Food Sci. Technol. Res., **10** (2), 152–156, 2004

DPPH Radical-Scavenging Activity and Polyphenol Content in Dried Fruits

K. ISHIWATA,[1] T. YAMAGUCHI,[2] H. TAKAMURA[2,3] and T. MATOBA[1,2*]

[1]Graduate School of Human Culture, [2]Department of Food Science and Nutrition, and [3]*KYOUSEI Science Center for Life and Nature, Nara Women's University, Kitauoya-Nishimachi, Nara, 630-8506 Japan*

Received August 26, 2003; Accepted January 20, 2004

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and polyphenol content of 25 dried fruits were evaluated and compared with fresh fruits. The contribution of ascorbic acid to this activity was also determined. All dried fruits contained DPPH radical-scavenging activity with hawthorn, apricot and blueberry having the highest. Other dried fruits containing rind also had high activity. The DPPH radical-scavenging activity and polyphenol content of dried fruits were highly correlated.

Keywords: dried fruits, fresh fruits, ascorbic acid, polyphenol, radical-scavenging activity

Recently it has become evident that free radicals and reactive oxygen species injure DNA and biological membrane, and consequently induce cancers, aging and life-style related diseases. Free radical-scavenging activities are detected in various kinds of foods (Arai *et al.*, 2001; Wang and Lin, 2000; Hirota *et al.*, 2000) and the results of clinical applications have been reported (Hibatallah *et al.*, 1999). In addition, the changes of free radical-scavenging ingredients during processing and cooking of vegetables have also become evident (Crozier *et al.*, 1997; Yamaguchi *et al.*, 2001). Therefore, it is anticipated that intake of these radical-scavenging ingredients in the daily diet can prevent cancers, aging and life-style related diseases.

Processed foods, and alcoholic and non-alcoholic beverages with added functional ingredients have been increasing on the market, and the production and consumption of these functional foods and beverages are also increasing (Chandan, 1999). There are many reports of the functional ingredients in vegetables and fruits. We previously reported the functional ingredients of alcoholic and non-alcoholic beverages (Ishiwata *et al.*, 2000, 2001a, b).

Since fresh fruits are rich in radical-scavenging compounds, dried fruits are also expected to be good sources of these compounds. However, these compounds may be decomposed during drying. Therefore, radical-scavenging compounds in dried fruits have to be determined in order to determine the food function of these fruits.

In this paper, we evaluated 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of various dried fruits as well as ascorbic acid and polyphenol contents, and compared them to those of fresh fruits.

Materials and Methods

Materials Dried fruits were purchased from local markets in Nara and Yokohama, Japan. Water content of each dried fruit was determined by drying method at 110°C.

Reagents DPPH, L-ascorbic acid, tris(hydroxymethyl)aminomethane (Tris), 2,4-dinitrophenylhydrazine, acetonitrile (HPLC grade) and Folin-Ciocalteu reagent solution were obtained from Nacalai Tesque Inc. (Kyoto). Ethanol and methanol (HPLC grade) were obtained from Wako Pure Chemical Industries (Osaka). The water used in this experiment was purified with Milli-Q Labo equipment (Millipore Japan, Tokyo).

Preparation of materials Dried fruits from the market were cut into small pieces, smashed in liquid nitrogen and freeze-dried (Vinson *et al.*, 1998). We used those freeze-dried fruit powders throughout this work.

Measurement of DPPH radical-scavenging activities Dried fruit powder was first extracted twice by ethanol, and then the residue was extracted twice by water. Before determination, extracts were filtered through a Cosmonice filter-W (0.45 μm, 25 mm i.d.). Measurement of DPPH radical-scavenging activities was carried out using the DPPH-HPLC method (Yamaguchi *et al.*, 1998). The HPLC analysis was carried out on a TSK gel Octyl-80Ts column (4.6×150 mm, Tosoh, Tokyo) equipped with a Hitachi L-7420 UV-VIS detector at room temperature. The mobile phase was methanol/water (70 : 30, v/v) and the flow rate was 1 ml/min. Detection wavelength was 517 nm.

