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八种水果中的多酚含量及其抗氧化性

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摘 要: 测定了苹果、石榴、橄榄、芒果、香蕉、菠萝、葡萄和龙眼的壳、肉及核中的多酚含量及其抗氧化性。以70%丙酮(v/v)为提取溶剂, 室温下超声波辅助浸提样品后得到提取液。采用普鲁士兰(Prussian blue)法测定了提取液中多酚和单宁的含量, 利用 FRAP法测定其抗氧化性。结果表明: 提取液中多酚和单宁的含量均与其抗氧化性成正相关关系; 石榴、橄榄、芒果、葡萄和龙眼等水果的壳与核有望成为天然抗氧化剂的新来源。

关键词: 多酚; 单宁; 抗氧化性; 水果皮; 果肉和种子

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Polyphenol Contents in Eight Fruits and Their Antioxidant Activities

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Abstract The contents of polyphenols of peel, pulp and seed fractions of apple, pomegranate, olive, mango, banana, pineapple, grape and longan and their total antioxidant activities were studied. After ultrasound-assisted extraction with 70% aqueous acetone at room temperature, polyphenols and tannins in the plant cells were separated and their contents were determined by Prussian blue assay. Antioxidant activities of the aqueous acetone extracts were evaluated by Ferric Reducing/Antioxidant Power (FRAP) assay. A positive linear correlation between antioxidant activities and phenolic contents was observed. The results showed that the fruit peel and seeds of pomegranate, olive, mango, grape and longan could be considered as potential sources of antioxidants rather than just discarded as waste.

Key words polyphenol; tannin; antioxidant; fruit peel; pulp and seed

Introduction

The research interest in polyphenolic antioxidants has increased remarkably in the last decade because of their free radical scavenging activities associated with various diseases [1]. Synthetic antioxidants require high manufacturing costs but show lower activities than natural antioxidants, and some of them may be toxic to human [2]. Therefore, a need is stimulated to identify natural and possibly more economic and effective antioxidants with potential to be used for foods industry. Antioxidant compounds have been identified in the apple pomace [3], banana [4], and in the seeds of grape [5], mango [6] and olive [7]. Besides, the pomegranate peel [8] and longan seed [2] also showed high antioxidant activity. However, to our knowledge, there are few studies relating to the antioxidant activity of fruit peel. Given the considerable amount of by-products arising from fruits-processing plants, improving the utilization of the fruit peel and seeds is a very important issue. One possible solution could be turning the fruit peel and seeds into a source of natural food additives and ingredients. Fruits have excellent antioxidant properties and these effects are mainly attributed to their phenolic constituents [1,2]. To find out the distribution of phenolic substance in different kinds of plant fruits and determine their antioxidant activities, apple, pomegranate, olive, mango, banana, pineapple, grape and longan were selected for this study. The objectives of the present study are to determine the total antioxidant capacities and the

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polyphenolic contents of peel, pulp and seed extracts of selected fruits and to further investigate the relationships between antioxidant activities and polyphenolic contents in different fruit parts.

Materials and Methods

Materials

Fruits

"Youtanben" longan was purchased from a local farm in Zhangzhou, Fujian Province, China. Apple (Fushi), pomegranate, olive, mango (Zihua), banana, pineapple, and grape were purchased from Wal-Mart supermarket in Xiamen, Fujian Province, China.

Chemicals

Polyvinylpolypyrrolidone (PVPP), L-ascorbic acid and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemicals. All other chemicals were commonly available reagents made in China.

Methods

Sample preparation

Organic solvent extraction is often used for isolation of antioxidants. Both extraction yield and antioxidant activity of extracts are strongly dependent on the solvent species [2]. Aqueous-acetone system is widely employed because of their high efficiency of polyphenol extraction [13]. Therefore, acetone-water system was selected for the extraction. The extraction process was followed by Makkar's [9]. 0.5–3 g of each part of the fruit (fresh tissue) was accurately weighed, and extracted with 50 mL aqueous acetone (70%, v/v) under ultrasonic treatment for 20 min (2 × 10 min with 5 min break in between) at room temperature. The solution was then transferred to centrifuge tubes and subjected to centrifugation for 10 min at 3000 g. The supernatant was collected and kept cooled on ice. The precipitate from centrifugation was then re-extracted with 50 mL aqueous acetone (70%, v/v) and the above procedures were repeated to maximize the extract. All extracts were concentrated on a rotary evaporator below 40 ℃ under reduced pressure. The aqueous residue (about 30 mL) was transferred into 50 mL volumetric flask.

