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REVIEW

Physiological Functionality of Purple-Fleshed Sweet Potatoes Containing Anthocyanins and Their Utilization in Foods

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Abstract

Studies on the physiological functionality of purple-fleshed sweet potatoes and their dominant anthocyanin pigments are described. The purple-fleshed sweet potato cultivar ‘Ayamurasaki’ contained anthocyanins, which consisted of mono- or di-acylated forms of cyanidin (YGM-1a, -1b, -2 and -3) and peonidin (YGM-4b, -5a, -5b and -6). It was also rich in anthocyanins with peonidin aglycon. The ‘Ayamurasaki’ extract and the purified YGM exhibited multiple physiological functions such as radical-scavenging, antimutagenic, angiotensin I-converting enzyme-inhibitory, and α-glucosidase-inhibitory activities *in vitro*. Moreover, they also showed an ameliorative effect on carbon tetrachloride-induced liver injury and decreased postprandial blood glucose levels in rats. In addition, their role in restoring the liver function and blood pressure levels to normal in volunteers with impaired hepatic function and/or hypertension was also confirmed. The acylated anthocyanins, which were the major radical scavengers in ‘Ayamurasaki’, were directly absorbed into the blood stream of rats and were present as intact acylated forms in the plasma, and could also enhance the plasma antioxidative capacity. Based on these evidences, the purple-fleshed sweet potato can be recommended as a superior source for the production of foods with health benefits. Some foods and beverages in Japan that utilize these characteristics of anthocyanin pigments are also introduced in this paper.

Discipline: Food
Additional key words: radical-scavenging activity, antioxidant, hepatic function, hypertension, blood glucose level

Introduction

The traditional food culture of Japan has attracted attention for its role in maintaining good health. However, the changes in the food habits during the past two or three decades have disturbed the body’s nutritional balance, leading to an increase in lifestyle-related diseases (chronic diseases) including obesity, arteriosclerosis, hepatitis, hypertension, hyperglycemia, constipation and colon cancer. Sweet potato, soybean, rice, buckwheat, green tea, vegetables, and fruits, which are a part of the daily diet in Japan, are spotlighted here as superior sources for the production of foods with a potential for the prevention of lifestyle-related diseases.

Sweet potato (*Ipomoea batatas* (L.) Lam.) is considered to be a nutritionally rich crop. Sweet potatoes are rich in vitamins (B_1, B_2, C and E), minerals (calcium, magnesium, potassium and zinc), dietary fiber, and non-fibrous carbohydrates[13]. Yellow-fleshed sweet potatoes are most common in Japan. However, recently, new varieties of sweet potatoes with white, deep yellow, orange, and purple flesh have been released from the National Agricultural Research Center for Kyushu Okinawa Region, (previously, Kyushu National Agricultural Experiment Station)[16,20]. Such sweet potatoes have the same nutritive value as yellow-fleshed sweet potatoes, and contain several types of beneficial and functional pigments, such as flavones, β-carotene and anthocyanins for deep yellow, orange, and purple-fleshed sweet potatoes, respectively. In particular, the purple-fleshed sweet potatoes, which contain anthocyanins, have attracted attention due to their multiple physiological functions such as radical-scavenging (or antioxidative), antimu-

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tagenic, hepato-protective, antihypertensive, and antihyperglycemic activities.

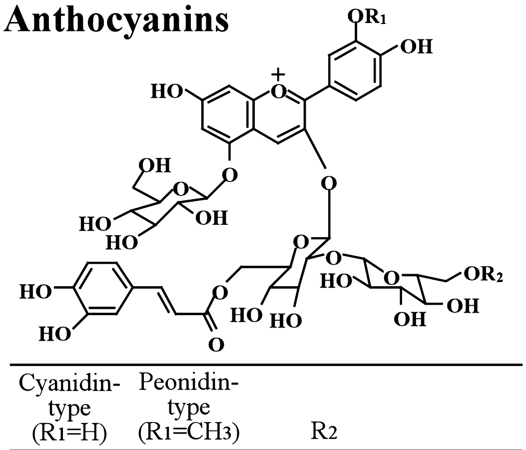
Elucidation of the physiological functionality of purple-fleshed sweet potatoes and their predominant anthocyanin pigments, has led to the development of a large number of processed foods from these sweet potatoes, which are now on sale all over Japan, by making use of the characteristics of the anthocyanin pigments. Thus, this paper introduces the physiological functionality of purple-fleshed sweet potatoes and their utilization in foods and beverages in Japan.

