RESEARCH ARTICLE

Guava, Papaya, Pineapple, and Pomelo Juices Inhibit Pancreatic Lipase Activity and Cholesterol Micelle Solubility

Kanittaporn Trisat[1], Nanteetip Limpeanchob[1], Anan Ounaroon[2]

[1] *Department of Pharmacy Practice and Center of Excellence for Innovation in Chemistry, Pharmacological Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand*
[2] *Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand*

Abstract

High fat consumption has been identified as one of the critical factors leading to obesity and several metabolic syndromes. Because restriction of diet is difficult for most people, an alternative dietary intervention is received special attention. This study aimed to investigate the potential of fruit juices for their capacity to reduce lipid digestion through the inhibitory activities against pancreatic lipase and micellar cholesterol solubility. Guava, papaya, pineapple and pomelo were selected for the study. Both effects were determined using freshly prepared and freeze-dried preparations of fruit juices. The result showed that fruit juices dose-dependently inhibited pancreatic lipase activity and solubility of cholesterol in lipid micelles. Among four species, papaya exhibited the highest pancreatic lipase inhibitory activity whereas guava showed the highest inhibition on micellar cholesterol solubility. Freshly prepared and freeze-dried juices were not statistically different by these two investigations. The results suggest that fruit juices consumption may potentially inhibit intestinal lipid digestion and consequently being helpful for weight and serum lipid controls.

Keywords: Fruit juice, pancreatic lipase, cholesterol micelle solubility, obesity

Address correspondence and reprint request to: Anan Ounaroon, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand. E-mail address: ananounaroon@yahoo.com

ศักยภาพของน้ำฝรั่ง มะละกอ สัปปะรด และส้มโอ ในการยับยั้งเอนไซม์แพนครีเอติกไลเปสในการย่อยไขมันและลดความสามารถการละลายของคอเลสเตอรอลไมเซลล์

ขนิษฐพร ไตรศรัทธ์[1], นันทีทิพ ลิ้มเพียรชอบ[2], อนันต์ อุ่นอรุณ[3]

[1] *ภาควิชาเภสัชกรรมปฏิบัติ, ศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี, และหน่วยปฏิบัติการวิจัยทางเภสัชวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก*

[2] *ภาควิชาเภสัชเคมีและเภสัชเวท คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก*

บทคัดย่อ

การบริโภคอาหารไขมันสูงเป็นตัวเหนี่ยวนำสำคัญตัวหนึ่งที่ทำให้เกิดโรคอ้วน การปรับพฤติกรรมการรับประทานอาหารเป็นทางเลือกแรกที่แนะนำ แต่ทั้งนี้การปรับพฤติกรรมการรับประทานอาหารนั้นทำได้ยากสำหรับคนทั่วไป ดังนั้น ผู้วิจัยจึงสนใจศึกษาศักยภาพของน้ำผลไม้ 4 ชนิด ได้แก่ ฝรั่ง มะละกอ สัปปะรด และส้มโอ ในการลดการย่อยอาหารไขมัน โดยทดสอบฤทธิ์ของน้ำผลไม้คั้นสดเปรียบเทียบกับสารสกัดผงแห้งในการยับยั้งการทำงานของเอนไซม์แพนครีเอติกไลเปสและการละลายของคอเลสเตอรอลไมเซลล์ ผลการทดลองพบว่า น้ำผลไม้ทุกชนิดสามารถยับยั้งเอนไซม์แพนครีเอติกไลเปสและการละลายของคอเลสเตอรอลไมเซลล์ได้ โดยฤทธิ์ดังกล่าวขึ้นกับความเข้มข้นของน้ำผลไม้ และเมื่อเปรียบเทียบผลไม้ทั้ง 4 ชนิด พบว่ามะละกอยับยั้งเอนไซม์แพนครีเอติกไลเปสได้ดีที่สุด ในขณะที่ฝรั่งยับยั้งการละลายของคอเลสเตอรอลไมเซลล์ได้ดีที่สุด นอกจากนี้ เมื่อเปรียบเทียบน้ำผลไม้คั้นสดกับสารสกัดผงแห้ง พบว่ามีฤทธิ์ไม่แตกต่างกันทางสถิติ ผลการทดลองนี้แสดงให้เห็นว่า การบริโภคน้ำผลไม้อาจมีผลยับยั้งการย่อยอาหารไขมันในทางเดินอาหาร และอาจมีประโยชน์ในการควบคุมน้ำหนักตัวและไขมันในเลือดได้