The radical-scavenging activities of ethanol and water extracts were determined separately and summation of the activities in the two extracts was used. A blank was run without sample, and Trolox was used as the positive control. Radical-scavenging activity was expressed as ascorbic acid equivalent.

Measurement of ascorbic acid Dried fruits were extracted by 5% metaphosphoric acid with or without 1% stannous chloride to determine [dehydroascorbic acid+diketogulonic acid] or [ascorbic acid+dehydroascorbic acid+diketogulonic acid], respectively. Ascorbic acid was measured by HPLC according to Kishida *et al.* (1992). HPLC analysis was carried out on a Cosmosil C18-AR-II column (4.6×250 nm, Nacalai Tesque) with a Hitachi L-7420 UV-VIS detector at room temperature. The mobile phase was acetonitrile/water (50 : 50, v/v) adjusted at pH 3.5 with 0.1% triethylamine and phosphoric

*To whom correspondence should be addressed.
E-mail: matoba@cc.nara-wu.ac.jp

Table 1. Radical-scavenging activity of dried and fresh fruits.

Fruit	Status	Country	Radical-scavenging activity (mg AsA eq/100 g dry weight)	AsA[a] contribution (%)	Water content (%)
Apple	D−r[b]	China	875±28[c]	0.1	34
	F−r[d]	Japan	4,754±69	0.1	84
Apricot	D+r[e]	China	3,846±96	0.0	19
	F+r[f]	Japan	29,413±172	0.0	88
Banana	D−r	PH[g]	295±18	0.0	10
	F−r	PH	547±20	0.0	77
Blueberry	D+r	USA	2,869±49	0.0	32
	F+r	USA	16,724±80	1.8	87
Cherry	D+r	China	1,010±33	0.7	30
	F+r	USA	6,529±33	0.1	78
Cranberry	D+r	USA	3,079±22	0.1	31
Fig	D+r	Turkey	1,087±11	0.1	21
	F+r	Japan	2,524±37	0.1	79
Grape	D+r	USA	1,346±25	0.1	14
	F+r	Japan	2,883±27	0.1	79
Hawthorn	D+r	China	5,439±68	0.2	31
Jujube	D+r	China	2,649±19	0.0	30
Kiwifruit	D−r	China	257±7	0.3	25
	F−r	NZ[h]	2,049±34	3.5	84
Kumquat	D+r	Japan	189±16	0.0	22
Mango	D−r	Thailand	456±24	1.6	31
	F−r	PH	6,841±92	1.2	84
Melon	D−r	China	440±14	0.9	20
	F−r	Japan	2,195±83	2.3	87
Muscat	D−r	China	1,106±6	0.1	19
	F−r	Japan	1,374±12	0.1	79
Papaya	D−r	PH	325±6	1.3	27
	F−r	PH	950±43	5.2	87
Peach	D−r	China	1,442±16	0.1	39
	F−r	Japan	1,949±88	0.7	91
Pear	D−r	China	1,301±6	0.1	34
	F−r	Japan	1,748±66	0.3	89
Pineapple	D−r	Taiwan	321±3	0.3	33
	F−r	Taiwan	798±38	1.1	79
Prune	D+r	USA	3,112±85	0.0	40
	F+r	USA	10,901±94	0.0	79
Rakanka	D+r	China	3,213±123	0.0	9
Strawberry	D−r	China	1,997±62	0.1	26

[a] AsA contribution, ascorbic acid content/radical-scavenging activity×100%. [b] D-r, Dried fruits without rind. [c] Data were means±SD of three determinations. [d] F-r, fresh fruits without rind. [e] D+r, dried fruits with rind. [f] F+r, fresh fruits with rind. [g] PH, Philippines. [h] NZ, New Zealand.

acid at the flow rate of 1 ml/min. Detection wavelength was at 505 nm. Ascorbic acid content was calculated by subtraction of [dehydroascorbic acid+diketogulonic acid] from [ascorbic acid+dehydroascorbic acid+diketogulonic acid].