Anthocyanins contained in purple-fleshed sweet potato

The purple-fleshed sweet potatoes contain a high level of anthocyanins, compared to white, yellow, and orange-fleshed ones, and the contents differ depending on the varieties[1]. The cultivar 'Ayamurasaki' is the second generation of a local Japanese sweet potato variety Yamagawa-murasaki which was developed at our research center in 1995 for use as a natural food colorant[20]. The anthocyanin content of this cultivar is about 0.6 mg of YGM-5b (peonidin 3-caffeoylsophoroside-5-glucoside, Pn 3-Caf·sop-5-glc) equivalent/g[1]. The anthocyanin pigments are mono- or di-acylated forms of cyanidin (YGM-1a, -1b, -2 and -3) and peonidin (YGM-4b, -5a, -5b and -6)[2,8,15] (Fig. 1), and the cultivar is rich in anthocyanins with peonidin aglycon. Among the 8 major anthocyanins in the purple-fleshed sweet potatoes, 2 (YGM-2 and YGM-5b) are mono-acylated by caffeic acid, and the others are di-acylated by caffeic acid alone (YGM-1b and YGM-4b), caffeic acid and *p*-hydroxybenzoic acid (YGM-1a and YGM-5a), or caffeic acid and ferulic acid (YGM-3 and YGM-6). Thus, all of the 8 major anthocyanins in the purple-fleshed sweet potatoes are characterized by binding at least one caffeoyl group, which is responsible for conferring a high radical-scavenging activity.

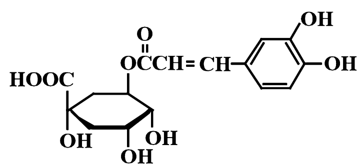
It is known that the color of anthocyanin pigments in vegetables and fruits is affected by the pH of the solution. In the case of purple-fleshed sweet potatoes, the extract exhibits brilliant red, purple and blue colors, under acidic, neutral and alkaline conditions, respectively (Fig. 2). Furthermore, the degree of color stability after heating and ultraviolet light irradiation has been reported to be correlated with the number of anthocyanin species, especially the acylated anthocyanin species[3]. HPLC analysis revealed that the sweet potato variety Yamagawa-murasaki shows 9 anthocyanin pigment peaks including 8 acylated peaks, and the sweet potato variety Tanegashima-murasaki shows 13 pigment peaks including 6 acylated peaks. Due to the presence of such a large number of acylated anthocyanins, purple-fleshed sweet potato anthocyanins belong to a group exhibiting the highest stability to heating and ultraviolet ray irradiation[3]. In fact, purple-fleshed sweet potato anthocyanins are stable compared to anthocyanins with a low level of acylation such as those in strawberry, raspberry, apple, and soybean with black seed coat[3].

Anthocyanins



Cyanidin-type (R_1=H)	Peonidin-type (R_1=CH_3)	R2
YGM-1b	YGM-4b	caffeic acid
YGM-1a	YGM-5a	*p*-hydroxy benzoic acid
YGM-2	YGM-5b	H
YGM-3	YGM-6	ferulic acid

Cinnamic acid-related compounds



chlorogenic acid
(5-caffeoylquinic acid)

iso-chlorogenic acid
(3,5-di-*O*-caffeoilquinic acid)
(4,5-di-*O*-caffeoilquinic acid)
(3,4-di-*O*-caffeoilquinic acid)

Vitamins

ascorbic acid
α-tocopherol

Fig. 1. Major antioxidants contained in purple-fleshed sweet potato

Fig. 2. Juices made from purple-fleshed sweet potato cultivar 'Ayamurasaki' and the pH-dependent color shift

Utilization

Purple-fleshed sweet potato is a good source of the stable purple pigments due to its high anthocyanin content and higher yield. Besides its use as a natural food colorant, the deep purple paste and flour made from 'Ayamurasaki' are used for the preparation of noodles, bread, jams, sweet potato chips, confectionery, juices (Fig. 2), and alcoholic beverages[17]. As a result of the development of foods and beverages using purple-fleshed sweet potato, many processed foods are now on sale in shops at stations, airports and tourist resorts in the Kyushu-Okinawa area. Our research center is breeding several other types of sweet potatoes to meet the increasing demand for new varieties of purple-fleshed sweet potatoes, including the bluish purple dominant clones with a high peonidin/cyanidin ratio, and the reddish purple dominant clones with a high cyanidin/peonidin ratio[21].

