

Two exotic fruits positively affect rat's plasma composition

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Abstract

The aim of this investigation was to determine the bioactive compounds and the antioxidant potentials of Snake fruit and Mangosteen and their influence on plasma lipid levels and antioxidant activity in rats fed cholesterol-containing diets. It was found that total polyphenols and antioxidant potentials of Snake fruit were significantly higher than those of Mangosteen ($P < 0.05$). A cholesterol-containing diet supplemented with the studied exotic fruits showed a positive affect on rat's plasma lipid levels and on the antioxidant activity during 30 days of feeding. In rat's plasma of the Chol/Snake diet group, the fibrinogen fraction showed a decrease in the amounts and compositions of electrophoretic protein bands in the range of 110 and 14 kDa. However, all the positive results of this experiment on animals could not be automatically applied to humans.

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1. Introduction

Tropical and subtropical fruits, such as red and white guava, green and ripe mango, banana, passion fruit, star fruit, rose apple, papaya, lime, passiflora, kumquat, pineapple, carambola, feijoa, kiwano, cherimoya, sapodilla, mamey, lychee and longan, are common ingredients of diets in North America and nowadays in Europe as well (Doyama, Rodrigues, Novelli, Cereda, & Vilegas, 2005; Dube et al., 2004; Kondo, Kittikorn, & Kanlayanarat,

2005; Luximon-Ramma, Bahorun, & Crozier, 2003; Ma et al., 2003; Mahattanatawee, Manthey, Talcott, Goodner, & Baldwin, 2005; Murcia, Jimenez, & Martinez-Tome, 2001; Nilsson et al., 2005; Talcott, Percival, Pittet-Moore, & Celoria, 2003; Wu et al., 2006; Yuka, Yumiko, Miyo, & Takashi, 2003).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities and polyphenol contents of some tropical dried fruits were evaluated and compared with fresh fruits. The qualities of persimmon, hawthorn and apricot were close to those of the dry fruits and they showed high DPPH radical-scavenging activity (Ishiwata, Yamaguchi, Takamura, & Matoba, 2004; Park et al., 2006).

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Others, namely Snake fruit (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*), are less known (Bunsiri, Ketsa, & Paull, 2003; Garcia, Magpantay, & Escobin, 2005; Leontowicz et al., 2006; Ramage, Sando, Peace, Carroll, & Drew, 2004; Wijaya, Ulrich, Lestari, Schippel, & Ebert, 2005). The published papers mostly describe the chemical composition of Mangosteen (Cao, Guo, & Cai, 2006; Garcia et al., 2005;) and the phylogenetic relationships among *Garcinia* species (Yapwattanaphun, Subhadrabandhu, Honscho, & Yonemori, 2004).

Mangosteen has been used for many years as a Thai indigenous medicine. Investigations suggested that a methanolic extract from the pericarp of *Garcinia mangostana* showed strong antiproliferation, potent antioxidation and induction of apoptosis. Thus, this substance can show different activities and has potential for cancer chemoprevention which are dose-dependent as well as exposure time-dependent (Moongkarndi et al., 2004). Polysaccharides from the pericarp of Mangosteen can stimulate phagocytic cells and kill intracellular bacteria (Chanarat, Chanarat, Fujihara, & Nagumo, 1997). In spite of the reviewed literature, a lack of knowledge of these two fruits is evident. The occurrence of different species of Mangosteen and their genomic composition are still unknown (Yapwattanaphun et al., 2004). For Snake fruit, even the composition is not well studied (Shui & Leong, 2005; Wijaya et al., 2005).

Therefore, in the present study, an experiment on laboratory animals is aimed to find whether these fruits, which possess good taste, have the necessary bioactive values to be recommended for disease-preventing diets. For this purpose the basal diets of cholesterol-fed rats are supplemented with the studied exotic fruits, and the influence of such diets on plasma lipids levels and antioxidant activity are evaluated. The results of this investigation should clarify whether these fruits could be a valuable supplement for disease-preventing diets. In order to achieve reliable data, it was decided to use a combination of two independent radical-scavenging antioxidant tests: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) and 2,4,6-tripyridyl-*s*-triazine (TPTZ).

As far as we know there are no published results of such investigations.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), FeCl₃ × 6H₂O, Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]), butylated hydroxyanisole (BHA), non-oxidized cholesterol and sodium dodecyl sulphate (SDS) were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water were used throughout.

