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Technical paper

Characteristics of Shodo Island Olive Oils in Japan: Fatty Acid Composition and Antioxidative Compounds

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The fatty acid composition, total phenol content and tocopherol content of olive oils from Shodo Island of Japan were analyzed for different varieties, maturation stages and extraction methods. Radical-scavenging activity of olive oils was also determined spectrophotometrically by measuring the disappearance of 1,1-diphenyl-2-picrylhydrazyl radical at 517 nm. The differences in fatty acids composition, total phenol and tocopherol content in virgin olive oils were not great among the varieties. The content of oleic acid was in range of 66–78%. Though virgin olive oils contained more phenolic compounds than hexane-extracted olive oils, tocophenol content was not very different. In hexane-extracted olive oils, the total phenol content decreased during olive maturation. The tocopherol content of three types of olive oils also decreased during maturation. Still, large amounts of phenolic compounds remained in the olive residue after hexane extraction. The contribution of tocopherols to radical-scavenging activity was 39–61% in virgin olive oil, which suggests that both tocopherols and phenolic compounds contribute to radical-scavenging activity.

Keywords: olive oil, fatty acid composition, phenol, tocopherol, radical-scavenging activity

Introduction

Olive oil, one of the oldest known vegetable oils, is prepared from olive fruit (*Olea europaea* L.) (Boekenoogen, 1968; Weiss, 1983). Virgin olive oil is rich in oleic acid, natural antioxidants (phenolic compounds and tocopherols) and aromatic compounds (Ranalli *et al.,* 1999; Cavalli *et al.,* 2003). Antioxidative components and polyunsaturated fatty acids in olive oil effect serum lipidic profile and decrease LDL susceptibility to oxidation (Visioli and Galli, 1998; Fitó *et al.,* 2000; Leenen *et al.,* 2002). Therefore, the demand of olive oil is increasing even in countries lacking a tradition of olive oil production, including Japan (Asakura, 1997).

The major olive oil producing countries in the world are located throughout the Mediterranean area. The climate in this region is suitable for producing high quality olive oil. The composition of olive oil is known differ with factors such as cultivar, variety, processing method, rainfall, irrigation, maturation and storage (Giovacchino *et al.,* 1994; Gutiérrez *et al.,* 1999; García *et al.,* 1996; Tovar *et al.,* 2001; Vierhuis *et al.,* 2001; Brenes *et al.,* 2000).

In Japan, olives grow on Shodo Island, which is separated from the main olive oil production area of the world. Although the history of planting and processing on Shodo Island is limited to about one hundred years, the characteristics of olive oil produced there have not yet been reported in detail (Shibasaki, 1999; Shibasaki and Hirai, 2000). Solvent extraction and pressing extraction are two of the main methods of preparing vegetable oils in the industry. There has been no report on the difference of oil characteristics prepared with the two methods. Considering the importance and benefit of olive products for health, the antioxidant characteristics of olive oil produced on Shodo Island should be better understood. Therefore, the purpose of this study is to assess the characteristics of olive oil produced on Shodo Island, Japan. In this study, we evaluated the fatty acid composition and the antioxidative compounds found in Shodo Island virgin olive oil and in hexane-extracted olive oil prepared from different varieties and at different ripening stages of olive fruits. For antioxidative compounds, we examined total phenol and tocopherol content in olive oils. We also determined radical-scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Materials and Methods

Materials Olive fruits (*Olea europaea* L.), *cv.* Manzanillo, Lucca, Nevadillo Blanco, and Mission were harvested during October and November 2003, in Uchinomi Town Olive Garden Public Co., Shodo Island, Kagawa, Japan.

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These cultivars are typical varieties grown on Shodo Island. Each cultivar was separated into three maturation stages (unripe, half-ripened and fully ripened) based on the fruit color (green, red, and black). Green: olives with intense or dark green skin, which were in the unripened stage (unripe). Red: olives with reddish spots or areas on the skin, which were in half-ripened stage (half-ripened). Black: olives with totally black or violet skin, which were in the ripened stage (fully ripened).

