, pumpkin, ginger, and cabbage (12-70 µg carotenoid/ g dw) reported by Inocent *et al.* (2007) and Kandlakunta *et al.* (2008). The ascorbic acid content of bael fruit pulps (26.17 mg/ 100 g dw) were also in the range of some varieties of herb and vegetable leaves (*Leucaena glauca* Benth and *Ocimum basilicum* Linn.) as well as berry and fruit seeds (*Nephelium lappaceum* Linn., *Parkia speciosa* Hassk., and *Tamarindus indica* Linn) (19.3-39.8 mg/ 100 g dw) reported by Maisuthisakul *et al.* (2008).

Volatile compounds

Twenty-eight compounds were characterized by SPME/ GC/MS (Figure 2 and Table 3). Among these components, monoterpenes and sesquiterpenes such as Limonene, p-Cymene, β-Phellandrene, and Dihydro-β-Ionone were recognized as the main components. These compounds are important contributors to fruit aroma showed in Table 4. In this study, the most important volatile compound in bael fruit pulp seems to be limonene because this compound had the highest percentage of peak area in chromatogram.

When the volatile compounds of Thai bael fruit and Sri Lankan bael fruit reported by MacLeod and Pieris (1981) are compared, the results showed that monoterpenes and sesquiterpenes seem to be the main volatile constituents of both Thai and Sri Lankan bael fruit. Among these components, limonene was one of the major constituents that produces the characteristic bael fruit flavor. Tokitomo *et al.* (1982) reported that Limonene, Δ^3-Carene, p-Cymene, Linalool oxide, Linalool, Benzyl acetate, Benzyl alcohol, 2-Phenyleyhanol, and β-Ionone are important constituents to produce the basic or fundamental flavor quality of bael fruit from Sri Lanka, and 3,7-dimethyl-1,5,7-octatrien-3-ol and its isomer are responsible for making the aroma of this fruit more attractive. It is interesting that the major components of bael fruit flavor were monoterpenes and sesquiterpenes, which is similar to citrus fruits of the Rutaceae family reported by Sawamura *et al.* (1990), Sawamura *et al.* (1991) and Choi (2003), especially limonene. Limonene is one of the bioactive compounds that provide health benefit in terms of anticancer (Bidlack, 2000; Shi *et al.*, 2002). Although the appearance of the fruit is like wood apple or quince, the aroma components are quite different from those of wood apple and quince whose aroma consists mainly of various esters (Schreyen *et al.*, 1979; MacLeod and Pieris, 1981).