2.2. Samples and preparation

Snake fruit (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*) of the 2005 harvest were purchased at the fruit market, Thailand, by one of the investigators. The fruit samples were lyophilized and then treated with solvents of different lipophilicity to obtain fractions for testing the antioxidant activity. To 10–100 mg of fruit powder, either 1 ml of water, methanol/water (70:30 v/v), or ethanol was added. After 24 h of incubation under agitation at 4 °C in the dark, all suspensions were centrifuged at 2570g for 10 min and the supernatants collected. The pellets were washed with 0.5 ml of solvent, left for 2 h at 4 °C in the dark and centrifuged. Supernatants from the same solvent were combined. The extracts were designated as fraction 1 (water), fraction 2 (methanol/water (70:30 v/v) and fraction 3 (ethanol). The clear supernatants and the solutions of the pure compounds were stored at –80 °C until used. A highly lipophilic fraction (fraction 4) was prepared from 1 g of fruit powder in 50 ml acetone/water (75:25 v/v) (Betancor-Fernández, Pérez-Gálve, Sies, & Stahl, 2003; Leontowicz et al., 2006).

2.3. Determination of total polyphenols

Total polyphenols [mg gallic acid equivalent (GAE) g⁻¹ dry weight (DW)] were determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) and measured at 765 nm.

2.4. Determination of antioxidant potential by 2,4,6-tripyridyl-*s*-triazine (TPTZ)

Ferric-reducing/antioxidant power (FRAP) potential (μmol Fe²⁺ g⁻¹ DW) measures the ability of the antioxidants contained in the fruit samples to reduce ferric-tripyridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺) which absorbs light at 593 nm. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against concentration of Fe²⁺ standard solution or trolox (Benzie & Strain, 1996; Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). This method was applied for determination of antioxidant potential of fruit samples and plasma antioxidant activity.

2.5. Determination of total antioxidant potential by radical-scavenging activity using 1,1-diphenyl-2-picrylhydrazyl radical method (DPPH[•])

The volume of fruit extracts in different test tubes was adjusted to 100 μl by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μl) to these tubes and shaken vigorously and stood at 27 °C for 20 min. The control was prepared as above without any fruit extract. MeOH was used for the baseline correction. Radical-scavenging activity (% inhibition) was expressed as the inhibition per-

centage and was calculated using the following formula: % radical-scavenging activity = (control OD – sample OD/control OD) × 100, where OD is optical density. Changes in the absorbance of the samples were measured at 517 nm. BHA was used for comparison (Singh, Chidambara, & Jayaprakasha, 2002).

2.6. Determination and separation of proteins

Plasma samples were dissolved in sample buffer: 2% SDS, 10% glycerol, 2% mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris–HCl, pH 6.8. Electrophoresis was performed with the Hoefer SE 600 vertical unit (Hoefer Pharmacia Biotech Inc., San Francisco, CA 94107, USA) according to Laemmli (1970) using polyacrylamide gels (resolving gel $T = 13.7\%$, $C = 1.7\%$, stacking gel $T = 3.8\%$, $C = 1.8\%$) with gel size of $180 \times 160 \times 1.5$ mm. Sample sizes were 2 and 8 μ l. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAP-SCAN 1236 (Agfa-Gevaert N.V. Belgium, Agfa SnapScan 1236s Color image scanner).

2.7. Rats and diets

The Animal Care Committee of the University had approved this investigation, which was conducted as previously described (Gorinstein et al., 2005).

Wistar male rats ($n = 20$), with mean weight 115 g at the beginning of the experiment, were divided into four diet groups, each of five and were named Control, Chol, Chol/Snake and Chol/Mangosteen. The animals were housed in individual plastic cages in an air-conditioned room (temperature 21–22 °C and relative humidity 55–65%). During 4 weeks of the experiment the rats of all four groups were fed basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed a BD only. To the BD of the other three groups 1% of cholesterol was added. The diets of the rats of Chol/Snake and Chol/Mangosteen groups were supplemented with 5% of the Snake fruit and Mangosteen, respectively. Non-oxidized cholesterol of analytical grade was used in the diets. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats.

The diets contained, as percentage of energy, 65% of carbohydrates, 26% of protein and 9% of fat. Their calculated energy was from 395.4 to 400.4 kcal/100 g, and the differences were not significant.

All rats were fed once a day at 10⁰⁰ h *ad libitum*. They had unrestricted access to drinking water. The feed intake was monitored daily, and body gains were measured every week. The blood samples were taken from the left atrium of the heart before and at the end of the experiment. Plasma was prepared and used for laboratory tests, which included

determination of plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), TC in liver and plasma antioxidant activity (Leontowicz et al., 2006).