Virgin olive oils were prepared with a continuous two-phase centrifugation olive oil processing plant (Oliomio 50, Toscana Enologica Mori, Italy) from randomly selected olive fruits of the four varieties indicated previously. The processing methods included selecting, crushing, kneading, pressure-centrifugation percolation and centrifugation, which separates virgin olive oil from the aqueous phase of the fruit.

Reagents Analytical grade *n*-hexane, methanol, ethanol, sodium methoxide, toluene, and HPLC grade *n*-hexane and 2-propanol were obtained from Wako Pure Chemical Industries (Osaka, Japan). DPPH, gallic acid, 1,4-dioxane and Folin-Ciocalteu reagents were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The water used in this experiment was purified with Mili-Q Labo equipment (Millipore Japan, Tokyo, Japan).

Tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol) and 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC) were from Eizai Co. (Tokyo, Japan).

Sample preparation Fresh olive fruits were first frozen with liquid nitrogen, and powdered by grinding with pestle and mortar. The obtained powder was lyophilized with a freeze dryer (VO-400F, Taitec, Japan). The dried powder was extracted with 10 times volume of hexane at 4℃ for 30 min in the dark. The suspension was centrifuged at 15,000×g at 4℃ for 20 min. The extraction step was repeated twice. The resulting supernatant was combined and evaporated with rotary evaporator. The oil obtained was sealed under argon gas and stored at −80℃ until analysis. The resulting residue was dried under vacuum to remove extra hexane. The obtained powder was ground into finer powder with a mortar with pestle and kept at −80℃ until analysis.

Water and oil contents Water content was determined by drying at 110℃ (American Oil Chemists' Society, 1989). Oil content was gravimetrically determined by extracting oil from lyophized fruit with *n*-hexane. The content was expressed as percentage of fresh weight (García *et al.,* 1996; Esti *et al.,* 1998).

Total phenol content The total phenol content was determined according to the method of Brenes *et al.* (2000) with little modification. Briefly, 1 g of oil sample was mixed with 1 ml of 60% methanol and 6 N HCl mixture (2: 1, v/v) at room temperature. The mixture was occasionally stirred for 4 h and then centrifuged at 2,000×g for 5 min; the aqueous phase was washed three times with 2-ml aliquots of hexane. The total phenol content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The content was expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

Fatty acid composition The fatty acid composition was determined after methylation according to the official method of the AOCS (American Oil Chemists' Society, 1989) using GLC (Shimadzu GC-17A, Kyoto, Japan) with a flame ionization detector (FID) and the capillary column (HR-SS-10, 25 m×0.25 mm, Ulbon, Shinwa Chemical Industries Ltd., Kyoto, Japan). The flow rate of argon gas was 0.6 ml/min, and the column oven temperature was programmed to increase from 160℃ to 220℃ at the rate of 2℃/min.

Tocopherol content Tocopherol content was determined according to the method of Ueda and Igarashi (1990) by HPLC coupled with fluorescence detector. The HPLC system consisted of a Cosmosil 5SL-11 column (4.6×250 mm), a L-6000 pump and a F-1000 fluorescence detector (Hitachi, Tokyo, Japan). Excitation and emission wavelengths were set at 295 nm and 325 nm, respectively. The mobile phase was *n*-hexane/1,4-dioxane/2-propanol (98.5/1/0.5, by vol.) pumped at a flow rate of 1.0 ml/min. Oil was diluted in *n*-hexane (between 50- and 100-folds) before analysis, and PMC was used as an internal standard. The content was expressed as mg of tocopherol per 100 g of oil.

DPPH radical-scavenging activity DPPH radical-scavenging activity was evaluated using the method of Blois (1958) and Espín *et al.* (2000). Oil samples and DPPH were dissolved in ethyl acetate. An aliquot of sample solution (160 μl) was mixed with the 100 mM DPPH solution (1840 μl). The mixture was left to stand for 30 min at room temperature in the dark. The absorbance by DPPH was measured at 517 nm by a UV-2100PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The DPPH radical-scavenging activity was determined from the difference in absorbance detected at 517 nm resulting from the decrease of the DPPH radical between the blank and each sample. Trolox was used as a control. The data are expressed as μ mol of Trolox equivalent of 100 g of each sample. The DPPH radical-scavenging activity of tocopherol standards (α-, β-, γ- and δ) was also analyzed according to the method stated.

