Anthocyanin Pigments of "Nama-shibazuke" Japanese Pickles in Package on Market

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ABSTRACT

The anthocyanin pigments in the packages of "nama-shibazuke" on the market were studied to compare with product (S)[1]. The product (S) is Japanese salted pickles made from vegetables such as eggplant (*Solanum melongena* L.) and perilla leaf (*Perilla frutescens* Britton var. *crispa* Decne) which was salted according to the traditional way for 20 days by the authors under the small-scale in Ohara, Kyoto. The anthocyanins in "nama-shibazuke" on the market were constituted of Cyanidin-3,5-diglucoside, Delphinidin-3-(p-coumaryl-L-rhamnosyl-glucoside)-5-glucoside [Nasunin], Cyanidin-3-(6-p-coumarylglucoside)-5-glucoside [Shisonin], Cyanidin-3-(6-caffeylglucoside)-5-(6-malonylglucoside) [Caffeylmalonylcyanin], Cyanidin-3-(6-p-coumarylglucoside)-5- (6-malonylglucoside) [Malonylshisonin] and three unknowns. The major anthocyanin pigment in "nama-shibazuke" on the market was nasunin and its color was exceptionally stable. These results were the same as the product (S). In addition, the sodium chloride content of various "nama-shibazuke" producted by makers on the market which were about 4%, and the results of Hunter's values (*L.a.b*) and pH showed the same values as the product (S). It was recognized that various "nama-shibazuke" on the market used in this work had a quality the same as Japanese traditional pickles by salted by the traditional way and the materials in Ohara, Kyoto.

INTRODUCTION

There are two types of "shibazuke" Japanese pickles on the market, one is called "nama-shibazuke" salted with the traditional way at Kyoto and the other is called "aji-shibazuke"[2] which is soaked again "nama-shibazuke" with some seasonings such as soy sauce, sugar and vinegar. The vegetables such as cucumbers may sometimes be added in it. Both shibazuke are stuffed into the small plastic bags respectively. They are sterilized by heating at 80 ℃ during 15 ~ 20 min[3][4] and then cooled in the cold water. For the plastic film, recently it is main substance to pack the pickles because the thermal conductivity is so high compared with the can and the bottle, Especially, the sterile condition at the temperature of below 80℃ is adapted to the film [5][6]. The price is the least expensive and the preservation of the material is kept in a good state.

The authors have previously reported that the main lactic acid bacterium in shibazuke is *Lactobacillus plantarum*, its activity during the ageing process for 20 days was very important tothe deepest magenta color and quality of shibazuke[7]. The main organic acid was lactic acid, followed by acetic acid [8]. And the main anthocyanin pigment in shibazuke was indentfied to be nasunin, using HPLC-mass spectrometry (LC/MS) [9].

This paper reports the main anthocyanin in "nama-shibazuke" on the market compared with the product (S) salted by the authors under the small-scale in Ohara, the northern district of Kyoto, in the traditional way of making "shibazuke" [1].

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MATERIALS AND METHODS

Materials and preparation of the analytical samples

The shibazuke goods named "nama-shibazuke" on the market made by various makers were obtained from department store. These samples were cut into small pieces, 20g was added to 60 ml of cold water, and then the mixture was homogenized in a blender for 1 min. After centrifuge at 3000 r.p.m. for 10 min, the supernatant was passed through a 0.45- μ m membrane filter and the filtrate was used for analysis. Five samples were prepared from various "nama-shibazuke" with the same procedure.

Analysis condition

The values for *L* (lightness), *a* and *b* (chromaticity) in the Hunter's system were determined by a photoelectric color meter (Nippon Denshoku Model ND-101 DC). The pH range was determined by a pH meter (TOA pH meter HM-40S). The method of Morl was carried out on the assay of sodium chloride.