Plasma fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized. The TPTZ test, as mentioned previously, was used for determination of the plasma antioxidant activity (Benzie & Strain, 1996), and the results are given as [mmol trolox equivalents (TE) l⁻¹]. The antioxidant activity was determined in the full plasma, as well as in methanol-precipitable fraction. As mentioned, the mobility and protein profile of plasma were determined by the Laemmli method (1970).

2.8. Statistical analyses

The results of this investigation *in vitro* are means \pm SD of three measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The P values of <0.05 were considered significant.

3. Results

3.1. *In vitro*

The efficacy of the Mangosteen extract (ME) to scavenge TPTZ was lower by about 32% than that of the Snake fruit sample (SE). The present data were slightly lower than for the same type of extracts from the same fruits of a previous harvest period (Leontowicz et al., 2006). The highest antioxidant capacity (μ molFe²⁺ g⁻¹ DW) was estimated in the first fraction, as 124 ± 12.4 , followed by the second fraction, 113 ± 11.6 , then third fraction, 45.2 ± 4.9 and finally the fourth fraction, 27.1 ± 2.8 (Fig. 1).

Corresponding fractions from Mangosteen were about 30% lower than the Snake fruit, and were lower than the harvest of 2004 (Leontowicz et al., 2006). The DPPH measurements corresponded with the results of TPTZ in the extracted fractions. Total phenols (mg GAE g⁻¹ DW), as well, were lower in this harvest than in the previous one: first fraction -13.9 ± 1.3 ; second fraction -9.90 ± 1.0 ; third fraction -5.82 ± 0.6 ; and fourth fraction -3.94 ± 0.5 . The amount of polyphenols in Mangosteen was about 28% less than that in the Snake fruit samples (Fig. 1), but there was a direct relationship between the amount of polyphenols and the antioxidant activity measured by FRAP and DPPH (Fig. 1). The contribution of total polyphenols to the antioxidant potential of Snake fruit and Mangosteen was relatively high, and the correlation coefficient was high as well (Leontowicz et al., 2006). For comparison, the radical-scavenging effects of trolox and BHA were measured and compared with those of fruit extracts.

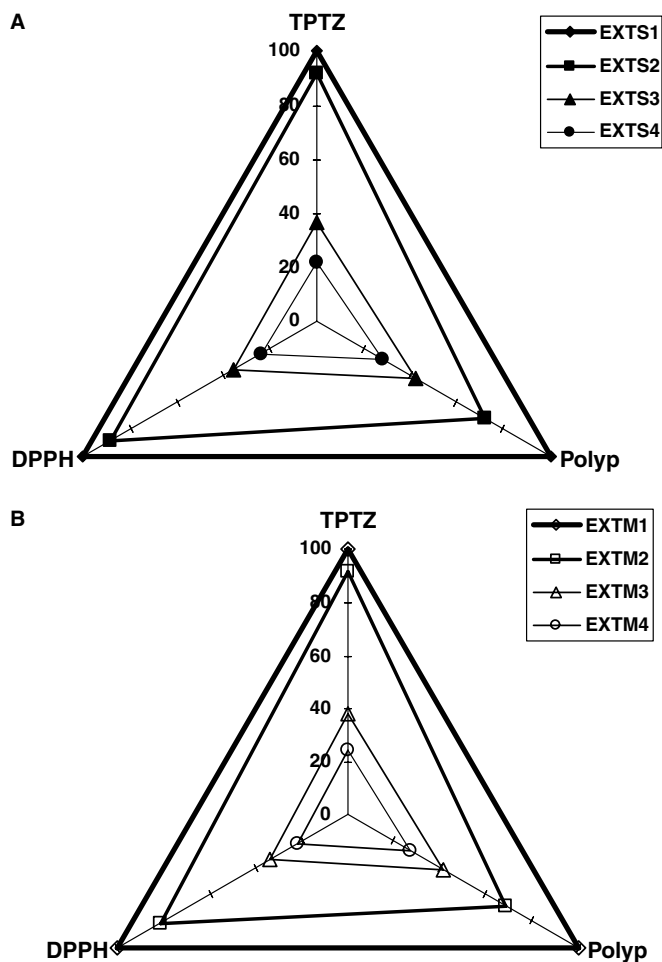


Fig. 1. Relative antioxidant activity and polyphenols (%) in extracts from Snake fruit (S): EXTS1, water; EXTS2, methanol/water; EXTS3, ethanol; EXTS4, acetone/water and from Mangosteen (M): EXTM1, water; EXTM2, methanol/water; EXTM3, ethanol; EXTM4, acetone/water; Polyp, polyphenols, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,4,6-triphenyl-*s*-triazine (TPTZ).