Measurement of polyphenol content Dried fruits were extracted by 80% methanol. Measurement of polyphenol content was carried out according to Singleton and Rossi (1965) and Kähkönen *et al.* (1999) using Folin-Ciocalteu reagent solution.

Results and Discussion

Radical-scavenging activities of dried and fresh fruits DPPH radical-scavenging activity of 22 kinds of dried fruits and 16 kinds of fresh fruits corresponding to the dried fruits are shown in Table 1. Among dried fruits, hawthorn contained the highest radical-scavenging activity. Dried fruits with rind such as apricot, cranberry, prune, and rakanka also contained high activity. However, the contribution of ascorbic acid was negligible in dried fruits, which suggests that the radical-scavenging activity may originate from other active compounds such as polyphenols. Table 1 also shows that the radical-scavenging activity of dried fruits was lower than that of the corresponding fresh fruits on a dry weight basis. On a fresh weight basis, however, dried fruits generally contained higher activity than fresh fruits. These results suggest that the dried fruits, especially those with rind, are a good source of radical-scavenging activity.

Radical-scavenging activity in rind and pulp of fresh fruits Since high radical-scavenging activity was found in dried fruits with rind, the activity in rind and pulp of fresh fruits was determined (Table 2). In all fruits except apricot, the activity of rind was higher than that of pulp. However, fresh fruits are usually eaten without the rind. Therefore, dried fruits with rind are suitable for efficient intake of radical-scavenging activity.

Leong & Shui (2002) reported that the contribution of ascorbic acid to radical-scavenging activity in fresh fruits was higher than our results. This may be due to the difference in the methods used, as previously reported by Murakami *et al.* (2002). They determined 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity, while we used DPPH radical.

Polyphenol content of dried and fresh fruits Polyphenol content of dried and fresh fruits is shown in Table 3. Among dried fruits, hawthorn contained the largest amount of total polyphenols, followed by blueberry, grape, and apricot. In addi-

Table 2. Radical-scavenging activity of pulp and rind of fresh fruits.

Fruit	Country	Part	Radical-scavenging activity (mg AsA eq/100 g dry weight)	AsA[a] contribution (%)	Water content (%)
Apple	Japan	rind	17,010±84[b]	0.1	71
	Japan	pulp	4,755±69	0.1	84
Apricot	Japan	rind	9,006±68	0.1	87
	Japan	pulp	20,516±135	0.0	88
Banana	PH[c]	rind	1,099±11	0.1	70
	PH	pulp	547±20	0.1	77
Fig	Japan	rind	3,420±100	0.1	75
	Japan	pulp	2,524±37	0.1	79
Grape	Japan	rind	11,126±42	0.0	77
	Japan	pulp	1,283±57	0.1	81
Kiwifruit	NZ[d]	rind	16,459±134	0.2	65
	NZ	pulp	2,049±34	3.5	84
Mango	PH	rind	24,802±78	0.0	75
	PH	pulp	6,841±92	0.6	85
Muscat	Japan	rind	3,458±80	0.1	80
	Japan	pulp	1,374±62	0.1	81
Papaya	PH	rind	4,056±77	0.7	82
	PH	pulp	950±42	5.2	87
Peach	Japan	rind	8,460±67	0.1	76
	Japan	pulp	1,949±88	0.7	91
Pear	Japan	rind	2,416±17	0.2	74
	Japan	pulp	1,748±66	0.3	79
Prune	USA	rind	4,906±80	0.0	70
	USA	pulp	1,375±6	0.1	84

[a] AsA contribution, ascorbic acid content/radical-scavenging activity×100%. [b] Data were means±SD of three determinations. [c] PH, Philippines. [d] NZ, New Zealand.

Table 3. Polyphenol content of dried and fresh fruits.

