Nationwide distribution of *Kyo-yasai* (heirloom vegetables in Kyoto) and the advantages of traditional farming methods with importance of ‘*Syun*’: a case of mizuna

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**Abstract**

**Introduction:** This study aimed to review the farming activity and the administrative measure to successfully increase the distribution of heirloom vegetable "mizuna" nationwide by examining the paradigm shift in the use of its young less-branched form for salad. We also discussed that breeding programs of the young form ironically resulted in reduction the flavor and the antimutagenicity of this vegetable.

**Methods:** Through hearing survey, we explored reasons for the successful nationwide distribution of heirloom vegetable, mizuna. Through chemical analysis, the fragrant ingredients in mizuna, their antimutagenicity and the changes in their amount for one year were determined.

**Results and discussion:** The primary factor for commencing the distribution of mizuna includes the new idea of using young less-branched form of mizuna as salad ingredient, which has been recognized by most who were involved in promoting the distribution of mizuna among farmers, distributors, and employees of agricultural extension section at Kyoto Prefecture office. The secondary factor is the fact that the primary factor coincides on a time axis with other two factors: the occurrence of the boom of Kyoto’s heirloom vegetables and the Vegetable Management Stabilization Project found as a measure of Kyoto Prefecture. We determined three fragrant compounds in mizuna, 3-butenyl isothiocyanate, 3-phenylpropionitrile, and phenylethyl isothiocyanate, of which their antimutagenic effects were also identified. Those amounts were highest at the best harvest season called ‘*Syun*’ in the past because of the cold temperature of Japanese winter in traditional open-field cultivation.

**Conclusion:** It implied that the successful nationwide distribution of mizuna resulted from the administrative measures based on the paradigm shift in the new use of mizuna for salad due to increase in demand of the vegetable.
Introduction

In Japan, the largest number of heirloom vegetables is distributed in 6 prefectures: Tokyo, Aichi, Nagano, Niigata, Yamagata, and Kyoto among 47 prefectures [1]. Heirloom vegetables in Kyoto are frequently referred to as “Kyo-yasai” or “Kyo-no-dentoyasai” in a less scientific language in Japanese pronunciation [2]. Kyo-yasai vegetables usually have more distinctive flavors than conventional vegetables. Since the 1970s, consumers have preferred milder flavors and odors than those of new heirloom vegetables varieties, and therefore some Kyo-yasai with strong flavors have greatly reduced in number and are thus facing extinction crisis. In fact, the two varieties currently exterminated are Kori-daikon (Kori Japanese white radish) and Taji-kabu (Toji-temple turnip) [3].

To preserve Kyo-yasai for the next generation, administrative measures were initiated in Kyoto Prefecture in 1974 to protect these heirloom vegetables from extinction, and to collect their various seeds from farmers and preserve them at the Horticultural Division of the Kyoto Prefectural Agriculture, Forestry, and Fisheries Technology Center (Kameoka, Japan) [4].

Mizuna (Brassica rapa var. japonica or Brassica juncea var. japonica) is one of the heirloom vegetables in Kyoto. The cultivation of mizuna in Kyoto was once in small quantity, but due to the administrative measures of Kyoto Prefecture, which proposed a new way of eating mizuna, it was successfully distributed nationwide and became one of the vegetables that is farthest from extinction crisis. Mizuna cultivation was recorded in a Japanese picture book, Shui-miyako-meisho-zue (1787; Fig. 1A). The picture book showed mizuna (Chinese characters read “mizuna” in Fig. 1B), and its place of origin in Kyoto city (Chinese characters read “Mibu” in Fig. 1C). It also showed mizuna to be in a well-branched form (Fig. 1D) in the past. However it is marketed in a young less-branched form in the present day (Fig. 1E). The place “Mibu” written in Fig. 1C still presents as “Mibu quarter” in the center of Kyoto city (Fig. 2). Mizuna in well-branched form (traditional type), namely well-grown form, is not suitable for raw consumption as the stems and leaves are tough. Before 1982 in Japan, mizuna in a well-branched form was usually used for the preparation of pickles “Shio-zuke”, by soaking the stems and leaves in salt from few days to several weeks, and finely chopping them with a kitchen knife before eating. It could also be made as simmered food “Harihari-nabe”, and cooked with whale flesh traditionally. However, currently, the well-branched form is rarely seen in supermarkets owing to the decrease in consumer demand.