Physiological functionality

Anthocyanins are widely distributed in fruits, beans, cereals, vegetables, and red wine, and humans ingest a considerable amount of anthocyanins daily in plant-based diets. In the past decade, anthocyanin-rich foods and preparations have attracted attention because of their health benefits in terms of prevention of some lifestyle-related diseases (chronic diseases). Three representative *in vivo* studies clearly demonstrate the health benefits of anthocyanin-containing foods in humans. They include the reports on the "French paradox" (a low incidence of coronary heart disease and arteriosclerosis despite a high-fat diet) of red wine[10], the ophthalmic activity of bilberry extract[7], and the hepatic function restorative activity of purple-fleshed sweet potato juice which we identified[11,12]. Here we describe the multiple physiological functions of purple-fleshed sweet potatoes and the acylated anthocyanins which they contain[13]. Some are *in vitro* studies such as radical-scavenging (or antioxidative), antimutagenic, and angiotensin I-converting enzyme (ACE) inhibitory activities. Others are *in vivo* studies as follows: studies on rats included those on the direct absorption of acylated anthocyanins, their ameliorative effect on carbon tetrachloride (CCl_4)-induced liver injury, and the suppressive effect on postprandial blood glucose level, while studies on humans investigated the role in the restoration of the liver function and blood pressure levels to normal in volunteers with impaired hepatic function and/or hypertension.

1. Radical-scavenging and antioxidative activity

Excess production of free radicals, such as the superoxide anion radical, hydroxyl radical, and alkylperoxyl radical, induces oxidative damage in cells. The damage is assumed to be associated with aging, cancer, arteriosclerosis, and other lifestyle-related diseases. In contrast, antioxidants, as scavengers of free radicals and inhibitors of lipid peroxidation, are assumed to play important roles in preventing such diseases. Thus, antioxidants are currently the most attractive topic for research because of their role in human health.

Purple-fleshed sweet potato cultivars showed a higher radical-scavenging (or antioxidative) activity than those with white, yellow, or orange flesh, in the assay system for evaluating the *tert*-butyl hydroperoxyl radical-scavenging activity[1], superoxide anion radical-scavenging activity[17], and lipid peroxidation-suppressing activity[1]. In a comparative study, a trifluoroacetic acid extract from the purple-fleshed sweet potato cultivar 'Ayamurasaki' showed a 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of 4.6–6.4 μmol expressed as Trolox equivalent/g fresh weight, and this activity was higher than that of other anthocyanin-containing agricultural products, as seen in soybean with black seed coat (0.62–0.76), black-hulled rice purchased at the local market (3.0–4.3), and eggplant (3.3–4.4) (unpublished data). Anthocyanins and other polyphenolic compounds (chlorogenic acid and isochlorogenic acid) contained in purple-fleshed sweet potato (Fig. 1) are highlighted as important candidates because their radical-scavenging activities are enhanced with the increase in the polyphenol content in sweet potatoes[1]. Ascorbic acid and α-tocopherol may also participate in the activity. Although the involvement of anthocyanins and other polyphenolic compounds in the total radical-scavenging activity varied with the types of purple-fleshed sweet potato cultivars, anthocyanins were the dominant radical scavengers in 'Ayamurasaki' and 'Murasakimasari'[9].

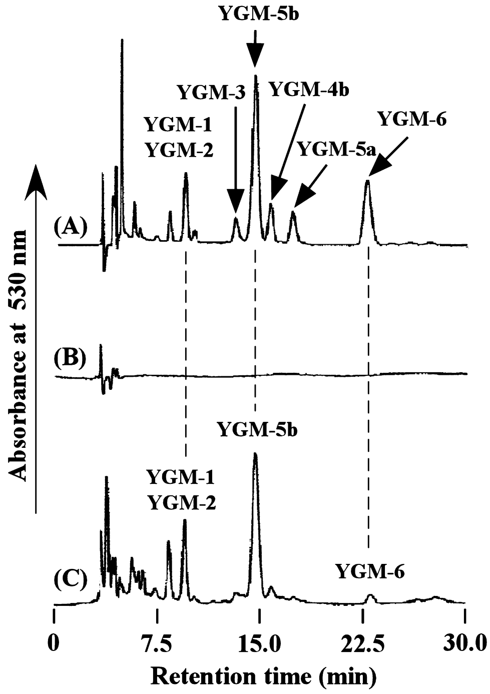


Fig. 3. HPLC chromatograms of purple-fleshed sweet potato anthocyanin (PSA) concentrate (A) and anthocyanins detected in rat plasma before (B) and 30 min after (C) PSA concentrate administration