Statistical Analysis The data are presented as mean ±standard deviation of three preparations. Student's t-test was accomplished using Microsoft Excel.

Results and Discussion

Water and oil contents of olive fruits The water and oil contents of olive fruits of different varieties and ripening stage are shown in Table 1. The water content was 68.6–79.4%. The skin color of fruit changes from "green" (the first stage of ripening) to "black" during ripening. The water content decreased 2.0〜13.1% in the course of ripening (Table 1). Esti *et al.* (1998) reported the same moisture decrease pattern for olive fruits in the Molise region. The oil content was different among the four varieties, ranging from 12.4–22.6% (Table 1). Lucca showed the highest oil content. However, significant differences for each ripening stage were not observed in all varieties. García *et al.* (1996) reported that the oil content of olive fruits in Spain did not change significantly during ripening.

Table 1. Water and oil contents in olive fruits.

Variety	Maturation stage	Water content (%)[a]	Difference (%)	Oil content (%)
Manzanillo	Green	79.4±3.7[b]	0	14.1±3.5
	Red	77.6±1.2	-2.0	14.8±1.3
	Black	74.9±1.7	-5.7	15.2±1.0
Lucca	Green	77.0±3.1	0	20.1±5.5
	Red	70.2±0.1	-8.8	17.6±3.2
	Black	69.3±1.4	-10.0	22.6±5.5
Nevadillo Blanco	Green	78.9±1.9	0	12.4±1.9
	Red	72.1±4.1	-8.6	16.7±2.1
	Black	68.6±0.4	-13.1	15.9±0.2
Mission	Green	79.1±1.5	0	14.5±2.0
	Red	76.1±1.6	-3.8	14.5±0.4
	Black	70.6±3.7	-10.8	17.1±1.4

[a] Weight percentage to the value for fresh materials.
[b] Values are means±standard deviations for three preparations.

Total phenol content The total phenol content of olive oil and residue is shown in Table 2. The oils analyzed were virgin and hexane-extracted olive oils from the olive fruits of various varieties and at different ripening stages. The total phenol content of virgin olive oil was 7.9–17.2 mg GAE/100 g oil. The total phenol content of olive oil in Italy, Spain, and Greece were reported to be 73–265 mg GAE/kg (Pellegrini *et al.,* 2001), 85.3–219.0 ppm as caffeic acid (89.0–228.5 mg GAE/kg) (Gutiérrez *et al.,* 2002) and 78–339 mg/kg as caffeic acid (74.1–353.8 mg GAE/kg) (Psomiadou and Tsimidou, 1998), respectively. The total phenol content in virgin olive oils of Shodo Island was at a level similar to those in Italy, Spain, and Greece.

The total phenol content of hexane-extracted oil was 0.7–7.9 mg GAE/100 g oil. Phenolic compounds present in olive oil can be classified as lipophilic and hydrophilic (Baldioli *et al.,* 1996). The total phenolic content of virgin olive oil was higher than that of hexane-extracted olive oil. This could be explained by the finding that hexane hardly extracted hydrophilic phenolic compounds from the fruit.

In the hexane-extracted olive oils, total phenol content was the highest in green olives and was significantly decreased during ripening, except in Lucca. This tendency agrees with the observation by Gutiérrez *et al.* (1999). This result suggests that the skin color of fruit can be a good index to evaluate total phenol content.

Next, we examined the total phenol content of residues after extraction of oil by hexane. The total phenol content was 514.7–1254.1 mg GAE/100 g in fresh olives (without pits), showing that the total phenol content of the residue about 100 times higher than that of virgin olive oils (Table 2). This suggests that the defatted residue, which has not been widely utilized, could be a good resource of phenolic compounds. Phenolic fractions of olive oils are being identified as an anti-oxidative stress in human cells (Manna, *et al.*, 2002). Dietary intake of natural antioxidants, including phenolic compounds, is expected to prevent lifestyle diseases, including cancer and cardiovascular diseases (Ames, 1983; Shukla *et al.,* 1996).

It is noteworthy that for defatted olive, which has been disregarded with respect to antioxidant properties, further investigation may be worthwhile.