For the analysis of anthocyanin pigments, an analytical high-performance liquid chromato- graphy (HPLC) system equipped with Waters Millennium™ 2020J 996 photodiode array detector in the range of 220-700 nm was used. The anthocyanins were separated with an Inertsil ODS-3 column (4.6 mm i.d x 250 mm) at 30 ℃, using the mobile phase consisted of the ratio of acetonitrile to 0.05% trifluoroacetic acid (TFA) at 1 : 4 (v/v) in isocratic mode at a flow rate 1.0 ml/min while monitoring at 525 nm. The fast atom bombardment mass spectroscopy (FAB-MS) were obtained by JNS-SX102A model mass spectroscopy (Nippon Denshi) to determine the molecular weight of the anthocyanin pigments with a 1N HCl-glycerine matrix. The relative content of individual anthocyanins in shibazuke is defined as (peak area) / (peak area on day 0 obtained from product S)[1].

RESULTS AND DISCUSSION

Chemical components and color tone

The values of pH, sodium chloride and color tone (*L.a.b*) in various shibazuke are shown in Table 1. A1 is the product matured for 180 days by A maker and just before packing. A2, B and C are packages of " nama-shibazuke" on the market. Each amount of pH and sodium chloride ranged from 3.42 to 3.55 and 4.19 % to 4.31 %. All of the *a* and *b* in the samples were located on the red-purple color side in hunter's diagrams. There was not great difference in the color tone between "nama-shibazuke" and product S[1]. It was found that various "nama-shibazuke" on the market have the deep magenta color which resulted from low pH to change the anthocyanin pigments into a deep magenta color. This would have resulted from the carbinol-base changed into the flavylium ion through the anhydro-base.[9]

Table 1 Chemical components and color in various "shibazuke"

	Sodium chloride (%)	pH	Hunter's values L	a	b
A1	4.30	3.42	40.96	24.50	-3.34
A2	4.31	3.46	48.51	22.04	-2.49
B	3.59	3.55	55.26	19.61	-3.14
C	4.19	3.54	56.16	24.23	-3.92

A_1: Product matured for 180 days by A maker (just before packing)
A_2: Package by A maker
B : Package by A maker
C : Package by A maker

Identification of the anthocyanins in "nama-shibazuke" on the market

HPLC traces of the anthocyanins in "nama-shibazuke" on the market is shown in Fig.1, which was the same as the result of products made by the authors. The anthocyanins were observed as 11 peaks on the chromatogram. Main 8 peaks of them were numbered according to the elution order. It was considered that the peak ①

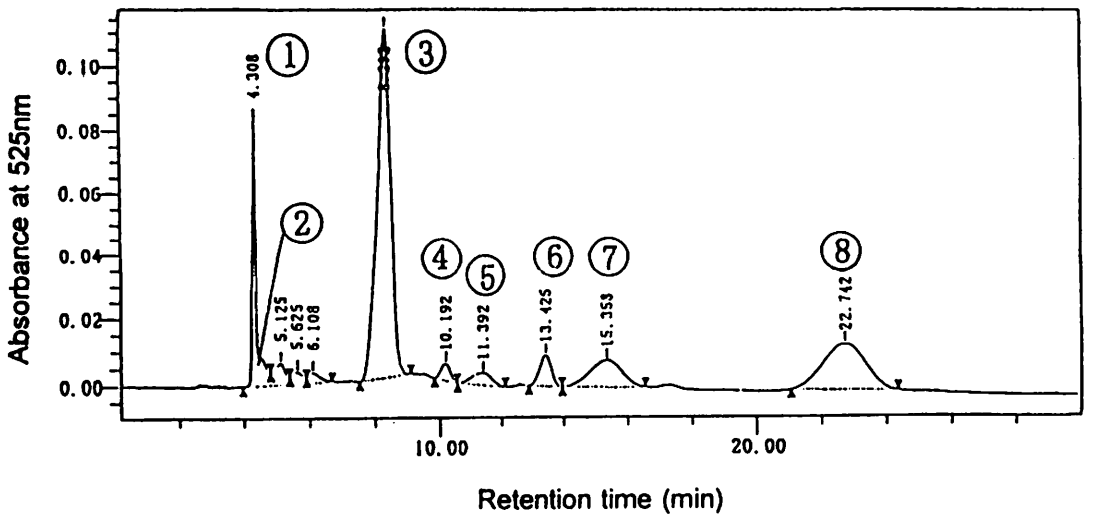

Fig. 1 HPLC traces of anthocyanins isolated from "nama-shibazuke" on the market

Analytical HPLC used an Inertsil ODS-3 column (4.6mm i.d. x250mm) at 25℃, a solvent system (acetonitrile-0.1%TFA=1:4 v/v) in isocratic elution mode at a flow rate of 1.0ml/min, and monitoring at 525nm. The volume of each injected shibazuke anthocyanin solution was 100 μl.