3.2. *In vivo*

Weight gains, feed consumption and feed efficiency ratio, in all four diet groups after the conclusion of the trial, varied (data not shown); however, this was not enough to be significant ($P > 0.05$). Diet supplemented with Snake fruit, and to a lesser degree, with Mangosteen (Fig. 2A) significantly hindered the rise in plasma lipids, which was connected with cholesterol feeding ($P < 0.05$). As expected, total cholesterol in liver after the experiment (Fig. 2B) was significantly higher in the rats of the Chol, Chol/Snake and Chol/Mangosteen groups than in Control (24.1 ± 1.7 , 17.9 ± 1.3 , 18.3 ± 1.4 and $5.25 \pm 0.7 \mu\text{mol g}^{-1}$, respectively). However, the increase in the TC liver concentration of the rats of the Chol/Snake and Chol/Mangosteen groups was significantly less than that in the rats of the Chol group. The TC concentration in rats of the Chol group was 4.59 times higher than that in the Control

group. In the Chol/Snake, and Chol/Mangosteen groups, these indices were only 3.41 and 3.49 times higher than that in the Control group, respectively ($P < 0.005$ in all cases). The above-mentioned data indicate that supplementation of diets with Snake fruit and Mangosteen significantly hindered the increase in TC liver concentration. A significant decrease in the plasma antioxidant activity values in the rats fed added cholesterol was found after the experiment (Fig. 3). The decrease in the antioxidant activity in the groups of rats fed diets supplemented with fruits (Snake and Mangosteen) was significantly less ($P < 0.05$) than that in the group of rats fed diet without fruits (Chol). The same relationship was revealed in the extracted plasma methanol fraction. The present results *in vivo* were similar to those discussed in the previous experiment, in spite of some slight changes of *in vitro* indices (Leontowicz et al., 2006).

The protein profile of serum samples showed that in the fibrinogen fraction of Chol/Snake fewer protein bands were detected and with lower intensity than in the Chol/Mangosteen group. The main patterns were located in the range of 40–65 kDa (Fig. 4). Two upper arrows, of ~ 110 kDa protein show that, for the Chol/Snake fibrinogen fraction, fewer protein bands were detected than in the same range of molecular weight for Chol/Mangosteen. The low arrow of ~ 14 kDa indicates some bands of Chol/Mangosteen, which were not allocated for the Chol/Snake diet group.

4. Discussion

Tropical and subtropical fruits, such as mango, guava, papaya, persimmon and many others, are well known in North America and Europe, and the scientific basis for their consumption is well founded (Dube et al., 2004; Garcia et al., 2005; Leontowicz et al., 2006). However, some others (Snake fruit and Mangosteen) are less well known and were investigated only *in vitro* (Shui & Leong, 2005; Wijaya et al., 2005; Yapwattanaphun et al., 2004). We did not find any publications on studies of Snake fruit and Mangosteen *in vivo*. Therefore, it was decided to investigate these exotic fruits both *in vitro* and *in vivo*. In our previous experiments, traditional citrus and tropical fruits were characterized by cholesterol-lowering and antioxidant properties performed on laboratory animals and investigations on humans (Gorinstein et al., 2006, 2005; Leontowicz et al., 2006).

Therefore, we recommended inclusion of these fruits in disease-preventing diets. In this study, we tried to discover whether the good taste of Snake fruit and Mangosteen also justified their inclusion in such diets.

The electrophoresis analysis has shown that the contents and profiles of protein compounds of both studied fruits were similar (Leontowicz et al., 2006). Our previous results *in vitro* (Leontowicz et al., 2006) and another report (Luximon-Ramma et al., 2003) showed that Snake fruit and Mangosteen were excellent sources of antioxidants. The lipophilic fraction was low in the investigated fruits. Most