Uchiyama et al. reviewed that branding of heirloom vegetables facilitates the transmission of the traditional products and relevant knowledge to future generations, and the vegetables are often “re-discovered”, following their distribution nationwide [1]. This has helped in improving successfully the distribution of heirloom vegetables in its young less-branched form (contemporary type), since newly “re-discovered” young form can be consumed raw as vegetable salad as the stems and leaves are soft. In this study, we reviewed farming activities and administrative measures adopted to increase the commercial cultivation of mizuna from 1982, and we demonstrated the paradigm shift in the new use of mizuna (young less-branched form) to increase the vegetable’s demand in contemporary dishes. It has been reported in many articles in traditional food including heirloom vegetables and traditional farming methods [5–14], and its basic information of local traditional knowledge is necessary in conservation, sustainable utilization, preservation, discovering health benefit in future [12]. We thus also discussed that breeding programs of the young form ironically resulted in reduction the flavor and the antimutagenicity of this vegetable.

Materials and methods

Hearing survey

We conducted face-to-face interviews with the former employees of agricultural extension section at Kyoto Prefecture office to distribute mizuna cultivation in Kyoto, and the following were verified: (1) the morphological shape of mizuna in the past and presently; (2) the paradigm shift in eating mizuna as salad; (3) the administrative measures in Kyoto Prefecture; (4) the original characteristics of disappearing mizuna.

Materials

3-Butenyl isothiocyanate (BUITC; <95% pure grade, CAS #3386-97-8), 3-phenylpropionitrile (PEN; <98% pure grade, CAS #645-59-0), phenylethyl isothiocyanate (PEITC; <97% pure grade, CAS #2257-09-2) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Mizuna samples (cultivated in greenhouse) were purchased from local supermarkets in Kyoto city. Mizuna samples (in open-field cultivation) were harvested in an

Keywords: Mizuna, Brassica, Heirloom vegetable, Antimutagen, Salad, Kyoto
open field at Katsura High School (Kyoto City: long. 135° 40′ E, lat. 34° 59′ N, altitude of 21 m), Japan. In open-field cultivation, 6 kind of lineages were used: two self-sufficient lineages maintained by Katsura High School, and by farmer in Katsura area, and four lineages of common varieties purchased from four different seed companies. Samples harvested in the open field carried to Kyoto Prefectural University within 1 day and analyzed immediately, while samples purchased from supermarkets were approximately harvested within 3 days, and also analyzed within 1 day of purchase. In our previous study, BUITC was produced in mizuna as same amount after 0, 1, 3, 7 days from the harvest day, and thus the difference of the days would not probably make a significant difference in ingredients if within 4 days.

**Identification of fragrant ingredients in mizuna**
Mizuna (100 g) was homogenized with deionized water (200 mL). The filtered extract was partitioned three times with *n*-hexane, chloroform, and ethyl acetate, in that
order, with 100 mL of solvent each time. The n-hexane, chloroform, and ethyl acetate layers were evaporated to dryness at < 35 °C. The n-hexane extract had mizuna-like fragrance as determined by a human sense of smell. A very specific mizuna-like fragrant fraction (10 mg) was obtained by fractionating the n-hexane extract of mizuna on a silica-gel column and mobile phase containing 1% acetone in n-hexane (named the fragrant fraction of mizuna). The fragrant fraction of mizuna was analyzed with gas chromatography-electron ionization-mass spectrometry (GC-EI-MS; JEOL JMS-AMSUN200 mass spectrometer, coupled on a Hewlett-Packard 6890 gas chromatograph, and Shimadzu GCMS-QP2010SE). The capillary column was a DB-5 (25 m × 0.2 mm, 0.33 μm film thickness; J&W Scientific, Folsom, CA, USA). The column oven temperature was held at 60 °C for 5 min and then was increased to 250 °C at a rate of 5 °C/min.