① Phenolic substance
② Cyanidin 3,5-diglucoside (Cy-3,5-glu)
③ Delphinidin-3-(*p*-counmaryl-L-rhamnosyl-D-glucoside)-5-glucoside [Nasunin]
④ Cyanidin 3-(6-*p*-coumaroylglucoside)-5-glucoside [Shisonin *cis* form]
⑤ Cyanidin-3-(6-caffeylglucoside)-5-(6-malonylglucoside) [Caffeylmalonylcyanin *trans* form]
⑥ Cyanidin-3-(6-*p*-counmarylglucoside)-5-(6-malonylglucoside) [Malonylshisonin *cis* form]
⑦ Shisonin (*trans* form)
⑧ Malonylshisonin (*trans* form)

corresponds to something like a phenolic substance because of the same retention time as in our previous work [1].

The mass spectrum of peak ③ with FAB- LC/MS is shown in Fig.2-a. The mass numbers of the molecular ions on the constituents of peak ③ was m/z 919 (M^+), an $[M-162]^+$ ion at m/z 757 (loss of glucose), an $[M-292]^+$ ion at m/z 627 (loss of *p*- coumarylrhamnose), an $[M-454]^+$ ion at m/z 465 (loss of *p*-coumarylrhamnose-glucose) and aglycone ion at m/z 303 (a delphinidin ion). So, the authors identified peak ③ to be nasunin and the structure is shown Fig.2-b. It has been identified Delphinidin-3-(*p*-coumaryl-L-rhamnosyl-D-glucoside)-5-glucoside by Watanabe *et al* [11]. The eggplant anthocyanins consisted of almost a single pigment of nasunin[1]. The other peaks were identified from retention time[10] and the same way of peak ③. The result is shown as Fig.1. They were identified that peak ② (m/z 611) was Cyanidin 3,5-diglucoside (Cy-3,5-glu) and peak ④ and ⑦ (m/z 757) were Cyanidin3-(6-*p*-coumarylglucoside)-5-glucoside [Shisonin *cis* form] and Shisonin (*trans* form) respectively. Peak ⑤ was Cyanidin-3-(6-caffeylglucoside)-5-(6-malonylglucoside) [Caffeylmalonylcyanin]. Peak ⑥ and ⑧ were Cyanidin-3-(6-*p*-coumarylglucoside)-5-(6-malonylglucoside) [Malonylshisonin *cis* form] and Malonylshisonin (*trans* form) respectively. These peaks have the origin in leaves of perilla since all of them were detected from HPLC of perilla pigments. We have not identified the remains of other peaks yet.

Main anthocyanin pigment in "nama-shibazuke" on the market

The relative content of pigments of the various "nama-shibazuke" on the market and the product (S) salted by the authors under the small-scale in Ohara, Kyoto is shown in Fig.3. The content of the total anthocyanin pigments of perilla origin on the product (S) were higher than that of the samples on the market. This was caused on the