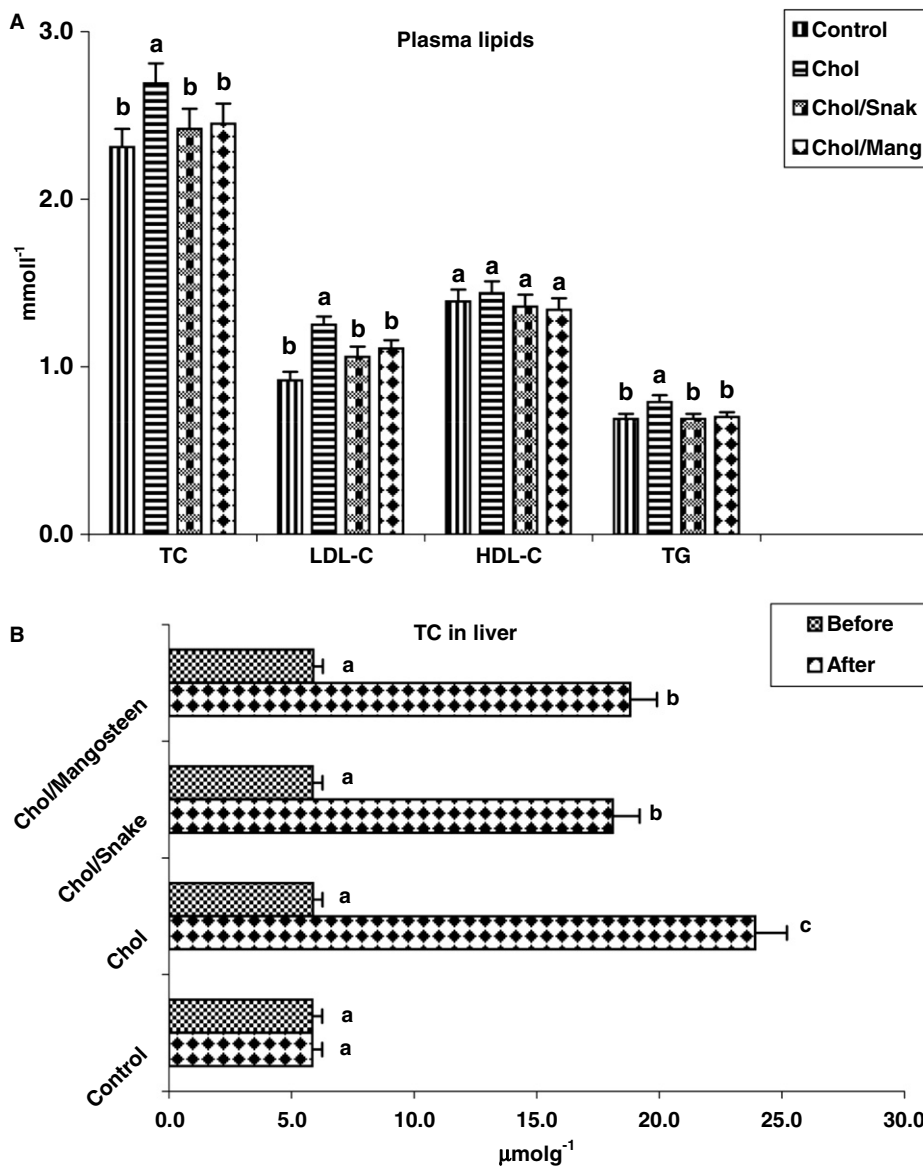


Fig. 2. Changes in (A) the plasma lipids (mmol l^{-1}): TC, total cholesterol; LDL, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides and (B) TC in liver ($\mu\text{mol g}^{-1}$) in the experiment ($n = 5$). Means \pm SD (A- vertical and B- horizontal lines). Means without superscript letter in common differ significantly ($P < 0.05$).

of the antioxidants were in the hydrophilic fraction, which also corresponds with reports of others (Ozgen et al., 2006). The same results were reported for passion fruit juice. Antioxidant activity was measured in juice and in two subfractions (hydrophilic and lipophilic) after processing and storage, but antioxidant values were non-additive. A significant chemical interaction affecting antioxidant capacity was found for hydrophilic juice components, but none was observed in the presence of lipophilic phytochemicals (Talcott et al., 2003).

The total antioxidant potential in Snake fruit was significantly higher than that in Mangosteem ($P < 0.05$). Results showed that the oxidation of linoleic acid was markedly inhibited by the alcohol-soluble extracts from all the fruits studied. Our results correspond to reported values (Garcia et al., 2005), showing that, among the fruits, durian and

mangosteem had the highest antioxidant activity, both in lipid peroxidation (linoleic acid) and the deoxyribose assay.