คำสำคัญ: น้ำผลไม้, เอนไซม์แพนครีเอติกไลเปส, การละลายของคอเลสเตอรอลไมเซลล์, โรคอ้วน

Introduction

Refined carbohydrate, high calorie, and high-fat diets lead to hyperlipidemia and obesity. Lifestyle changes are generally recommended for controlling body weight and serum lipid levels. However, for most people long-term adherence to these behavioral changes are often poor. Given that behavioral changes often fail, we need alternative treatments to curb excess nutrient intake. Currently, there are few efficacious anti-obesity drugs, so a treatment based on food itself may be a better way of reducing excess weight. Therefore, natural products with minimal side effects in the form of a traditional remedy or dietary supplement have been intensively investigated and show promise as anti-obesity and cholesterol lowering agents with a reduction in the associated diseases.[1,2]

One strategy is to reduce lipid digestion by inhibiting pancreatic lipase and/or disruption of lipid micellization.[3,4] Pancreatic lipase hydrolyses dietary triglycerides.[5] Inhibition of this enzyme is one of the most widely studied mechanisms for anti-obesity agent and orlistat has long been used in this way. Several food and plant extracts also demonstrate inhibition of pancreatic lipase.[1] Alternatively, disruption of lipid micellization also curtails dietary fat absorption. Many natural products including sunflower protein hydrolysates, insoluble buckwheat protein and soya protein prevent cholesterol incorporation into lipid micelles thus decreasing cholesterol solubility.[6-8] These agents were derived from a variety of foods and herbs but the role of common fruits in reducing fat absorption has received limited attention.

Generally, fruit is regarded as part of a healthy diet because of their nutrient and fiber content. Some fruits possess lipid lowering actions in animal[9] and human studies[10] but the mechanism of this effect is unclear. To show whether fruit juices have the potential to interfere with lipid digestion, their inhibitory effects on pancreatic lipase activity and cholesterol solubility were determined. Four types of fruits were selected for this study; guava *(Psidium guajava* L.), papaya (*Carica papaya* L.), pineapple (*Ananas comosus* Merr.), and pomelo (*Citrus maxima* Merr.), since they are available throughout the year in Thailand. For this type of testing, lyophilized samples were commonly used and might not reflect the activity of fresh fruit. Thus, freshly prepared fruit juices were compared with those reconstituted from lyophilized powders.

Materials and Methods

Chemicals

Folin-Ciocalteu's reagent, gallic acid, lipase from porcine pancreas, 1,2-di-*O*-lauryl-rac-glycero-3 glutaric acid 6′-methylresorufin ester, orlistat, cholesterol, l-α-phosphatidylcholine, taurocholic acid sodium salt hydrate, hydrazine hydrate solution, 3-α-hydroxysteroid dehydrogenase and β-nicotinamide adenine dinucleotide were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate (Na_2CO_3) and sodium chloride (NaCl) were obtained from Merck (Darmstadt, Germany). Cholesterol test kit was purchased from HUMAN GmbH (Wiesbaden, Germany).

Preparation of fruit juices

The fresh ripe guava (Pan-see-thong variety), papaya (Holland variety), pineapple (Phuket variety) and pomelo (Khao-tang-kwa variety) were sourced in Phitsanulok, Thailand from three separate markets to offset variations in growing sites. All fruits were weighed, washed, peeled (except guava) and chopped into small pieces. Juices were prepared by a juice extractor without water added (Moulinex, France) and the pulp was discarded. Half of the clear juice was lyophilized, and the powder was stored at -20ºC. The remaining juice was tested immediately.