Tocopherol content Table 3 shows that for tocopherol content of olive oils, α-tocopherol is the major component and δ-tocopherol is found in trace amounts in both virgin and hexane-extracted olive oils. The tocopherol content of each type of virgin oil was similar among the varieties, showing α-tocopherol content of 20.1–23.9 mg/100 g. The α-tocopherol content of virgin olive oil in foreign countries was reported as follows; 64.1–146.6 mg/kg (Ranalli *et al.,* 1999) and 121–369 mg/kg in Italy (Pellegrini *et al.,* 2001) 156–253 ppm in Spain (Gutiérrez *et al.,* 2002) and 184–284 mg/kg in Greece (Psomiadou and Tsimidou, 1998). These results indicate that the α-tocopherol content of Shodo Island olive oil is comparable to olive oils of the Mediterranean region.

Next, the effect of ripening on tocopherol content was examined using the hexane-extracted oils. α-Tocopherol content of Nevadillo Blanco, Mission and Manzanillo decreased 33%, 31% and 27%, respectively, during the maturation from "green" to "black". However, α-tocopherol content of Lucca did not change in this pattern. Gutiérrez *et al.* (1999) reported that the tocopherol content of olive oil in Spain decreased during the ripening. The farmers usually determine olive fruit maturation stage by fruit color. Therefore, this may offers a relatively simple way for farmers to get higher quality olive oil with higher antioxidant compound content such as tocopherols.

Fatty acid composition The fatty acid composition of various olive oils is showed in Table 4. The oils analyzed were virgin olive oils and hexane-extracted olive oils from the fruits of different varieties and at different ripening stages. The content of oleic acid (18: 1), a major fatty acid of olive oil, was in range of 66～78% in the virgin oils. The content of docosaenoic acid (22: 1) of the virgin oils

Table 2. Total phenol content in olive oils and residue.

Oils and residue	Variety	Maturation stage	Total phenol content (mg gallic acid eq./100 g)[a]
Virgin olive oil	Manzanillo		10.8±0.4
	Lucca		7.9±0.8
	Nevadillo Blanco		16.5±0.3
	Mission		17.2±0.6
Hexane-extracted olive oil	Manzanillo	Green	7.9±0.8
		Red	6.3±0.1*
		Black	3.2±0.6***
	Lucca	Green	5.1±0.5
		Red	3.5±0.5**
		Black	3.7±2.1
	Nevadillo Blanco	Green	4.3±0.2
		Red	2.6±0.4***
		Black	0.7±0.3***
	Mission	Green	7.4±0.6
		Red	4.4±0.2***
		Black	2.4±0.7***
Residue after hexane extraction	Manzanillo	Green	1050.3±60.5
		Red	857.4±29.1**
		Black	845.3±60.1**
	Lucca	Green	791.7±2.6
		Red	563.2±33.1***
		Black	514.7±7.4***
	Nevadillo Blanco	Green	1254.1±190.2
		Red	1043.3±34.4
		Black	891.7±26.0*
	Mission	Green	1112.1±120.3
		Red	1044.5±146.0
		Black	907.9±66.1*

[a] Values are means±standard deviations for three preparations and expressed as mg equivalent of gallic acid for olive oil and fresh olive (without seed) after oil extraction.
* Significantly different from green sample ($p<0.1$).
** Significantly different from green sample ($p<0.05$).
*** Significantly different from green sample ($p<0.01$).

was higher than that of the hexane-extracted oils. The overall fatty acid composition of olive oils in Shodo Island (Table 4) was similar to that of the oils reported previously (Cinquanta *et al.* 2001; Gutiérrez *et al.* 1999; Beltrán *et al.,* 2004).

Radical-scavenging activity The DPPH radical-scavenging activity of virgin and hexane-extracted olive oils is shown in Table 5. The activity of virgin oils was similar among the three varieties other than Lucca. The DPPH radical-scavenging activity of Lucca olive oil was lower than that of other olive oils (Table 5). The lower phenol (Table 2) and tocopherol content (Table 3) in Lucca olive oil than in other olive oils was responsible for the lower DPPH radical-scavenging activity. The activity of each hexane-extracted oil obtained from various varieties of fruits was very similar to that of each virgin olive oil.