Fruit	Status	Country	Total polyphenols (mg GAE[a]/100 g dry weight)	Aglycons (mg GAE/100 g dry weight)	(%)
Apple	D-r[b]	China	916±7[c]	103±1	11.3
	F-r[d]	Japan	2,168±38	470±28	21.7
Apricot	D+r[e]	China	2,256±13	634±12	28.1
	F−r[f]	Japan	3,062±25	2,330±52	76.1
Banana	D−r	PH[g]	413±3	25±0	6.1
	F−r	PH	804±5	244±3	30.3
Blueberry	D+r	USA	2,832±4	185±2	6.5
	F+r	USA	3,590±31	2,390±14	66.6
Cherry	D+r	China	828±7	226±14	27.3
	F+r	USA	1,017±28	265±51	26.1
Cranberry	D+r	USA	1,819±8	332±6	18.2
Fig	D+r	Turkey	1,234±41	136±16	11.0
	F+r	Japan	1,699±24	361±3	21.3
Grape	D+r	USA	2,414±7	354±5	14.7
	F+r	Japan	3,202±50	939±11	29.3
Hawthorn	D+r	China	3,563±11	1,138±5	31.9
Jujube	D+r	China	1,152±4	350±4	30.4
Kiwifruit	D−r	China	758±6	276±4	36.4
	F−r	NZ[h]	930±11	676±15	72.7
Kumquat	D+r	Japan	530±4	193±5	36.5
Mango	D−r	Thailand	885±9	323±6	36.5
	F−r	PH	1,448±91	544±24	37.6
Melon	D−r	China	555±8	1±0	0.2
	F−r	Japan	1,042±17	138±2	13.2
Muscat	D−r	China	1,161±43	163±10	14.0
	F−r	Japan	1,558±24	304±2	19.5
Papaya	D−r	PH	389±2	284±3	73.0
	F−r	PH	510±8	236±2	46.3
Peach	D−r	China	1,260±29	222±16	17.6
	F−r	Japan	1,833±13	398±2	21.7
Pear	D−r	China	1,196±21	316±7	26.4
	F−r	Japan	1,328±14	321±6	24.1
Pineapple	D−r	Taiwan	802±5	293±3	36.5
	F−r	Taiwan	1,248±13	349±10	27.9
Prune	D+r	USA	1,032±6	183±3	17.7
	F+r	USA	1350±7	509±5	37.7
Rakanka	D+r	China	2,007±20	697±13	34.7
Strawberry	D−r	China	1,162±17	382±6	32.8

[a] GAE, gallic acid equivalent. [b] D-r, dried fruits without rind. [c] Data were means±SD of three determinations. [d] F-r, fresh fruits without rind. [e] D+r, Dried fruits with rind. [f] F+r, fresh fruits with rind. [g] PH, Philippines. [h] NZ, New Zealand.

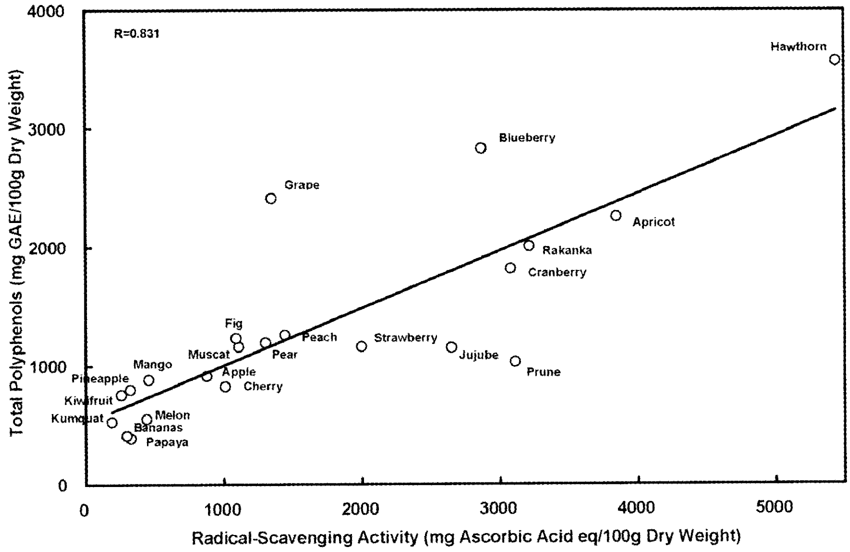

Fig. 1. Correlation between radical-scavenging activity and total polyphenols in dried fruits. GAE, gallic acid equivalent.

