Difficulty for consumers in choosing commercial bilberry supplements by relying only on product label information

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ABSTRACT

Background: Various kinds of bilberry supplements have recently become available on the market. However, it is doubtful whether consumers receive accurate information to be able to compare different supplements. **Objective:** We aimed to investigate whether consumers can obtain the expected benefits by relying only on the information printed on the product labels of commercial bilberry supplements. **Materials and Methods:** The quality of 20 supplements was investigated by the spectrophotometric method and ultra high performance liquid chromatography (UHPLC). Each peak was identified by liquid chromatography–mass spectrometry and quantified using an external standard. The percentage of the actual measured value relative to the indicated value on the product label was determined using the spectrophotometric method. The daily dosage was calculated from the total amount of anthocyanins quantified by UHPLC and information on the product label. **Results:** In 14 of 20 supplements, the total anthocyanin content expressed as delphinidin equivalents was within 20% of the labeled value. However, the extent of degradation could not be determined by the spectrophotometric method. In fresh bilberry fruit, anthocyanidins were barely detected. In 8 of 20 supplements, the anthocyanidin content was >1.0%. The daily dosage of anthocyanins varied by about 66-fold among supplements, and the dosage of 6 supplements was less than the recommended level in Japan. **Conclusions:** Consumers cannot always obtain the expected benefits by relying only on product label information. Therefore, new rules concerning product label information are required to make it possible for consumers to take the equivalent amounts of anthocyanins for whichever bilberry supplement they choose.

Key words: Anthocyanidins, bilberry anthocyanins, consumer, product label information, quality

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INTRODUCTION

Various kinds of blueberry supplements are available on the market, advertising a large number of health benefits. The main active constituents in blueberry are anthocyanins, which exhibit a wide range of beneficial effects on visual capacity,[1] brain cognitive function,[2] obesity,[3] ulcer protection,[4] cardiovascular disease risk,[5] and cancer prevention.[6] Blueberry cultivars are mainly highbush blueberry and rabbiteye blueberry in Japan. In contrast, they are predominantly lowbush blueberry in North America and bilberry in Europe. Among these cultivars, anthocyanins are especially abundant in bilberry (*Vaccinium myrtillus* L.),[7] which is therefore extensively used in supplements.

Anthocyanins occur as glycosides, and their aglycones as anthocyanidins are derived from the 2-phenylbenzopyrylium cation belonging to the vast group of flavonoids. Until date, there have been reports of more than 650 different anthocyanins[8] and 23 anthocyanidins.[9] Recently, we reported that the beneficial effects of bilberry supplements might be due to anthocyanins and not anthocyanidins.[10,11] Anthocyanin-containing products are prescribed as medicines in Italy, New

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Zealand, and Korea, but are classified as health foods in Japan and the United States. Due to lax regulations of health foods in the latter countries, the ingredients are variously described on product labels as "anthocyanins," "anthocyanidins," and "extract of bilberry." This makes it difficult for consumers to know whether the amount of anthocyanins contained in the product meets the recommended daily dosage. Moreover, anthocyanins are very sensitive to thermal conditions and are easily degraded to anthocyanidins,[12] so it is doubtful whether consumers take sufficient amounts of anthocyanins for whichever bilberry supplement they choose.

The objective of this study was to investigate the daily dosage of anthocyanins in commercial bilberry supplements to determine whether consumers can obtain the expected benefits by relying only on the information printed on the product label.

MATERIAL AND METHODS

Reagents

Bilberon-25, a concentrated extract of bilberry, was generously donated by Tokiwa Phytochemical Co. Ltd. (Chiba, Japan). Malvidin chloride, cyanidin chloride, peonidin chloride, and delphinidin chloride (ChromaDex, Irvine, CA) and petunidin chloride (Tokiwa Phytochemical Co. Ltd.) were used as anthocyanidin standards. Cyanidin 3-*O*-galactoside (Sigma-Aldrich, St. Louis, MO), cyanidin 3-*O*-glucoside (Fujicco Co. Ltd., Kobe, Japan), and cyanidin 3-*O*-arabinoside chloride (Extrasynthese, Lyon, France) were used as anthocyanin standards. All other chemicals and reagents used in this study were of analytical grade and obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) or Junsei Chemical Co. Ltd. (Tokyo, Japan). Millipore-MilliQ distilled–deionized water (Millipore SAS, Molsheim, France) was used for all experiments.

Preparation of extracts of fresh bilberry

Frozen bilberry fruits were generously donated by Tokiwa Phytochemical Co. Ltd. The fruits were pounded in acidified methanol (2% hydrochloric acid in methanol). After filtration with a syringe filter (Whatman, Kent, UK), the solution was analyzed by ultra high performance liquid chromatography (UHPLC).

Commercial dietary supplements

A total of 20 commercial supplements were purchased at a store or via the Internet. Of these, 18 were available in Japan and 2 were obtained from other countries. These supplements were marketed as bilberry extract-containing supplements and were formulated as soft capsules (n = 9), hard capsules (n = 3), tablets (n = 7), and granule (n = 1).