Good correlations, *in vitro*, of phenols from methanolic extracts with their antioxidant activities [DPPH, FRAP and ABTS-TEAC (trolox equivalent antioxidant capacity)] were achieved in the present report, as well as in our very recent studies; $R^2 = 0.96 - 0.94$ (Leontowicz et al., 2006) and completely corresponded with Mahattanatawee et al. (2005), confirming the correlation of 0.96. Others indicated that there were strong correlations between antioxidant activity (assessed by both DPPH and FRAP) and the total phenolics (Luximon-Ramma et al., 2003; Yuka et al., 2003). The DPPH radical-scavenging activity and polyphenol content of dried fruits were highly correlated as well (Ishiwata et al., 2004; Park et al., 2006). DPPH-radical scavenging activity in the studied fruits is generally linked

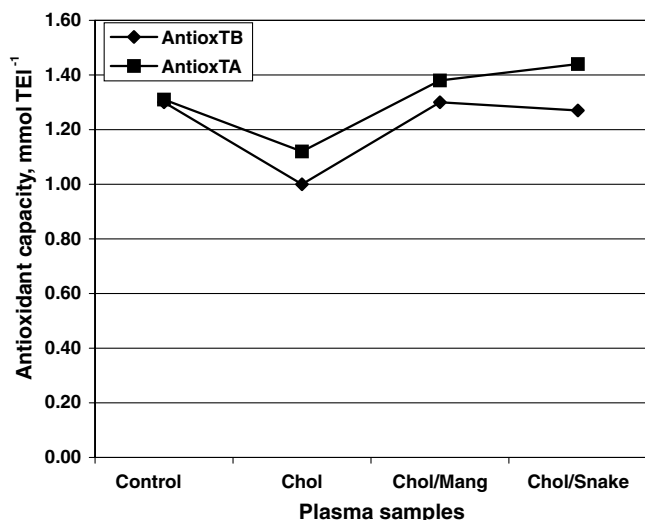


Fig. 3. Changes in the plasma antioxidant activity (TA) and methanol fraction (TB) after completion of the experiment ($n = 5$).

with total phenolics, as was shown in present study and in the previous one (Leontowicz et al., 2006), but the DPPH in papaya was associated with ascorbic acid concentrations (Kondo et al., 2005).

As previously mentioned, knowledge of Snake fruit and Mangosteen is limited; therefore the obtained results in this report can be compared with those of other tropical fruits. The future reader could ask: where is the place of the two studied exotic fruits among other tropical fruits?

Murcia et al. (2001) showed the order of efficiency, as antioxidant scavengers, between tropical fruits to be: passion fruit > lime > passiflora > kumquat > avocado > pineapple > physalis > papaya fruit > carambola > mango > banana. Luximon-Ramma et al. (2003) showed that the antioxidant activities (FRAP) of the fruits ranged (from 0.3 to 34 $\mu\text{mol TE g}^{-1}$ FW and from 2.86 to 323 $\mu\text{mol Fe}^{2+} \text{g}^{-1}$ DW) and total phenolics in the fruits ranged from 118 to 5638 $\mu\text{g g}^{-1}$ FW and from 0.66 to 31.5 mg g^{-1} DW). The highest antioxidant capacities were observed in red and yellow *Psidium*