Next, the contribution of tocopherols to DPPH radical-scavenging activity in olive oils was calculated (Table 5). The contribution was 39–61% in virgin olive oils and 44–64% in hexane-extracted olive oils. Therefore, both tocopherols and phenolic compounds are the main antioxidants in olive oil. This result is in accordance with those reported by Baldioli *et al.* (1996) and Espín *et al.* (2000). Further investigation is underway to confirm the effect of phenolic compounds to DPPH radical scavenging activity.

Conclusion

Fatty acid composition, total phenol, and tocopherol content of olive oils differed among the four varieties produced on Shodo Island, Japan. The quality of these oils was comparable with virgin olive oils produced in the other countries, such as Spain and Italy. There were more phenolic compounds in the virgin olive oils than in

the hexane-extracted oils. Large amounts of phenolic compounds remained in the residue after extraction by hexane, which indicates that the residue is a source of phenolic compounds to be developed such as natural antioxidant additive.

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Table 3. Tocopherol content in olive oil (mg/100 g oil).

Olive Oil	Variety	Maturation stage	Tocopherol (mg/100 g)[a]			
			α	β	γ	Total
Virgin olive oil	Manza nillo		23.9±0.1[b]	1.2±0.0	2.3±0.0	27.4±0.1
	Lucca		20.6±0.7	0.4±0.0	0.4±0.0	21.4±0.7
	Nevadillo Blanco		22.8±0.8	0.4±0.0	1.6±0.1	24.8±0.7
	Mission		20.1±0.2	0.8±0.0	0.6±0.0	21.5±0.2
Hexane-extracted olive oil	Manza-nillo	Green	28.1±3.8	3.3±0.5	1.6±0.0	33.0±4.3
		Red	20.9±0.1	2.1±0.1	2.2±0.2	25.2±0.4
		Black	20.6±1.9	2.0±0.0	2.0±0.3	24.6±2.2
	Lucca	Green	23.1±3.8	0.6±0.1	0.3±0.1	24.0±4.0
		Red	19.9±3.0	0.5±0.1	0.3±0.0	20.7±3.1
		Black	22.3±3.9	0.8±0.4	0.4±0.2	23.5±4.5
	Nevadillo Blanco	Green	29.1±2.2	2.4±0.5	0.9±0.0	32.4±2.7
		Red	23.7±1.1	1.6±0.1	1.2±0.1	26.5±1.3
		Black	19.6±2.0	1.0±0.3	1.2±0.2	21.8±2.5
	Mission	Green	28.9±0.9	3.0±0.5	0.6±0.1	32.5±1.5
		Red	24.5±0.9	2.1±0.1	0.9±0.1	27.5±1.1
		Black	19.9±3.9	1.4±0.2	0.9±0.4	22.2±4.5

[a] δ-Tocopherol was not detected in virgin olive oils. The amount of δ-tocopherol in hexane-extracted olive oils was less than 0.1%.
[b] Values are means±standard deviations for three preparations.

Table 4. Fatty acid composition of olive oils.