Pancreatic lipase inhibitory activity

Pancreatic lipase activity was measured using the method of Aubry et al.[11] Lyophilized juices were reconstituted to its original volume with distilled water. Various concentrations; 25, 50, 75, and 100%, of lyophilized and fresh juices were prepared with reaction buffer pH 8.0 (0.8 M Tris-HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$). Five hundred microliters of each diluted juice were centrifuged at 10,000 rpm for 1 min and their supernatants were collected. Twenty-five microliters of each supernatant was mixed with reaction buffer (40 µL) and 50 U/mL pancreatic lipase (25 µL) in 96-well plate (black-side and clear-bottom). Finally, the reaction was started by adding 10 µL of 400 µM substrate (1,2-di-*O*-lauryl-rac-glycero-3 glutaric acid 6'-methylresorufin ester) and run at 37ºC in the dark for 60 min. Amount of product, methylresorufin, was measured by spectrofluorometer at Ex 535 nm and Em 595 nm. Orlistat was used as a positive control.

Cholesterol micellar solubility

Cholesterol micelle solubility assay was adapted from Kirana et al.[12] Lipid micelles were prepared with the final concentrations of 1 mM cholesterol, 1 mM sodium taurocholate, and 0.6 mM phosphatidylcholine. Micelle solutions were sonicated, mixed with fresh or lyophilized juices, and incubated at 37ºC for 3 h. The precipitated cholesterol was separated from the intermicellar cholesterol by filtering through a 0.22 µm syringe filter. The concentration of cholesterol remaining in the intermicelles was determined by the cholesterol assay kit.

Determination of total phenolic content

Total phenolic contents were determined using Folin-Ciocalteu method. Lyophilized powders of juices were suspended in water to the initial volume of juices. Juice samples were centrifuged at 10,000 rpm for 1 min and supernatants were collected. Supernatants (20 µL), Folin-Ciocalteu reagent (100 µL) and 0.1 N Na_2CO_3 (80 µL) were added in 96-well plate. The mixtures were incubated at 50ºC for 5 min and subsequently at room temperature for 30 min before measuring the absorbance at 750 nm. Gallic acid (GA) was used as a standard phenolic compound. Total phenolic content was calculated and expressed as milligram gallic acid equivalent (GAE).

Statistical analysis

All data are expressed as mean±standard error of mean (SEM) and analyzed using the one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. Differences were considered to be significant when P-values are less than 0.05.

Results and Discussion

Total phenolic content of juice

A vast range of phytochemicals inhibit pancreatic lipase including various classes of polyphenols which apart from having readily oxidizable phenolic groups appear to have little stereochemistry in common.[13] However, lipase inhibition may be a property of phenolics. There was also published research reported that poly-phenolic compounds showed the ability to inhibit the formation of cholesterol micelles.[14] We therefore sought to test the "total phenolic content" of the 4 fruit juices and relate this with their inhibitory actions.

Our study showed that guava juice had the highest total phenolic content while papaya, pineapple and pomelo had uniformly lower phenolic contents irrespective of whether the juice was fresh or lyophilized (Table 1).

Table 1. Total phenolic content of fresh and lyophilized fruit juices

Fruit	Variety	Total phenolic content (mg GAE/100 ml of juice)	
		Fresh juice	Lyophilized juice
Guava	Pan-see-thong	3.47±0.16*	3.52±0.04*
Papaya	Holland	1.04±0.02	1.06±0.03
Pineapple	Phuket	0.94±0.07	1.01±0.03
Pomelo	Khao-tang-kwa	0.9±0.02	1.10±0.03

Note: Data are mean±SEM from three plantation lots of fruits. GAE = gallic acid equivalent.
* means significantly different ($p<0.05$) from the other fruits.