Production of BUITC, PEN, and PEITC in mizuna

The bunch of mizuna samples were collected, and 40 g was homogenized with 80 mL of deionized water in an automatic juicer mixer (SM-R50, Sanyo). The BUITC, PEN, and PEITC production was determined 15 min after the homogenizing step, because the maximum amount of BUITC, PEN, and PEITC produced in homogenized mizuna was found to be 10 min after homogenizing and thereafter did not change for another 15 min. The homogenized mizuna was incubated for 15 min at 25 °C, all of the homogenized mizuna was filtered, and the residue was extracted twice with 30 mL of deionized water. The water extracts were combined and diluted to 250 mL to prepare an aqueous crude sample solution and subjected to analysis of BUITC, PEN, and PEITC concentration. To avoid degradation of BUITC, PEN, and PEITC in alkaline pH and quench the activity of myrosinase prior to analysis of the amount of BUITC, PEN, and PEITC, 10 μL of 6 M HCl was added to 1.0 mL of the aqueous sample extract, and the solution was extracted three times, each with 200 μL of n-hexane. The n-hexane extracts were combined and BUITC, PEN, and PEITC were measured by GC-EI-MS. The GC-EI-MS in the selected ion monitoring (SIM) mode using selective fragment ions was used to quantify each fragrant ingredient. EI-MS m/z (relative intensity) of BUITC, PEN, and PEITC are presented in the following: BUITC 113 [M]+ (72), 85 (13), 72 (100), 55 (24); PEN 131 [M]+ (21), 91 (100), 65 (12), 51 (7); PEITC 163 [M]+ (32), 105 (15), 91 (100), 65 (12). In the SIM mode, integration of selected ion intensity (m/z 113 for 10.0–13.0 min in BUITC, 131 for 19.0–21.5 min in PEN, 163 for 25.0–27.5 min in PEITC) were recorded.

Assay of antimutagenicity

The assay was carried out as described by Nakamura et al. [15]. Briefly, the detection of antimutagenicity was based on UV-induced mutation that is mainly caused by the bulky DNA product, thymine dimer in E. coli B/r WP2 (trpE65, repair-proficient). The antimutagenicity was evaluated by determining the relative mutagenic activity (RMA, percent of control). To identify an active sample, we used a criterion “IC₅₀” determining the lowest dose needed to acquire a 50% RMA, which was calculated from a linear regression derived from at least 15 points taken over five doses. A fraction was determined active if it met this criterion.

Results and discussion

Hearing survey

Hearing survey of Kyo-yasai in general has been conducted a detailed so far by Rath and de St Maurice [16, 17]. In this study, a face-to-face interview was conducted at Kyoto Prefectural University with two former employees of agricultural extension section of Kyoto Prefecture office based on their actual experience, in chronological order from mizuna cultivation in tradition to nationwide spread. One of the two was 68 years old on the day of the interview. He was engaged in work in Kyo-yasai including mizuna at Kyoto Prefecture office from 1977 and 2013, and then at Public Interest Group Corporation.
Kyo-Branded Products Association (Kyo-no-furusato-sanpin-kyokai in Japanese pronunciation) from 2013 to 2018. The other was 65 years old on the day of the interview. He worked at Kyoto Prefecture office from 1978 and 2017, and was engaged in Kyo-yasai including mizuna between 1984 and 2002. All the interviews were the work conducted by the authors, and the outcome has thus been newly revealed information in mizuna in this study.

Morphological shape of mizuna in the past and presently

The morphology of mizuna during shipment is shown in Fig. 3. Mizuna in well-branched form (traditional type; Fig. 3A) and young less-branched form (contemporary type; Fig. 3B) are sometimes referred to as “Oo-kabu mizuna” and “Ko-kabu mizuna” (in Japanese pronunciation), respectively. In the past, “Oo-kabu mizuna” was cultivated at open-field traditionally (Fig. 4A–C), and was scraped root before shipment (Fig. 4D, E). When seeding mizuna in October, a longer period (> 90 days) is needed to harvest the well-branched form, whereas the young less-branched form can be harvested after 40 days. In July and August (summer season in Japan), young less-branched form is usually harvested shorter than 30 days, and this allows continuous cropping in same crop field.