Determination of total polyphenol contents

Total polyphenol contents of the aqueous acetone extracts were evaluated by the Prussian blue assay [10]. Diluted extracts (3.0 mL) were mixed with 1 mL of 0.016 mol/L $K_3Fe(CN)_6$ in a tube, then 1 mL of 0.02 mol/L $FeCl_3$ in 0.1 mol/L HCl was added. The contents were mixed well and kept at (24 ± 1) ℃ for 15 min. Then 3 mL of 6.03 mol/L H_3PO_4 was added, and the reagents in the tube were mixed well. After 2 min, 2 mL of 1% gum acacia was added and the color density was measured at 700 nm against a reagent blank. The amount of total polyphenols was calculated as a tannic acid equivalent from the calibration curve of tannic acid standard solutions (covering the concentration range between 3 and 15 mg/L), and expressed as mg tannic acid equivalent (TAE)/g fresh plant material. All measurements were performed in triplicate.

Determination of tannins

Tannin content was determined by Prussian blue assay as above, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone, PVPP) [11]. Insoluble, cross-linked PVPP (approximately 250 mg) was weighed and mixed with 10 mL diluted extracts in test tubes. After 15 min at 4 ℃, tubes were vortexed and centrifuged for 10 min at 4350 g. Aliquots of supernatant were transferred into test tubes and non-absorbed polyphenols determined as described above. Calculated values were subtracted from total polyphenol contents and total tannin contents expressed as mg tannic acid equivalent (TAE)/g fresh plant material. All measurements were performed in triplicate.

Determination of total antioxidant activity

Total antioxidant activity of investigated aqueous acetone extracts was evaluated by the modified FRAP (Ferric Reducing/Antioxidant Power) assay [12]. Diluted extracts (0.1 mL) were transferred into test tubes and 3.0 mL of freshly prepared FRAP reagent (25 mL acetate buffer, 300 mmol/L, pH 3.6 + 2.5 mL 10 mmol/L TPTZ in 40 mmol/L HCl + 2.5 mL 20 mmol/L $FeCl_3 \cdot 6H_2O$) were added. After 5 min, the absorbance was measured at 593 nm against a blank containing 0.1 mL of solvent. Relative activities were calculated from the calibration curve of L-ascorbic acid standard solutions (0.1–1 mmol/L) under the same experimental conditions and expressed as μmol ascorbic acid equivalents (AAE) /g of fresh plant material. All measurements were performed in triplicate.

Results and Discussion

Phenolic contents

Total polyphenol and tannin contents of peel, pulp and seed fractions of eight selected varieties of fruits were measured by the use of Prussian blue method. As shown in Table 1, total polyphenol and tannin contents varied considerably from one kind of variety to another. In addition, they were found to be different in different parts of the fruits. Mean total polyphenol and tannin contents varied from 0.28 to 71.93 mg/g and from 0.05 to 50.94 mg/g of fresh matter, respectively. A good linear relationship between total polyphenol and tannin contents was found (r^2 = 0.9901, P < 0.001, n = 22) (Fig 1). The slope of curve in Fig 1 indicated that the mean tannin content value corresponded to 71% of the mean total polyphenol content value.

Table 1 Total antioxidant activities and contents of polyphenol and tannin of peel, pulp and seed extracts

Sample		Total polyphenols[a]	Tannins[a]	FRAP values[b]
Apple	Peel	1.52±0.03	1.27±0.05	10.07±0.33
	Pulp	0.47±0.06	0.40±0.02	3.01±0.17
	Seed	1.41±0.10	0.86±0.01	3.55±0.07
Pomegranate	Peel	71.93±7.84	50.94±0.33	571.92±11.14
	Pulp	0.72±0.06	0.53±0.02	3.22±0.18
	Seed	2.27±0.42	1.08±0.29	18.52±3.44
Olive	Peel	14.47±0.28	10.61±0.08	75.21±2.23
	Pulp	14.39±0.64	10.84±0.57	73.15±5.91
	Seed	12.80±0.60	10.67±0.41	69.54±1.92
Mango	Peel	16.08±0.40	7.83±0.14	52.22±4.50
	Pulp	1.17±0.06	0.92±0.05	4.79±0.26
	Seed	27.44±0.76	17.82±0.40	87.42±6.56
Banana	Peel	1.05±0.11	0.58±0.08	7.63±0.53
	Pulp	0.47±0.04	0.31±0.08	3.53±0.27
Pineapple	Peel	0.95±0.01	0.63±0.02	6.00±0.30
	Pulp	0.42±0.00	0.24±0.00	2.46±0.14
Grape	Peel	4.78±0.13	3.50±0.08	29.05±0.85
	Pulp	0.28±0.02	0.05±0.01	4.05±0.12
	Seed	15.22±0.42	11.38±0.44	91.80±4.43
Longan	Peel	21.11±0.99	16.13±0.58	118.34±5.49
	Pulp	0.90±0.06	0.56±0.06	5.15±0.16
	Seed	26.72±1.95	21.84±1.32	155.32±4.78