Pancreatic lipase inhibitory activity of fruit juices

Pancreatic lipase is the principal lipolytic enzyme in the small intestine and plays a key role in the digestion of triglycerides. In this experiment, orlistat was used as a positive control to inhibit pancreatic lipase with IC_{50} = 10.36 nM (Figure 1). The pancreatic lipase inhibitory activities of all fruit juices were increased when the concentration increased. The effects of reconstituted juice from lyophilized powder mirrored those of the fresh juices and there was no statistically significant difference between both forms (Figure 2). Among tested fruits, papaya juice showed most effective pancreatic lipase inhibitor (~40%) whereas guava, pineapple, and pomelo juices exhibited similar pancreatic lipase inhibitory activities (~30%). It was reported that many polyphenols from oolong tea efficiently inhibited pancreatic lipase activity.[15] According to our result, guava juice had the highest total phenolic content but papaya juice showed the highest pancreatic lipase inhibitory activity. This suggests that pancreatic lipase inhibition of fruit juices may not be the result of total phenolic compounds.

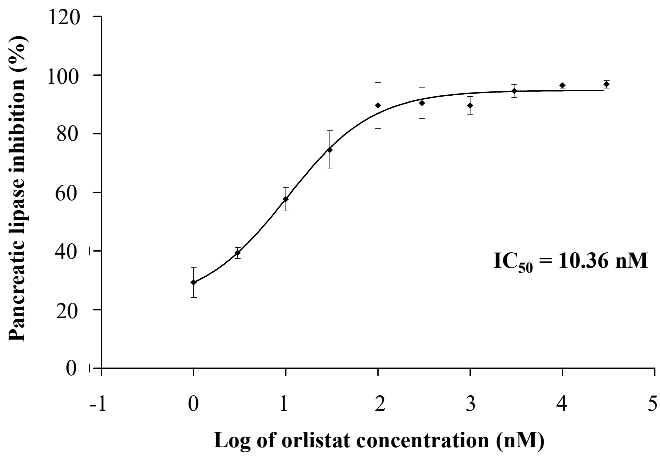

Figure 1. Dose-response curve of orlistat on pancreatic lipase activity. Results were presented as mean±SEM (n=3).