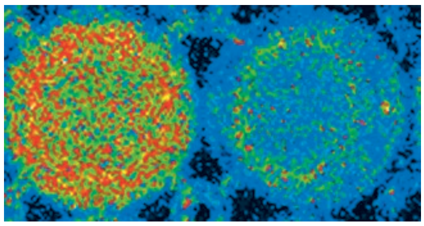


Fig. 4. Image of chemiluminescence generated by reaction between *t*-butyl hydroperoxyl radical and luminol in the presence of rat plasma before (left) and 30 min after (right) PSA administration

A low chemiluminescence (blue) indicates a high radical-scavenging activity.

2. Absorption of acylated anthocyanins into the rat blood stream

An experiment on the absorption of the acylated anthocyanins contained in purple-fleshed sweet potatoes into the animal body is important to determine whether the acylated anthocyanins can exert a physiological function *in vivo*. We prepared a purple-fleshed sweet potato anthocyanin (PSA) concentrate from 'Ayamurasaki' flesh, and administered it orally to rats by direct stomach intubation[14]. Typical HPLC profiles of anthocyanins detected in the rat plasma after the administration of the PSA concentrate are shown in Fig. 3. A major peak detected in the rat plasma corresponded to YGM-5b. Other anthocyanins were also detected as minor peaks. Here, it is interesting to note that acylated anthocyanins were detected in the plasma as intact forms, because this evidence suggests that the absorbed acylated anthocyanins reach the target organs through the circulatory system, and exert their physiological functions as in the case of the *in vitro* assay system. Actually, the rat plasma showed a higher radical-scavenging activity after the administration of PSA concentrate than before the administration (Fig. 4). Furthermore, acylated anthocyanins may exert potent activities against a wide range of organs and tissues, because the PSA concentrate and purified acylated anthocyanins showed inhibitory effects on the oxidation of plasma, liver and brain, the hemolysis of erythrocytes, and the oxidation of LDL, microsomes and erythrocyte membrane ghosts.

3. Ameliorative effect on liver injury

The CCl_4-treated animal is frequently used as a suitable *in vivo* model for induced liver injury through a free radical-mediated reaction. In such animals, the metabolite of CCl_4, trichloromethyl radical ($CCl_3^{\bullet}$), causes an oxidative liver injury which results in the release of GOT and GPT from the liver into the blood stream. Pretreatment with juice made from the purple-fleshed sweet potato cultivar 'Ayamurasaki' orally for 5 consecutive

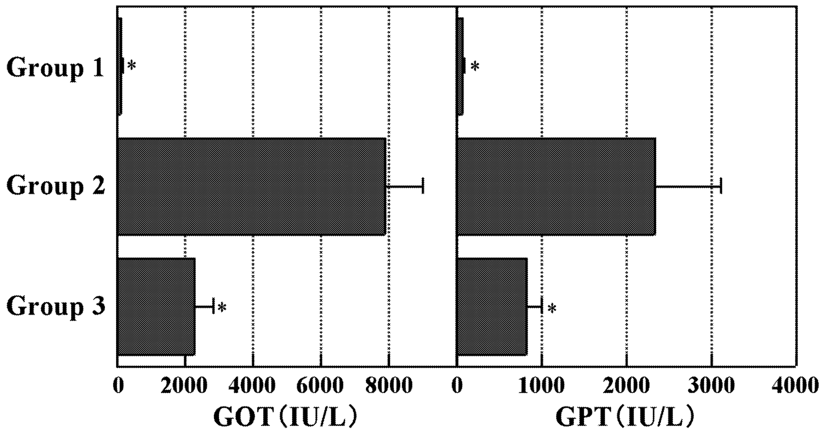


Fig. 5. Effects of juice made from purple-fleshed sweet potato cultivar 'Ayamurasaki' on the serum GOT and GTP levels in CCl_4-treated rats

Group 1: Tap water & olive oil.
Group 2: Tap water & CCl_4 in olive oil.
Group 3: 'Ayamurasaki' juice & CCl_4 in olive oil.
'Ayamurasaki' juice was administered orally (0.8 mL/100 g rat/day) for 5 consecutive days. CCl_4 in olive oil (0.05 mL/0.2 mL/100 g rat) was given orally 12 h after administration of the last dose of juice. Rats were sacrificed at 24 h after the administration of CCl_4, and serum GOT and GPT levels were measured as indices of liver injury. Values are means ± SD, n = 8. *Significant differences from Group 2 (p<0.001).