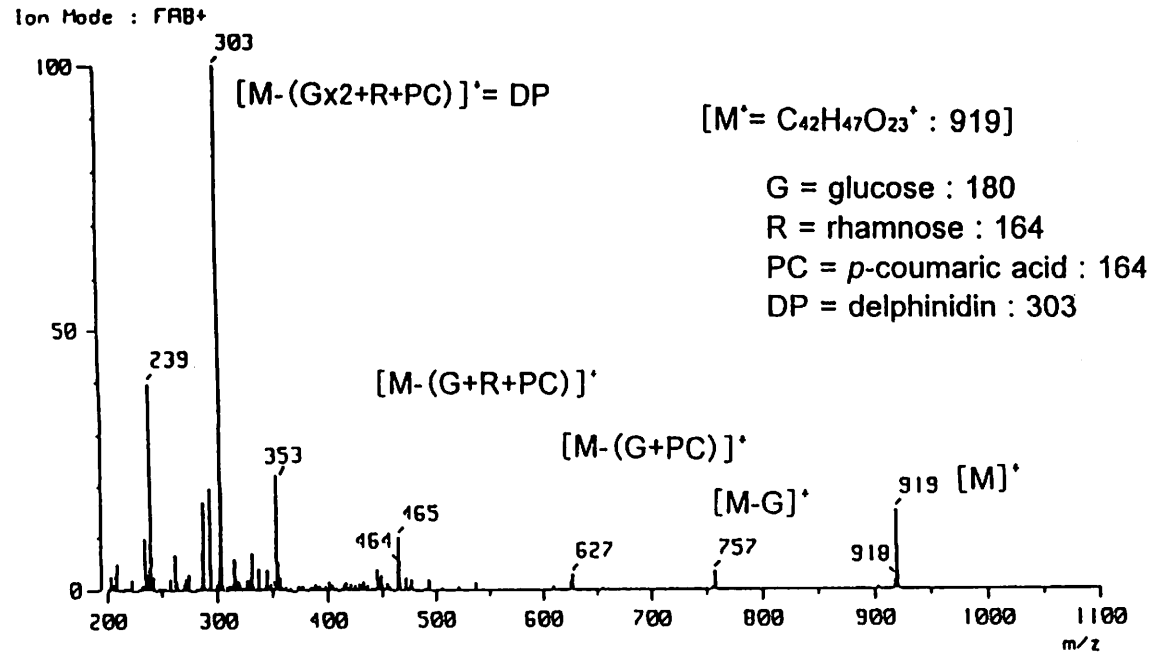

Fig. 2-a Mass spectrum of peak ③ with FAB-LC/MS

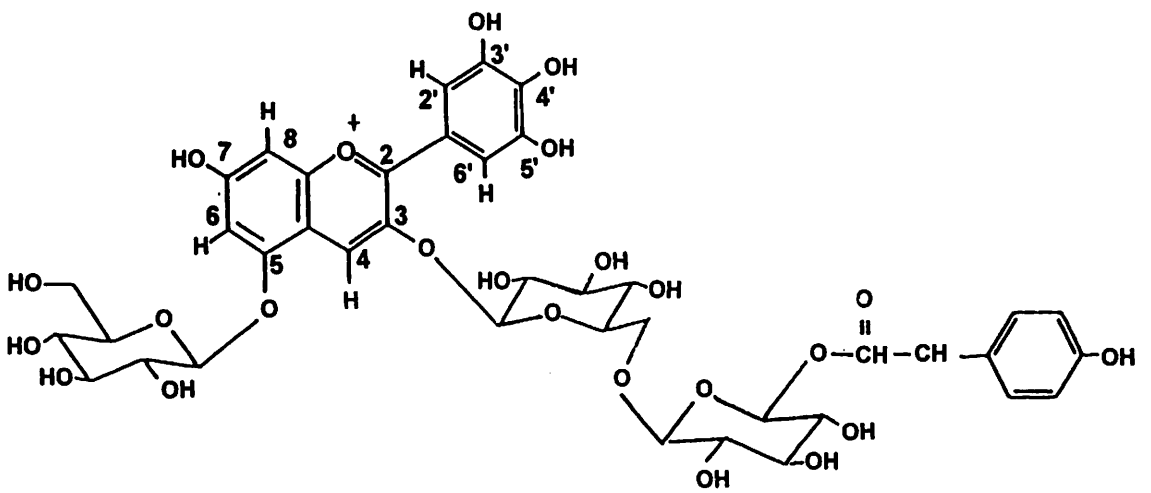

Fig. 2-b Structure of delphinidin-3-(*p*-coumaryl-L-rhamnosyl-D-glucoside)-5-glucoside [Nasunin]

treatment of the perilla leaves, so called the perilla-cover, which covered over the materials of shibazuke-pickles. Usually perilla-cover is removed just before packing, but the authors did not remove it for the product (S).