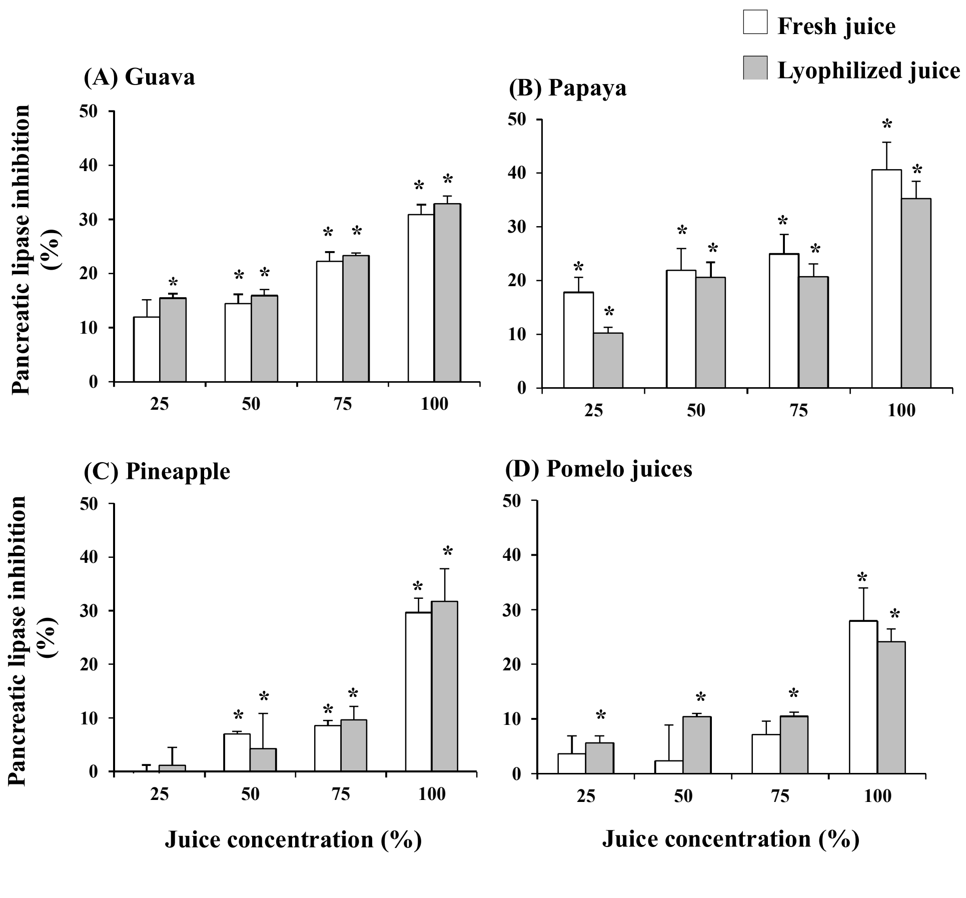

Figure 2. Comparison the pancreatic lipase inhibitory effect between fresh and lyophilized of guava (A), papaya (B), pineapple (C), and pomelo (D) juices. Results were presented as mean±SEM from 9 repeats (each fruit source was separately tested 3 times and there are total 3 sources).
* $p<0.05$ compared to control (water).

Fruit juices reduce cholesterol micellar formation

The pancreas is also the main source of bile salts which aids fat dispersion and micelle formation. In the present study, all four types of fruit juices displaced cholesterol from micelles (Figure 3). For guava, there was a significant complete or nearly complete inhibition of cholesterol micellar formation at every concentration tested (Figure 3A). Again, the actions of the fresh and reconstituted juices produced very similar actions.