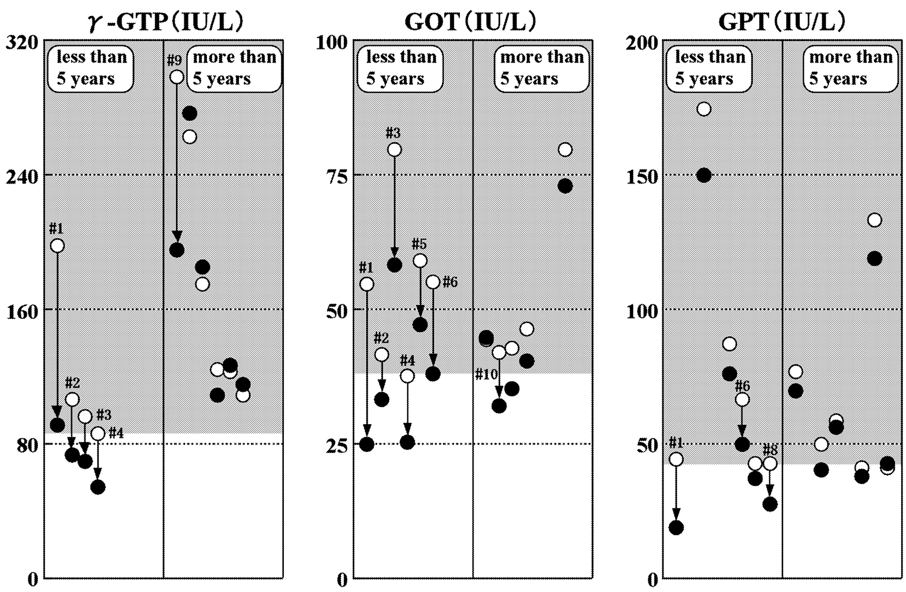


Fig. 6. Ingestion effects of juice made from purple-fleshed sweet potato cultivar 'Ayamurasaki' on the serum γ-GTP, GOT and GPT levels in volunteers with impaired hepatic function

Serum γ-GTP, GOT and GPT levels in volunteers were measured before (○) and after 44 days (●) of continual ingestion (23.7 mg of YGM-5b equivalent/120 mL/day) of 'Ayamurasaki' juice. Subjects were divided into two groups, those with high levels of these hepatic enzymes for less than 5 years (left) and those with high levels for more than 5 years (right). Arrow indicates the case when the value decreased by more than 20% compared to the initial one.

days prior to CCl_4 treatment effectively reduced the levels of serum GOT and GPT[11] (Fig. 5), suggesting the ameliorative effect of purple-colored sweet potato juice with high anthocyanin content on CCl_4-induced liver injury. The anthocyanins contained in purple-fleshed sweet potato seem to be the principal contributor to the prevention of radical-mediated liver injury, because the ameliorative effect was exhibited in rats administered anthocyanin-rich sweet potato juice but not in those administered β-carotene-rich sweet potato juice. Furthermore, we confirmed their ameliorative effect on the hepatic function in humans[12] (Fig. 6). Namely, serum γ-GTP, GOT and GPT levels in some volunteers with impaired hepatic function became normal after 44 days of continual ingestion of the 'Ayamurasaki' juice with high contents of anthocyanins (23.7 mg of YGM-5b equivalent/120 mL/day). As additional information, most of the subjects whose liver function was restored, belonged to a group with a less than 5-year history of impaired hepatic function, suggesting that the ameliorative effect of 'Ayamurasaki' juice is exerted in patients with mild hepatitis.

4. ACE-inhibitory activity and antihypertensive effect

Angiotensin I-converting enzyme (ACE) plays an important physiological role in regulating blood pressure; it catalyzes the conversion of the inactive angiotensin I to the potent vasoconstrictor angiotensin II, and inactivates the vasodilator, bradykinin. Therefore, the specific inhibition of ACE exerts therapeutic and preventive effects on hypertension. Water extracts from the purple-fleshed sweet potato cultivar 'Ayamurasaki' showed a higher ACE-inhibitory activity than those from white- or orange-fleshed sweet potatoes[17]. The 'Ayamurasaki' anthocyanins also showed an ACE-inhibitory activity with an IC_{50} (concentration of ACE inhibitor required to inhibit 50% of ACE activity) of 0.16 mg/mL for YGM-3 or YGM-6. Furthermore, the 'Ayamurasaki' juice lowered the systolic blood pressure in volunteers with hypertension toward normal levels. Among 12 subjects with systolic blood pressure beyond 140 mm Hg, 2 subjects showed a blood pressure decrease over 20 mm Hg, and 4 subjects showed a decrease in the range of 10–20 mm Hg[12] (Fig. 7).