Means of six determinations±SD (standard deviation)
[a] Expressed as mg tannic acid /g fresh plant material
[b] Expressed as μmol AAE /g fresh plant material

The peel and seed extracts contain more polyphenolic and tannin contents than the pulp extract. Some previous studies[2,5,6] focused on phenolic compounds of the seeds of subtropical and tropical fruits, but there are few reports on the phenolic contents of the peel extracts. The results obtained showed that the polyphenolic content of pomegranate peel was the highest, 71.93 mg/g followed by the peel of longan (21.11), mango (16.08), olive (14.47), grape (4.78), apple (1.52), banana (1.05), and pineapple (0.95).

Antioxidant activities

FRAP assay is widely employed to evaluate the total antioxidant activity of plant materials[14]. As shown in Table 1, FRAP values of different fruits are very different, ranging from 2.46 to 571.92 μmol AAE /g of fresh plant materials. The FRAP values vary considerably from one kind of variety to another. In addition, they are found to be different for different parts of the fruits. On the basis of the wet weight, longan seed had the highest antioxidant activity, followed by the seeds of grape, mango, olive, pomegranate and apple. For the peel portion, pomegranate peel showed the highest antioxidant activity, followed by longan, olive, mango, grape, apple, banana and pineapple. And for the pulp fraction, olive showed much higher antioxidant activity than the seven other fruits. Overall, fruit peel and seed extracts showed a much higher antioxidant capacity and polyphenolic contents than the pulp extracts of the tested fruits.

As illustrated in Fig 2 and Fig 3, good correlations were found between FRAP values and total polyphenol content (r^2 = 0.9123, P < 0.001, n = 21) and between FRAP values and tannin content (r^2 = 0.9678, P < 0.001, n = 21). This result strongly suggested that polyphenol content should be considered as an important feature of the fruits and their high levels of antioxidant activities were mainly attributed to the presence of phenolic constituents.

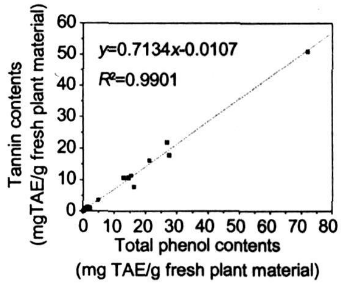

Fig. 1 Correlation of total polyphenol and tannin contents of peel, pulp and seed extracts

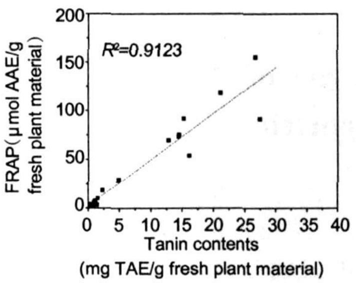

Fig. 2 Correlation of FRAP and total polyphenol contents of peel, pulp and seed extracts

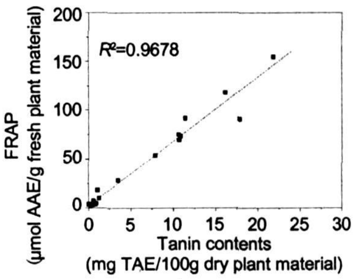

Fig. 3 Correlation of FRAP and tannin contents of peel, pulp and seed extracts

Overall, the observed antioxidant activities and polyphenol contents were greatly dependent on different extracts of different fruit varieties. Interestingly, the peel extracts also exhibited high polyphenolic content and antioxidant activity, especially of the subtropical fruit, as well as the seed extracts. As phenolic compounds with different structures are likely to have different chemical and biological properties, it is important to clearly identify the individual phenolic compounds so that their single or synergistic effect can be further studied.

Conclusions

A great variation of polyphenolic contents and FRAP values in different extracts of different fruit varieties was observed. Total antioxidant capacities and polyphenolic contents of fruit peel and seed extracts were found to be significantly higher than those of the pulp extracts. Approximately 71% of the total polyphenols of eight determined fruits were tannins, which were the major antioxidant components of fruit. Due to the elevated values of their antioxidant activity, the peel and seed extracts of pomegranate, olive, mango, grape and longan could be considered as new potential sources of antioxidants. The further study on their chemical properties and processing technologies, the identification of polyphenolic structures and stability of polyphenols, will be of importance.

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