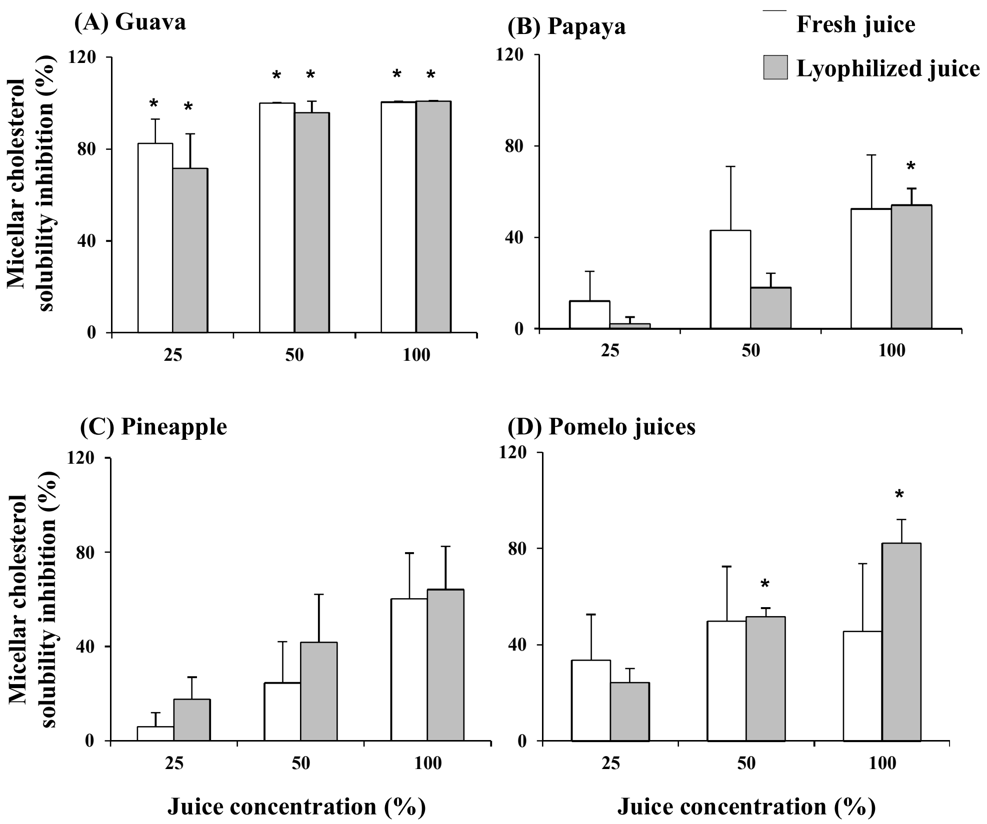

Figure 3. Comparison of micellar cholesterol solubility inhibition between fresh and lyophilized of guava (A), papaya (B), pineapple (C), and pomelo (D) juices. Results were presented as mean±SEM from 9 repeats (each fruit source was separately tested 3 times and there are total 3 sources). * $p<0.05$ compared to control (water).

This action might be through fruit juice components. Binding to either cholesterol or the bile salts or to both direct interactions to cholesterol was proposed for such activity. Since bile acids are essential for micelle formation, binding to bile acids could also interrupt the solubility of cholesterol in lipid micelles. One study showed *in vitro* that bile acids could bind to components of freeze-dried banana, grape, pineapple and others.[16] From our study, phenolic compounds might be responsible for this action since guava juice having the highest total phenolic content exhibited the highest inhibition on micellar cholesterol solubility. Previous study also reported the inhibitory activity of polyphenolic compounds on the formation of cholesterol micelles,[14] but their mechanism of action is unclear.

Recently, pomelo was reported to have antihyperlipidemic effect via several activities including pancreatic lipase inhibition, reduction of micelle formation and binding to bile acids.[17] Taken all data together, papaya and guava juice showed the highest potential as antihyperlipidemic and/or anti-obesity product through the inhibition of pancreatic lipase and micellar cholesterol solubility. Our data could support the mechanisms of cholesterol lowering action of guava previously reported.[3] Decreased serum total cholesterol and triglycerides levels were observed after 12 weeks of guava intake in essential hypertension patients.[18] In diabetic rats, aqueous extract of guava fruit peel was also shown to reduce serum total cholesterol, LDL, and triglycerides.[19] We expected guava juice, also other three juices, could be beneficial in improving lipid profile.

General considerations

It is clear that the potency profiles on lipase and micelle formation are quite different. However, these *in vitro* experiments cannot replicate the complex environment of the small intestine and the down-stream changes in the blood lipid profile may reflect other actions of the fruit juice. Thus, whether the same interactions occur in the intestine requires *in vivo* experiments. Additional actions in the gut may include the influence of fiber, particularly in the whole fruit, the influence of polyphenols on creating a more favorable gut microbiota and their antioxidant action preventing lipid oxidation in the gut.[3]

Conclusion

Our study shows that guava, papaya, pineapple, and pomelo juices dose-dependently inhibit pancreatic lipase activity and solubility of cholesterol in lipid micelles. Their polyphenolic compounds may be involved in inhibitory activity of cholesterol micellization but not in pancreatic lipase inhibition. Ingesting of these fruits may potentially inhibit intestinal lipid digestion and consequently being helpful for controlling body weight and serum lipid levels. Among all tested fruits, papaya and guava exhibit the most beneficial effect for weight and/or serum lipid control. In addition, freeze-drying process does not change total phenolic content as well as above mentioned activities.

Acknowledgements

The authors thank Dr. Norman Scholfield for manuscript preparation. This work was financial supported by Thailand Research Fund (grant number RDG5420070).

References

1. Yun JW. Possible anti-obesity therapeutics from nature-a review. Phytochemistry. 2010;71(14-15):1625-41.
2. Nijjar PS, Burke FM, Bloesch A, Rader DJ. Role of dietary supplements in lowering low-density lipoprotein cholesterol: a review. J Clin Lipidol. 2010; 4(4):248-58.