Determination of total anthocyanin content by a spectrophotometric method

For sample preparation, the contents of hard and soft capsules were emptied into glass vials, and tablets were pounded using pestle and mortar. Samples (10 mg) were dissolved in acidified methanol and heated for 30 min on a water bath (TYPE 6; AS ONE Co. Ltd., Osaka, Japan). The solution was allowed to cool and then diluted by adding acidified methanol to reach the concentration needed for measurement of the UV-visible spectrum. The UV-visible spectrum of the solution was measured from 200 nm to 600 nm using a spectrophotometer (UV-2450; Shimadzu Co. Ltd., Kyoto, Japan). The absorbance at the λ_{max} was measured and the content of anthocyanins was expressed as delphinidin equivalents. This method was based on the Japan Health and Nutrition Food Association (JHFA) quality standard.[13] Anthocyanin content was measured in triplicate and presented as the percentage of the actual measured total anthocyanins relative to the amount indicated on the product label.

Chromatographic analysis

We developed a UHPLC method using a high-resolution YMC-UltraHT Pro C18 (3.0 i.d. × 100 mm, 2 μm) column (YMC Co. Ltd., Kyoto, Japan), and the separation was performed using the ACQUITY UPLC system (Waters Co. Ltd., Milford, MA). The gradient started with 100% A to reach 82% in 6 min, from 82% to 70% in 8 min, from 70% to 35% in 4 min, from 35% to 0% in 0.04 min, and isocratic for 1.96 min. The injection volume of the samples was 4 μL, the sample cooler temperature was 4°C, and the detector was set at 535 nm. The flow rate was 0.43 mL/min, column temperature was set to 40°C, and total run time was 20 min.

Quantification of anthocyanin content in supplements

The contents of hard and soft capsules were emptied into glass vials, and the tablets were pounded using pestle and mortar. For each supplement and Bilberon-25, 10 mg of the samples was added to 10 mL acidified methanol and dissolved using ultrasonic waves. After filtration with a syringe filter (Whatman), the solution was subjected to UHPLC. Anthocyanin content was measured in triplicate.

Each anthocyanin and anthocyanidin peak was identified using the Exactive liquid chromatography–mass spectrometry system (Thermo Fisher Scientific Inc., Waltham, MA). In addition, five types of anthocyanidin and three glycoside forms of cyanidin (3-*O*-glucoside, 3-*O*-galactoside, and 3-*O*-arabinoside) were identified by comparison of the retention times and were quantified using an external standard. Anthocyanins other than those listed above were quantified using the predictive sensitivity coefficient, by multiplying the sensitivity coefficient of

the identified anthocyanidin by that of cyanidin glycoside. The amount of anthocyanidins in each supplement was presented as a percentage. Daily dosage was calculated from the measured weight of a capsule/tablet, the percentage of total anthocyanins per weight, and the number of capsules/tablets recommended per day according to the product label.

RESULTS AND DISCUSSION

The percentage of actual measured total anthocyanins relative to the amount indicated on the product labels was investigated using the spectrophotometric method [Figure 1]. The results varied by about 8-fold, from 21.6% for sample D to 155.9% for sample M. The actual measured value of 6 of 20 supplements (i.e., samples B, D, J, K, M, and O) was >20% or <20% than the labeled value. The spectrophotometric method is generally used in the food industry to determine the total anthocyanin content as it is a simple procedure and can screen products rapidly. However, this method has two disadvantages. First, the heating procedure as part of the method alters the original composition by degrading anthocyanins to anthocyanidins. Second, it is impossible to identify and quantify each individual anthocyanin by this method. Therefore, the extent of degradation and the presence of anthocyanins cannot be determined by the spectrophotometric method.

On the other hand, UHPLC is a useful tool for the identification and quantification of individual anthocyanins. Chromatograms of fresh bilberry fruits and Bilberon-25 are shown in Figure 2 (a). In fresh bilberry, anthocyanidins were barely detected (<0.1%), and all detected peaks were confirmed as anthocyanins. The percentage of anthocyanidins of the 20 supplements and Bilberon-25 is shown in Figure 2 (b). The value of anthocyanidins in bilberry extract is specified not to exceed 1.0% in the USP, Italian, and European Pharmacopoeias. Similar to fresh bilberry, the anthocyanidin content of Bilberon-25 was <1.0%. By contrast, 8 of 20 supplements contained >1.0% anthocyanidins. The predominant forms present in fresh fruits were glycosides, and the anthocyanidin content was increased by degradation during the manufacture and storage of products. We recently reported that anthocyanin-rich bilberry extract alleviates pruritus, whereas anthocyanidin-rich extract does not have this effect.[10,11] Hence, the beneficial effects of bilberry supplements may be caused by anthocyanins and not by anthocyanidins. In addition, anthocyanidins are unstable under physiological conditions and are rapidly degraded to protocatechuic acid.[14]

We investigated the actual daily dosage of anthocyanins, excluding all anthocyanidins [Figure 3]. The dosage varied considerably among the 20 supplements by about 66-fold, from 2.3 mg/day for sample D to 151 mg/day for sample N. In Japan, a daily dose of at least 29 mg of anthocyanins is recommended,[13] but 6 of the 20 tested supplements did not comply with this recommendation. These results suggest that consumers will not receive equivalent amounts of anthocyanins if they choose different supplements.