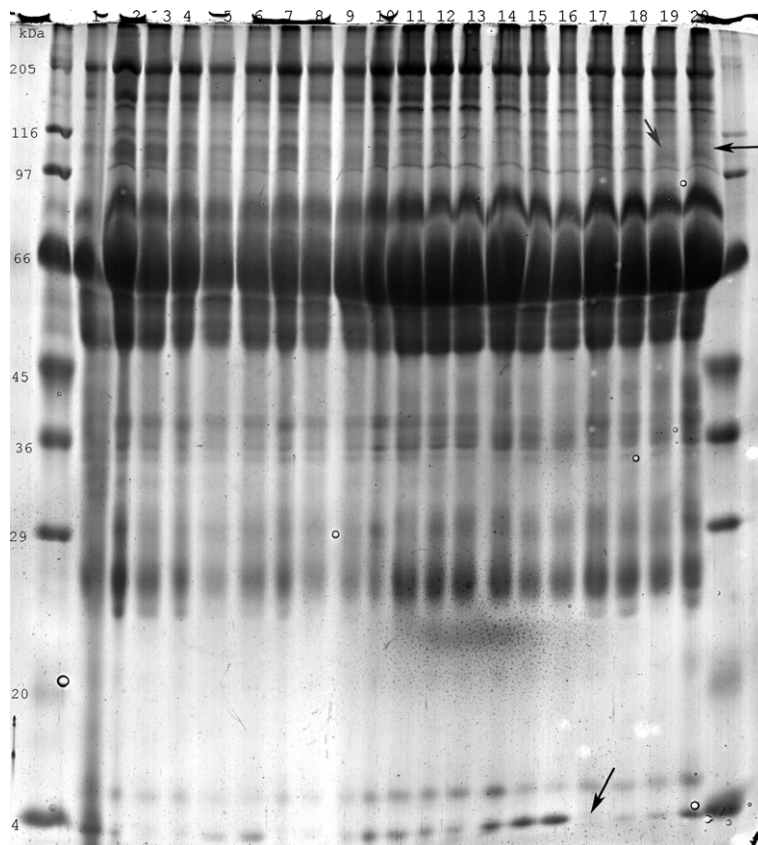


Fig. 4. Comparison of the band intensities of serum proteins after different diets are dissolved with sample buffer containing SDS and 2-ME and separated by SDS-PAGE; molecular markers (kDa): (205)-myosin; (116)- β -galactosidase; (97)-phosphorylase b; (66)-albumin; (45)-ovalbumin; (36)-glyceraldehyde-3-phosphate dehydrogenase; (29)-carbonic anhydrase; (24)-trypsinogen, PMSF treated; (20)-trypsin inhibitor, (14)- α -lactalbumin; Lanes 1–10 full serum, loading 2 μl and 8 μl ; 11–20 methanol precipitated; lanes 1–2 and 11–12 Standard diet groups; lanes 3–4 and 13–14 Control diet groups; lanes 5–6 and 15–16 Cholesterol diet groups; lanes 7–8 and 17–18 Chol/Mangosteen; lanes 9–10 and 19–20 Chol/Snake. Two upper arrows of ~ 110 kDa protein show that, in this zone, this feature is characteristic for Chol/Snake methanol-precipitated samples; there are fewer protein bands; the low arrow of ~ 14 kDa indicates a band of Chol/Mangosteen methanol-precipitated samples. This band has less protein and is also thinner than others with the same mobility.

cattleianum Sabine “Chinese guava”, sweet and acid *Averrhoa carambola* L. “starfruit”, *Syzygium cumini* L. *Skeels* “jamblon”, and white *Psidium guajava* L. “guava”. Our results of FRAP (from 124 to 87 $\mu\text{mol Fe}^{2+} \text{g}^{-1} \text{DW}$) and total phenolics (from 13.9 to 10.4 $\text{mg GAE g}^{-1} \text{DW}$), put, according to Luximon-Ramma et al. (2003), Snake fruit and Mangosteen between star fruit and white guava. Mahattanatawee et al. (2005) reported (for red guava, white guava, carambola, red pitaya, white pitaya, mamey, sapodilla, lychee, longan, green mango, ripe mango and green papaya) the values of phenols, ORAC and DPPH ranged (from 205 to 2316 $\mu\text{g GAE g}^{-1}$ puree; \sim from 1.15 to 12.9 $\text{mg GAE g}^{-1} \text{DW}$), from 0.02 to 16.9 $\mu\text{mol TE g}^{-1}$ puree; \sim from 0.11 to 94.6 $\mu\text{mol TE g}^{-1} \text{DW}$ and from 2.08 to 620 $\mu\text{g GAE g}^{-1}$ puree; \sim from 11.6 to 3465 $\mu\text{g GAE g}^{-1} \text{DW}$, respectively. The antioxidant activities, evaluated by both ORAC and DPPH, showed similar trends where red guava and carambola had the highest and sapodilla and green papaya had the lowest. The present results confirmed that Snake fruit and Mangosteen can be placed between guava and carambola. Nilsson et al. (2005), for the water-soluble fraction, using two methods ($\mu\text{mol TE g}^{-1} \text{FW}$), 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS^+) and FRAP, reported the following results: guava: [16.9 and 10.2 ($\mu\text{mol g}^{-1} \text{FW}$); \sim 94.4 and 57.1 ($\mu\text{mol g}^{-1} \text{DW}$)]; and mango: [4.0 and 1.67 ($\mu\text{mol g}^{-1} \text{FW}$); \sim 21.8 and 9.1 ($\mu\text{mol g}^{-1} \text{DW}$)]. The results of antioxidant activity by FRAP in the present report correspond with the previous data by ABTS^+ (Leontowicz et al., 2006). Analyses of Snake fruit and Mangosteen in the present and previous reports and in Nilsson et al. (2005) indicated the order of efficacy as scavengers, in these two methods, between guava and mango. According to Talcott et al. (2003), total soluble phenolics of about 457 mg l^{-1} (\sim 2.55 $\text{mg g}^{-1} \text{DW}$) in passion fruit were lower than in the present study, but the antioxidant capacity of $17.2 \pm 0.25 \mu\text{M TE ml}^{-1}$ (\sim 96.1 $\mu\text{M TE g}^{-1} \text{DW}$) completely matched the results obtained for Snake fruit of 72.9 $\mu\text{M TE g}^{-1} \text{DW}$ (Leontowicz et al., 2006). The reviewed data of the total phenolic contents (Wu et al., 2006) of flesh in red pitaya (42.4 $\text{mg GAE } 100 \text{ g}^{-1} \text{FW}$; \sim 2.36 $\text{mg GAE g}^{-1} \text{DW}$) and antioxidant activity (28.3 $\mu\text{mol TE g}^{-1} \text{FW}$, \sim 158 $\mu\text{mol TE g}^{-1} \text{DW}$) show that our results in polyphenols and antioxidants were lower, by as much as twice, than the discussed ones for red pitaya.