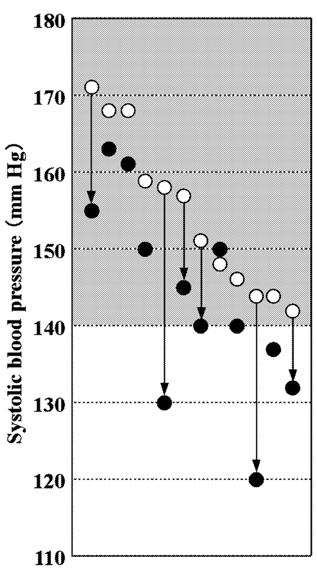


Fig. 7. Ingestion effects of juice made from purple-fleshed sweet potato cultivar 'Ayamurasaki' on the systolic blood pressure in volunteers with hypertension

Blood pressure in volunteers was measured before (○) and after 44 days (●) of continual ingestion (23.7 mg of YGM-5b equivalent/120 mL/day) of 'Ayamurasaki' juice. Arrow indicates the case when the systolic blood pressure decreased by more than 10 mm Hg compared to the initial one.

5. Antimutagenic activity

Antimutagenic activity of foods has been studied for their role in reducing the risks of mutagenesis and carcinogenesis. Water extracts from 'Ayamurasaki' and 'Ayamurasaki' anthocyanins YGM-3 and YGM-6 effectively inhibited the reverse mutation induced by mutagens such as Trp-P-1, Trp-P-2, and IQ[18,19].

6. α-Glucosidase-inhibitory activity and decrease of postprandial blood glucose level

The effective management of diabetes mellitus, in particular non-insulin-dependent diabetes mellitus (NIDDM) involves the prevention of excessive postprandial rise of the blood glucose level. α-Glucosidase, which is a membrane-bound enzyme located in the epithelium of the small intestine, catalyzes the cleavage of glucose from disaccharides. A recent study[4,5] indicated that the 'Ayamurasaki' anthocyanins YGM-3 and YGM-6 displayed potent α-glucosidase (maltase) inhibitory activities with IC_{50} values of 200 μM, which were more than 5 times higher than that of D-xylose (IC_{50} = 1,190 μM). In a study in rats[6], when the 'Ayamurasaki' anthocyanin YGM-6 (100 mg/kg) was administered following maltose (2 g/kg), the maximal blood glucose level at 30 min significantly decreased by 16.5% compared to the control group. This result reveals that 'Ayamurasaki' anthocyanins, retained in the small intestine without absorption into the blood stream, exert an antihyperglycemic effect through maltase activity inhibition.

Conclusion

Purple-fleshed sweet potatoes were revealed to contain a high level of anthocyanins, which consisted of mono- or diacylated forms of cyanidin (YGM-1a, -1b, -2 and -3) and peonidin (YGM-4b, -5a, -5b and -6). Since these acylated anthocyanins were directly absorbed into the blood stream of rats and were present as intact forms in the plasma, it is strongly suggested that the absorbed anthocyanins could directly exert their physiological functions on the target organ in the same way as in the *in vitro* assay system. The extract from the purple-fleshed sweet potato 'Ayamurasaki' and/or the purified YGM exhibited multiple physiological functions such as radical-scavenging, ACE-inhibitory and α-glucosidase-inhibitory activities *in vitro*, and also hepato-protective, antihypertensive and antihyperglycemic effects *in vivo*. Such evidences indicated that purple-fleshed sweet potato was a superior source for the production of foods with health benefits. Among the purple-fleshed sweet potato cultivars, 'Ayamurasaki' and 'Murasakimasari' are the most suitable sources for anthocyanin pigments due to their high anthocyanin content and higher yield, and also their higher color stability. We expect that more products using these purple-fleshed sweet potato cultivars will be developed in the near future and exert their health benefits by helping prevent lifestyle-related diseases (chronic diseases).

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