Olive oil	Variety	Maturation stage	Fatty acid (%)[a]								
			16:0	16:1n-9	18:0	18:1n-9	18:2n-6	18:3n-3	20:0	22:0	22:1n-9
Virgin olive oil	Manzanillo		14.8±0.3[b]	2.0±0.0	2.9±0.5	66.9±5.6	7.1±1.0	0.5±0.1	0.3±0.0	tr[c]	5.5±0.1
	Lucca		13.9±0.1	1.3±0.0	1.8±0.0	70.1±0.1	10.0±0.0	0.4±0.0	0.3±0.0	0.3±0.0	2.2±0.0
	Nevadillo Blanco		13.5±0.1	1.2±0.0	1.6±0.0	68.2±0.3	12.4±0.1	0.3±0.0	0.3±0.0	tr	2.5±0.2
	Mission		10.2±0.3	0.7±0.0	1.8±0.0	75.3±0.2	8.6±0.1	0.3±0.0	0.3±0.0	tr	2.8±0.1
Hexane-extracted olive oil	Manzanillo	Green	10.2±0.3	0.7±0.0	1.8±0.0	75.3±0.2	8.6±0.1	0.3±0.0	0.3±0.0	tr	2.8±0.1
		Red	5.3±0.3	2.0±0.0	2.9±0.5	66.6±5.6	6.9±1.0	0.5±0.1	0.2±0.0	tr	1.1±0.0
		Black	15.4±0.4	2.1±0.3	3.0±0.5	67.1±4.8	8.5±3.0	0.4±0.0	0.2±0.0	tr	1.0±0.1
	Lucca	Green	14.9±0.5	1.1±0.0	1.8±0.1	70.9±1.1	10.0±0.6	0.4±0.0	0.3±0.0	tr	0.5±0.1
		Red	15.0±0.5	1.4±0.1	1.8±0.1	69.9±1.3	10.9±0.8	0.4±0.0	0.3±0.0	tr	0.4±0.0
		Black	15.1±0.4	1.5±0.2	2.0±0.1	68.6±2.0	11.9±0.0	0.4±0.0	0.3±0.0	tr	0.4±0.0
	Nevadillo Blanco	Green	15.1±1.1	1.4±0.3	1.7±0.0	69.4±0.3	10.9±1.1	0.4±0.0	0.3±0.0	tr	0.8±0.1
		Red	14.4±0.5	1.1±0.1	1.6±0.0	69.1±0.8	12.6±0.3	0.3±0.0	0.3±0.0	tr	0.5±0.0
		Black	13.4±0.2	1.1±0.2	1.6±0.1	70.1±0.7	12.6±0.7	0.3±0.0	0.3±0.0	tr	0.5±0.1
	Mission	Green	11.1±0.3	0.7±0.1	1.7±0.0	78.2±0.9	7.0±1.3	0.3±0.0	0.3±0.0	tr	0.6±0.1
		Red	11.4±0.8	0.8±0.1	1.6±0.1	75.4±2.5	9.5±1.6	0.3±0.0	0.3±0.0	tr	0.6±0.0
		Black	11.2±0.7	0.8±0.1	1.8±0.0	73.7±1.0	11.4±0.1	0.3±0.0	0.3±0.0	tr	0.5±0.1
Previous Reports		1[d]	16.5±0.9	1.6±0.1	2.5±0.2	69.1±0.7	9.0±0.2	0.7±0.0	-	-	-
		2[e]	9.8±0.1	0.5±0.0	3.1±0.0	79.2±0.1	7.3±0.0	0.8±0.0	0.4±0.0	tr	-
		3[f]	9.9±0.2	0.7±0.0	2.5±0.1	81.0±0.3	3.4±0.1	0.5±0.0	0.4±0.0	tr	-

[a] Weight percentage.
[b] Values are means±standard deviations for three preparations.
[c] Trace amount.
[d] Cinquanta *et al.* (2001).
[e] Gutiérrez *et al.* (1999)
[f] Beltran *et al.* (2004)

Table 5. Radical-scavenging activity and tocopherol content in olive oils.

Olive oil	Variety	Maturation stage	Radical-scavenging Activity (μmol Trolox eq./100 g)	Activity due to Tocopherols (μmol Trolox eq./100 g)	Contribution of tocopherols (%)
Virgin olive oil	Manzanillo		115.0±1.6[a]	59.4±0.2	51.6
	Lucca		78.0±0.5	47.4±1.7	60.8
	Nevadillo Blanco		117.0±12.0	54.3±1.7	46.4
	Mission		119.0±23.1	46.6±0.5	39.2
Hexane-extracted olive oil	Manzanillo	Green	148.7±2.3	71.3±9.4	48.0
		Red	123.5±40.7	54.1±0.5	43.8
		Black	102.0±9.8	52.9±4.4	51.9
	Lucca	Green	88.8±12.3	53.2±9.3	59.9
		Red	84.3±6.2	45.9±7.2	54.4
		Black	93.6±1.6	51.9±9.8	55.5
	Nevadillo Blanco	Green	133.8±1.5	70.8±5.7	52.9
		Red	89.9±12.3	57.8±2.9	64.3
		Black	83.4±5.9	47.6±5.1	57.0
	Mission	Green	119.7±14.2	71.0±2.5	59.3
		Red	106.0±7.0	60.0±2.0	56.6
		Black	87.8±5.6	48.5±9.4	55.2

[a] Values are means±standard deviations for three preparations.

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