Table 4. Polyphenol content of pulp and rind of fresh fruits.

Fruit	Country	Part	Total polyphenols (mg GAE[a]/100 g dry weight)	Aglycons (mg GAE/100 g dry weight)	Aglycons (%)
Apple	Japan	rind	3,555±35[b]	2,335±27	65.7
	Japan	pulp	2,168±38	470±28	21.7
Apricot	Japan	rind	1,390±38	971±23	69.9
	Japan	pulp	3,063±24	2,330±35	76.1
Banana	PH[c]	rind	2,799±13	1,724±9	61.6
	PH	pulp	804±5	244±3	30.3
Fig	Japan	rind	2,092±17	798±6	38.2
	Japan	pulp	1,699±24	361±3	21.3
Grape	Japan	rind	1,450±24	1,280±14	88.3
	Japan	pulp	442±3	74±6	16.7
Kiwifruit	NZ[d]	rind	1,282±24	1,068±10	83.3
	NZ	pulp	930±11	676±15	72.7
Mango	PH	rind	16,475±189	14,157±127	85.9
	PH	pulp	1,448±91	544±24	37.6
Muscat	Japan	rind	3,418±52	2,061±12	60.3
	Japan	pulp	1,558±24	304±2	19.5
Papaya	PH	rind	744±9	12±3	1.6
	PH	pulp	510±8	236±2	46.3
Peach	Japan	rind	2,258±30	1,013±15	44.9
	Japan	pulp	1,833±13	398±2	21.7
Pear	Japan	rind	2,013±24	1,958±8	97.3
	Japan	pulp	1,328±14	321±6	24.1
Prune	USA	rind	4,608±55	3,730±20	81.0
	USA	pulp	1,800±38	1,424±33	79.1

[a] GAE, Gallic acid equivalent. [b] Data were means±SD of three determinations. [c] PH, Philippines. [d] NZ, New Zealand.

tion, the total polyphenol content of dried fruits with rind was high. The fruits containing high DPPH radical-scavenging activity tended to contain a large amount of polyphenols; as shown in Fig. 1, these two factors are highly correlated in dried fruits. These results suggest that the contribution of polyphenols to radical-scavenging activity is large in these fruits.

Table 3 also shows that the polyphenol content of dried fruits was lower than that of the corresponding fresh fruits on dry weight basis. The ratio of aglycons was also lower in dried fruits. Aglycons probably became more decomposed than glycosides during preparation of the dried fruits, since aglycons are less stable than glycosides (Rice-Evans & Miller, 1996; Chuda *et al.*, 1998).

In this study, individual polyphenolic compounds could not be determined, however, polyphenols in some fresh fruits have been reported: cyanidin and peonidin-3-galactose in cranberry (Kähkönen *et al.*, 2001); cyanidin and chlorogenic acid in blueberry (Tsushida, 1998) and prune (Donovan *et al.*, 1998); and catechin, epicatechin, gallic acid and malvidine-3-glucoside in grape (Iwashina, 1994). Further study on individual compounds in fresh and dried fruits is necessary.

Polyphenol content of rind and pulp of fresh fruits Polyphenol content of rind and pulp of fresh fruits was determined (Table 4). In all fruits except apricot, the polyphenol content of rind was higher than that of pulp, as true of the radical-scavenging activity. Since fresh fruits are usually eaten without rind, eating dried fruits with rind are a good means of efficient intake of polyphenols as well as radical-scavenging activity.

Conclusions

Among the dried fruits examined, hawthorn, apricot and blueberry had the highest radical-scavenging activity. Though ascorbic acid did not contribute to this activity, polyphenol content was highly correlated with it. Since dried fruits, especially with rind, contain high radical-scavenging activity and polyphenol content, they as well as fresh fruits are considered healthy and suitable for preventing life-style related diseases.