Paradigm shift in eating mizuna as salad

In Japan, the number of nuclear family households has increased in 1970s, and the demand accordingly increased for smaller vegetables in size. In response to this, the miniaturization of vegetables had started progressing such as Chinese cabbage and daikon. When breeding the well-branched form “Oo-kabu mizuna,” thinning is performed at the initial stage of the cultivation, wherein most seedlings are thinned out and discarded. This is performed because too dense plants hinder the growth of other plants. In early 1980s, a new idea of using thinned-out seedlings called “Ko-kabu mizuna” for salad ingredient was recognized by most who were involved in promoting the distribution of mizuna to farmers, distributors, and employees of agricultural extension section at the Kyoto Prefecture office. At the time of promoting mizuna by consumers, it was thereby appealed that “you can eat “Ko-kabu mizuna” even salad”, and the salad menu was solicited at the cooking contest held by Kyo-no-furusato-sanpin-kyokai (in Japanese pronunciation), an outer organization of Kyoto Prefecture office that promotes the sales of Kyo-yasai, and the award-winning menus were publicized by posting the cooking recipe on the pamphlet [Kajitani, Personal communication].
Administrative measures in Kyoto Prefecture

The administrative goal of promoting the vegetable in Kyoto Prefecture was to greatly strengthen the vegetable-producing areas and to improve the income of farmers. In 1982, new measures of greenhouse cultivation were launched for medium-sized vegetable farm. Mizuna was selected as one of the vegetables, and its cultivation was progressively promoted in greenhouses at the suburbs of Kyoto Prefecture [4, Kajitani, Personal communication]. The effect of greenhouse was not only to regulate the temperature and light rays, but also to suppress the invasion of pests by attaching a near-ultraviolet ray removing film to the greenhouse surface. In addition, it can properly manage soil moisture by covering with a lawn cloth after sowing.

Fig. 4 Traces of mizuna cultivation in the past: A cultivation field of mizuna in well-branched form “Oo-kabu” (traditional type), B magnification of a part of the cultivation field, C Root, stems and leaves overall, D Old-fashioned style in scraping root in 1982, E mizuna in well-branched form with the scraping (left), and without the scraping (right)
As a result, the cultivation environment was improved and contributed to the improvement of the quality of mizuna.

Since 1985, Kyo-yasai boom has occurred mainly in Tokyo metropolitan area, Japan. When this was featured in the media, Kyo-yasai including mizuna became well-known throughout Japan. At the same time, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center (Kameoka, Kyoto; former name Kyoto Prefectural Agricultural Research Institute), selected varieties of mizuna appropriate for securing stable supply to the Japanese market [Kajitani, Personal communication].

In 1986, the Vegetable Management Stabilization Project in Kyoto Prefecture mandated that farmers who wish to cultivate new vegetables must form a group of at least three farmers [18]. The prototype of this system was promulgated for conventional vegetables in 1960 in Kyoto, and it started in 1966 for Japan overall. This raised the awareness of farmers and created a sense of security in which they could consult for agricultural products, and thereby promoted stable production of agricultural products. The project also guaranteed the price decline for farmers, even if the market price of vegetables decreased. This encouraged new farmers of Kyo-yasai to enter the market, and it was a measure unique to Kyoto Prefecture that was applied to mizuna.

In 1987, Kyoto Prefecture started selecting the crop field of young less-branched form of mizuna “Ko-kabu” for salad. The initial crop field was 2500 m² at Wachi quarter (a region in central Kyoto Prefecture), and “Ko-kabu” was harvested for salad by a female group of Wachi-farmers in June, 1988. A product of mizuna packed in a small plastic bag was produced, and the initial shipping price was 350 yen for 1 kg, which was a satisfying price for farmers at that time.