The DPPH radical- and OH radical-scavenging activities of the polyphenol extracted with 80% ethanol in feijoa was equivalent to, or higher than that in the other fruits: kiwano, cherimoya, papaya and mango (Yuka et al., 2003). The total polyphenol content of feijoa (edible part) (59.2 $\text{mg}/100 \text{ g}^{-1} \text{FW}$; \sim 3.3 $\text{mg g}^{-1} \text{DW}$) was similar to that of cherimoya. Comparison with these results put the two investigated fruits in the established order of papaya and mango. The antioxidant activity of sapodilla fruit has been reported (Ma et al., 2003) to be very high in the ABTS assay (3396 mg kg^{-1} ; \sim 76 $\mu\text{mol TE g}^{-1} \text{DW}$) and this exactly corresponds with our data of Snake fruit.

The comparison of our present and recent data on Snake fruit and Mangosteen show that these fruits, according to DPPH, ABTS and TPTZ scavenging activities of the polyphenols extracted with solvents of different lipophilicities (water, methanol/water or ethanol) were equivalent to, or better than other fruits: papaya, mango, sapodilla and guava. This conclusion, *in vitro* is very important in terms of the antioxidant properties and consumption of the less studied Snake fruit and Mangosteen.

The investigation *in vivo* has shown that, after 30 days of different feeding, the diet supplemented with Snake fruit and, to a lesser degree with Mangosteen, hindered the rise in plasma lipids and the decrease of plasma antioxidant activity in rats fed a cholesterol-containing diet. Such changes appeared not only in the total antioxidant activity, but also in the methanol-extracted fraction. No significant changes in the plasma lipid levels or antioxidant activity were observed in the Control group.

Electrophoretic bands of the fibrinogen fraction showed a difference between the Snake and Mangosteen diets: in Chol/Snake, the 110 kDa-electrophoretic bands had less protein than in Chol/Mangosteen. No information exists about the changes in the separated proteins after exotic fruits or other plant diets; therefore, it is impossible to compare our data with other reports. Hawley, Tamura, and Miles (2001) mostly describe the use of electrophoretic separation for characterization of a profibrinolytic plasminogen-binding protein. In the present report, differences in the protein composition in plasma of rats after fruit diets were found. The reviewed data of the activity of fibrinogen were found around the range of 40–54 kDa (Hawley et al., 2001) and, as can be seen (Fig. 4), most proteins were located in this region, but the differences in this study were found to be in low (14 kDa) and high (110 kDa) molecular weight proteins. The decrease of fibrinogen in the detected electrophoretic fractions reduces the potential risk of atherosclerosis and blood coagulation (Gorinstein et al., 2006). Our data corresponded with others who found improvement of rat blood fluidity and inhibitory effects on the thrombin-induced conversion of fibrinogen to fibrin using the Japanese apricot (Kubo, Yamazaki, Matsuda, Gato, & Kotani, 2005). The supplementation of rat's diet with these studied fruits was similar to the supplementation with *Passiflora alata* extract administered orally in rats at a dose of 1000 mg kg^{-1} . In two cases there was an increase in high-density lipoprotein cholesterol (Doyama et al., 2005).

5. Conclusions

1. Snake fruit and Mangosteen have high contents of total polyphenols and high antioxidant potential. The results indicate that both fruits are rich in polyphenols and are good sources of antioxidants in comparison with other tropical fruits. These fruits, in future, should draw more attention of Thai growers, not only because of their taste and economic value, but also their antioxidative activity from polyphenol contents.

2. Diets supplemented with Snake fruit, and to a lesser degree with Mangosteen, positively affect plasma lipid levels and plasma antioxidant activity in rats fed cholesterol-containing diets.
3. The positive results of this experiment on animals cannot automatically be applied to humans.

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