In these products (A1, A2, B and C), it was found that the major anthocyanin is nasunin and which is stable in "nama-shibazuke" because a large proportion of anthocyanins derived from the peelings of eggplant. Because the color of nasunin is intensified when cy-3,5-glu and other anthocyanins such as shisonin, caffeylmalonylcyanin and malonylshisonin from perilla are combined with nasunin. This effect is known as intermolecular stacking due to hydrophobic bonding between anthocyanin moieties and aromatic acid residues in poly-acylated anthocyanin [12].

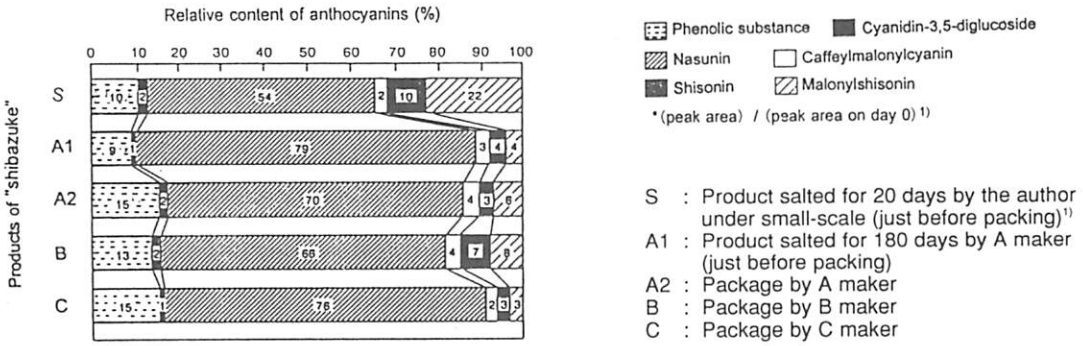

Fig. 3 Relative content of various "nama-shibazuke"

This will be also supposed through that the eggplants pickles without perilla leaves salted by the authors did not exhibit a magenta color even at the pH of 3.4.

In conclusion, the main anthocyanin pigment in "nama-shibazuke" on the market was nasunin, which remained stable. It was recognized that various "nama-shibazuke" on the market used in this work had a quality the same as Japanese traditional pickles salted by the traditional way in Ohara, Kyoto.

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要　旨

今日市販されている「袋詰生しば漬」中のアントシアニン色素について、既に著者等が報告した「しば漬Ｓ」[1]中のアントシアニン色素と比較・検討した。

「しば漬Ｓ」は京都大原で行われている伝統的な手法に従い、著者らにより漬けられた日本古来の伝統発酵漬物であり、ナス（*Solanum melongena* L.）とシソ（*Perilla frutescens* Britton var. *crisa* Decne）をミニプラントで20日間漬けたものである。

実験の結果、市販の「袋詰生しば漬」中のアントシアニン色素は、シソ由来のシアニジン-3,5-ジグルコシド、カフェイルマロニルシアニン、シソニン、マロニルシソニン、そしてナス由来のナスニンと、シソ由来の3個の不明なアントシアニンとで構成されていることが確認された。

また、市販の「袋詰生しば漬」中の主要アントシアニン色素はナスニンであることが判り、その色調は「しば漬Ｓ」中のアントシアニン色素と同様にしば漬中で安定していた。これらの結果は「しば漬Ｓ」中のアントシアニン色素構成および色調結果と全く同様であった。さらに、本実験に用いた製造業者の異なる幾つかの市販の「袋詰生しば漬」の食塩濃度は約4%前後で、pHおよびハンター尺度の*L.a.b*値のいずれにおいても「しば漬Ｓ」と比較して大きな差異は認められなかった。

すなわち、今回実験に用いた市販の「袋詰生しば漬」も京都大原で行われている伝統的な手法および原料を用いて漬けられており、日本古来の伝統発酵漬物と同じ品質を有していることが認められた。