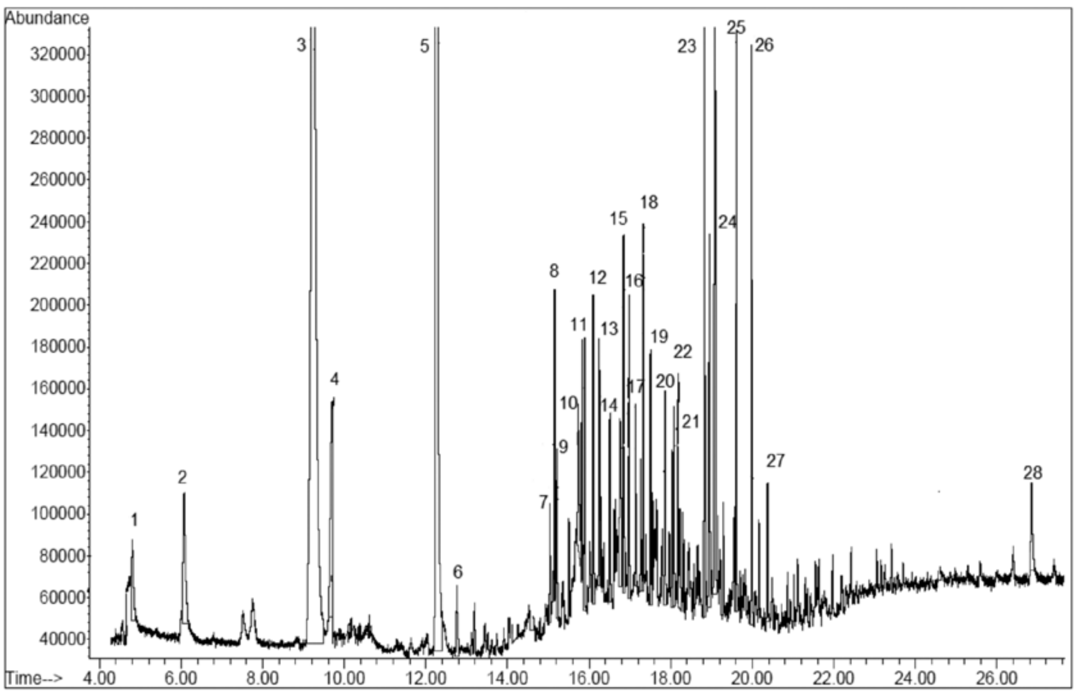


Figure 2: Chromatogram of volatile compounds identified from SPME/GC/MS

Table 3: Volatile compounds identified from SPME/GC/MS

Peak No.	RI[a]	Compound	% Area
1	1093	Hexanal	0.93
2	1147	Isoamyl acetate	2.13
3	1202	Limonene	32.48
4	1217	β-Phellandrene	3.13
5	1279	p-Cymene	27.19
6	1295	Acetoin	0.70
7	1379	(E)-2-Octenal	0.60
8	1401	(E,E)-2,4-Heptadienal	1.08
9	1414	Dehydro-p-cymene	1.47
10	1425	Linalool oxide	0.88
11	1432	3,5-Octadiene-2-one	1.44
12	1463	α-Cubebene	1.75
13	1470	trans-p-Mentha-2,8-dienol	0.71
14	1488	Citronellal	1.42
15	1558	β-Cubebene	1.18
16	1594	β-Caryophyllene	1.88
17	1600	Hexadecane	1.71
18	1665	Pulegone	1.71
19	1680	α-Humulene	1.08
20	1729	Verbenone	1.25
21	1751	Carvone	0.98
22	1759	Carvyl acetate	0.82
23	1825	Dihydro-β-Ionone	3.95
24	1840	(E)-6,10-dimethyl-5,9-Undecadien-2-one	2.16
25	1947	β-Ionone	2.95
26	1999	Caryophyllene oxide	2.57
27	2015	Humulene oxide	0.78
28	2860	Hexadecanoic acid	0.92

[a] RI = Kovat's retention indices

Table 4: Examples of dominant volatile compounds of "Matoom Kai", their chemical groups and other sources

Chemical groups	Volatile compounds	Sources
monoterpene	Limonene	orange, mango, grape, lemon,
	β-Phellandrene	blackcurrant
	p-Cymene	mango, citrus, lime oil
	Linalool oxide	orange, berry
	Pulegone	blackberry
	β-Ionone	raspberry, mango
	Dihydro-β-Ionone	raspberry
sesquiterpene	β-Cubebene	citrus
	β-Caryophyllene	lemon, guava, mango, grape, blackcurrant

References: MacLeod and Snyder (1985), Maarse (1991), Sawamura *et al.* (1991), Píry *et al.* (1995), Pino *et al.* (2005), Wenguang *et al.* (2007), Harb *et al.* (2008)

CONCLUSION

The physicochemical properties of Thai bael fruit are similar to Indian bael fruit. Bioactive compounds of bael fruit in this study contain relatively high content of dietary fiber, ascorbic acid, total phenolics, total flavonoids, total carotenoids, and also strong antioxidant activities. The volatile compounds of Thai bael fruit were comparable to bael fruit from Sri Lanka. The main components were monoterpenes and sesquiterpenes. Among these components, limonene was the major constituent producing the characteristic bael fruit flavor. This compound was also found in citrus fruits. This study indicates that Thai bael fruit may impart health benefits when it is used in functional food products and should also be regarded as a potential nutraceutical resource in the future. In addition, it can be used as food additive because of its typical color, flavor, and texture. These results are useful for developing and improving the quality of bael fruit cultivar in order to provide more value addition and usefulness from bael fruit.

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