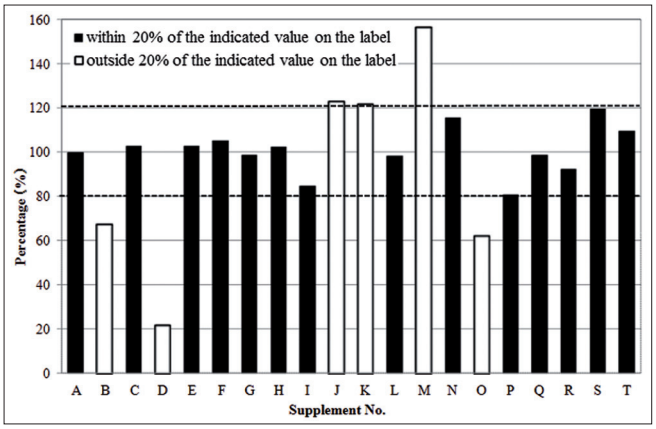

Figure 1: The percentage of actual measured total anthocyanins relative to the indicated value on the product label. Dashed lines represent 80% and 120%

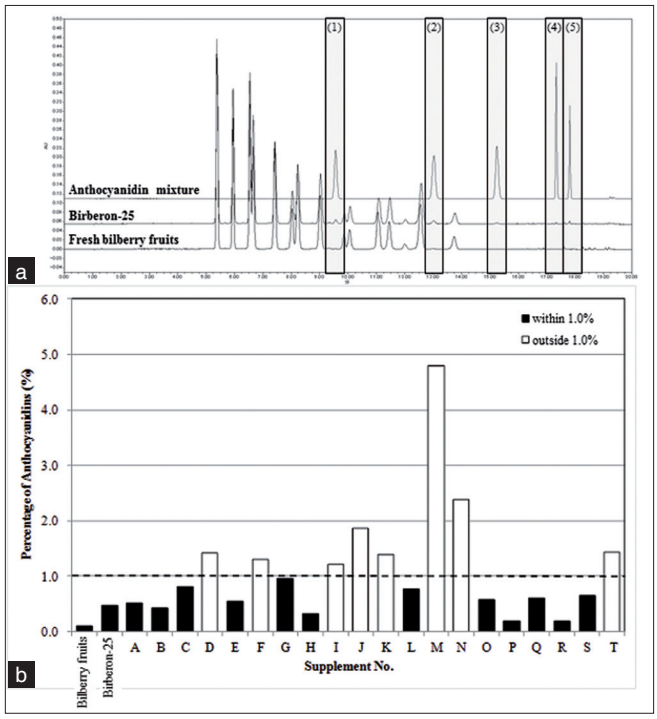

Figure 2: Anthocyanidins in fresh bilberry fruits and supplements measured by UHPLC. (a) Chromatograms of fresh bilberry fruits and Bilberon-25. Peak identities: (1) delphinidin, (2) cyanidin, (3) petunidin, (4) peonidin, and (5) malvidin. (b) Anthocyanidin content of 20 bilberry supplements. Dashed line represents 1.0% anthocyanidins in a sample

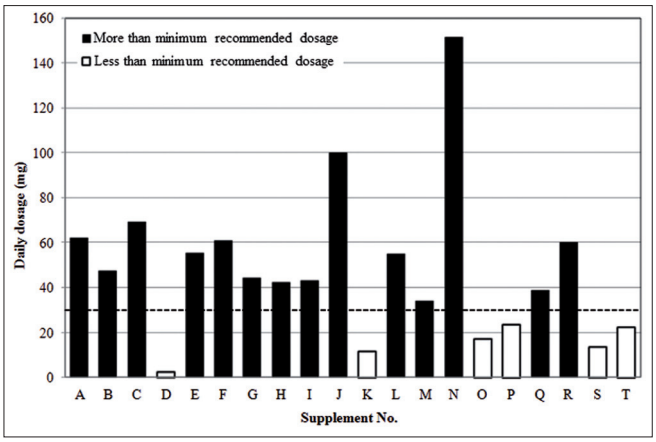

Figure 3: Actual daily dosage of total anthocyanins based on the indicated dosage printed on the product label. Dashed line represents 29 mg as the minimum recommended dosage of anthocyanins by the JHFA

CONCLUSION

Anthocyanidins, which were barely present in fresh bilberry fruits, were detected in bilberry supplements as a result of degradation. Approximately half of the supplements investigated in this study contained >1.0% anthocyanidins. Furthermore, the actual amount of anthocyanins ingested by following the indicated daily dosage printed on the product labels strongly differed between supplements on the market examined in this study. Therefore, consumers cannot always expect to obtain the expected benefits by relying only on the information printed on the product labels of commercial bilberry supplements. In conclusion, new rules concerning product label information and the quality control of the anthocyanin content are required to make it possible for consumers to take the equivalent amounts of anthocyanins, especially in the case of bilberry supplements, for whichever commercial supplement they choose.

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