References

Arai, S., Osawa, T., Ohigashi, H., Yoshikawa, M., Kaminogawa, S., Watanabe, M., Ogawa, T., Okubo, K., Watanabe, S., Nishino, H., Shinohara, K., Esashi, T. and Hirahara, T. (2001). A mainstay of functional food science in Japan—history, recent status, and future outlook. *Biosci. Biotechnol. Biochem.*, **65**, 1–13.

Chandan, R.C. (1999). Enhancing market value of milk by adding cultures. *J. Dairy Sci.*, **82**, 2245–2256.

Chuda, Y., Suzuki, M., Nagata, T. and Tsushida, T. (1998). Contents and cooking loss of three quinic acid derivatives from garland (*Chrysanthemum coronarium* L.). *J. Agric. Food Chem.*, **46**, 1437–1439.

Crozier, A., Lean, M.E.J., McDonald, M.S. and Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *J. Agric. Food Chem.*, **45**, 590–595.

Donovan, J.L., Meyer, A.S. and Waterhouse, A.L. (1998). Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *J. Agric. Food Chem.*, **46**, 1247–1252.

Hibatallah, J., Carduner, C. and Poelman, M.C. (1999). *In-vivo* and *in-vitro* assessment of the free-radical-scavenger activity of ginkgo flavone glycosides at high concentration. *J. Pharm. Pharmacol.*, **51**, 1435–1440.

Hirota, A., Taki, S., Kawaii, S., Yano, M. and Abe, N. (2000). 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging compounds from soybean miso and antiproliferative activity of isoflavones from soybean miso toward the cancer cell lines. *Biosci. Biotechnol. Biochem.*, **64**, 1038–1040.

Ishiwata, K., Takamura, H. and Matoba, T. (2000). DPPH radical-scavenging activity of commercial beverages. *J. Cook. Sci. Jpn.*, **33**, 483–493.

Ishiwata, K., Murakami, M., Takamura, H. and Matoba, T. (2001a). Changes in the DPPH radical-scavenging activity and ascorbic acid content of commercial beverages during preservation. *J. Cook. Sci. Jpn.*, **34**, 68–72.

Ishiwata, K., Takamura, H. and Matoba, T. (2001b). DPPH radical-scavenging activity of commercial alcoholic drinks. *J. Cook. Sci. Jpn.*, **34**, 407–417.

Iwashina, T. (1994). Flavonoids of fruits. *Food Ind.*, **37**, 52–69.

Kähkönen, M.P., Hopia, A.I. and Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.*, **49**, 4076–4082

Kähkönen, M.P., Hopia, A.I., Vuorelam, H.J., Rauham, J.-P., Pihlaja, K., Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, **47**, 3954–3962.

Kishida, E., Nishimoto, Y. and Kojo, S. (1992). Specific determination of ascorbic acid with chemical derivatization and high-performance liquid chromatography. *Anal. Chem.*, **64**, 1505–1507.

Leong, L.P. and Shui, G. (2002). An investigation of fruits in Singapore markets. *Food Chem.*, **76**, 69–75

Murakami, M., Yamaguchi, T., Takamura, H. and Matoba, T. (2002) A comparative study on the various in vitro assays of active oxygen scavenging activity in foods. *J. Food Sci.*, **67**, 539–541

Rice-Evans, C.A. and Miller, N.J. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Biochem. Soc. Trans.*, **24**, 790–795.

Singleton, V.L. and Rossi, J.A., Jr. (1965). Phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic.*, **16**, 144–158.

Tsushida, T. (1998). Physiological Functionality of Blueberry. *Food Ind.*, **31**, 5–8.

Vinson, J.A., Hao, Y., Su, X. and Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.*, **46**, 3630–3634.

Wang, S.Y. and Lin, H.S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.*, **48**, 140–146.

Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.*, **62**, 1201–1204.

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J., Kanazawa, K., Takamura, H. and Matoba, M. (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Sci. Technol. Res.*, **7**, 250–257.