3. Slanc P, Doljak B, Kreft S, Lunder M, Janes D, Strukelj B. Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. Phytother Res. 2009;23(6):874-7.

4. Adisakwattana S, Intrawangso J, Hemrid A, Chanathong B, Mäkynen K. Extracts of edible plants inhibit pancreatic lipase, cholesterol esterase and cholesterol micellization, and bind bile acids. Food Technol. Biotech. 2012;50(1):11-6.

5. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today. 2007;12(19-20):879-89.

6. Megias C, Pedroche J, Del Mar Yust M, Alaiz M, Giron-Calle J, Millan F, et al. Sunflower protein hydrolysates reduce cholesterol micellar solubility. Plant Foods Hum Nutr. 2009;64(2):86-93.

7. Metzger BT, Barnes DM, Reed JD. Insoluble fraction of buckwheat (*Fagopyrum esculentum* Moench) protein possessing cholesterol-binding properties that reduce micelle cholesterol solubility and uptake by Caco-2 cells. J Agric Food Chem. 2007;55(15):6032-8.

8. Nagaoka S, Miwa K, Eto M, Kuzuya Y, Hori G, Yamamoto K. Soy protein peptic hydrolysate with bound phospholipids decreases micellar solubility and cholesterol absorption in rats and caco-2 cells. J Nutr. 1999;129(9):1725-30.

9. Daher CF, Abou-Khalil J, Baroody GM. Effect of acute and chronic grapefruit, orange, and pineapple juice intake on blood lipid profile in normolipidemic rat. Med Sci Monit. 2005;11(12):BR465-72.

10. Alvarez-Parrilla E, De La Rosa LA, Legarreta P, Saenz L, Rodrigo-Garcia J, Gonzalez-Aguilar GA. Daily consumption of apple, pear and orange juice differently affects plasma lipids and antioxidant capacity of smoking and non-smoking adults. Int J Food Sci Nutr. 2010;61(4):369-80.

11. Aubry S, Aubert G, Cresteil T, Crich D. Synthesis and biological investigation of the beta-thiolactone and beta-lactam analogs of tetrahydrolipstatin. Org Biomol Chem. 2012;10(13):2629-32.

12. Kirana C, Rogers PF, Bennett LE, Abeywardena MY, Patten GS. Naturally derived micelles for rapid in vitro screening of potential cholesterol-lowering bioactives. J Agric Food Chem. 2005;53(11):4623-7.

13. de la Garza AL, Milagro FI, Boque N, Campion J, Martinez JA. Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta Med. 2011;77(8):773-85.

14. Vermeer MA, Mulder TP, Molhuizen HO. Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. J Agric Food Chem. 2008;56(24):12031-6.

15. Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, et al. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. J Agric Food Chem. 2005;53(11):4593-8.

16. Kahlon TS, Smith GE. In vitro binding of bile acids by bananas, peaches, pineapple, grapes, pears, apricots and nectarines. Food Chem. 2007;101(3): 1046-51.

17. Makynen K, Jitsaardkul S, Tachasamran P, Sakai N, Puranachoti S, Nirojsinlapachai N, et al. Cultivar variations in antioxidant and antihyper-lipidemic properties of pomelo pulp (*Citrus grandis* [L.] Osbeck) in Thailand. Food Chem. 2013;139(1-4):735-43.

18. Singh RB, Rastogi SS, Singh R, Ghosh S, Niaz MA. Effects of guava intake on serum total and high-density lipoprotein cholesterol levels and on systemic blood pressure. Am J Cardiol. 1992;70(15):1287-91.

19. Rai PK, Mehta S, Watal G. Hypolipidaemic & hepatoprotective effects of Psidium guajava raw fruit peel in experimental diabetes. Indian J Med Res. 2010;131:820-4.