In 1989, Kyoto Prefecture also named Brand-Kyo-yasai, which reflects Kyoto’s image, and thus is a certificate of good quality, individuality, and superiority for outside Kyoto Prefecture, securing stable supply to the market. After Kyoto Prefecture titled mizuna as Brand-Kyo-yasai, the price did not decrease but increased surprisingly, because the demand always exceeded the supply. In addition, when there was shortage of leafy vegetables during summer, the price of mizuna was sometimes particularly higher, which should be the result of the evaluation of the rarity of mizuna salad products. Therefore, the cultivation of mizuna spread to other quarters in Kyoto Prefecture, and it became a hit product in Kyoto Prefecture that resulted in successful increase in the income of farmers.

From 1989 to 1990, the market demand of mizuna became so high that the supervisor of the agricultural extension section at Kyoto Prefecture office who was working on the spread of mizuna implemented “Fill the crop field with mizuna, leaving only the scaffolding.” However, after that, when the production of mizuna started in large-scale crop fields outside Kyoto Prefecture, its share increased in the Japanese vegetable market, and the Kyoto’s image of mizuna faded accordingly. Nevertheless, it was certain that Kyoto Prefecture’s measure resulted in a great success in “creating high-demand hit products” and “returning income to farmers”.

To summarize the above commencement of the nationwide distribution of mizuna, the primary factor was a new idea of using mizuna as salad ingredient recognized by farmers, distributors, and employees of Kyoto Prefecture office. The secondary factor was the fact that the primary factor coincides on a time axis with the other two factors that caused the boom of Kyoto’s heirloom vegetables and the Vegetable Management Stabilization Project found as a measure used by Kyoto Prefecture.

Original characteristics of disappearing mizuna
Some Kyo-yasai seeds that can adapt to these requirements were selected for Brand-Kyo-yasai. As a result, mizuna seeds were also selected from the commercially available seeds that can be supplied in sufficient amounts to farmers and concluded to be producer driven preferences for higher yield. However, the seeds collected from farmers at Hazukashi quarter, Kyoto city had better retention of deeply cut and fringed leaves, which is the original characteristic of mizuna as shown in Fig. 1A and D. Ironically, the seed having this characteristics was not a candidate for Brand-Kyo-yasai due to insufficient seed supply to farmers and yield per unit area. It is considered that these administrative measures in Kyoto Prefecture were also one of the reasons the original mizuna was suspended from distribution in the markets in Japan as drawn in the ancient paintings (Fig. 1A). It might be the period the varieties changed from heirloom varieties that retained the old-fashioned characteristics to cultivars.

Identification of fragrant ingredients in mizuna
Fragrant ingredients fraction (10 mg) was obtained from mizuna by fractionating the n-hexane extract of mizuna on a silica-gel column and mobile phase containing 1% acetone in n-hexane. The fragrant fraction contained mainly three compounds as indicated on the total ion chromatogram obtained from GC–EI–MS analysis (Fig. 5A). Compound 1, appearing at $t_R$ 12.8 min showed ion peak at $m/z$ of 113 [M]$^+$ and prominent fragment ions having masses of 85, 72, and 55. Compound 2, appearing at $t_R$ 20.7 min showed ion peak at $m/z$ of 131 [M]$^+$ and prominent fragment ions having masses of 91, 65, and 51. Compound 3, appearing at $t_R$ 26.8 min showed ion peak at $m/z$ of 163 [M]$^+$ and prominent fragment ions having masses of 105, 91, and 65. To validate
our tentative identification, we used authentic BUlITC, PEN, and PEITC that had the same retention times and m/z ratio of the ion peak as each of the tentatively identified compounds. Thus, compounds 1–3 in the fragrant fraction of mizuna were successfully identified (Fig. 5B).

**Antimutagenic effect of compounds 1–3**
We determined the antimutagenic effect of compounds 1–3 (using authentic chemicals) as a function of their ability to inhibit UV-induced mutation in repair-proficient *E. coli* B/r WP2. The assay detects the enhancing effect of mutation suppression either by increasing the level of error free DNA-repair for UV-induced DNA lesions (thymine dimers), or by increasing the opportunity for DNA repair by delaying DNA replication and mutation-fixation [15]. Compound 1 (BUlITC), compound 2 (PEN), and compound 3 (PEITC) were antimutagenic (Fig. 6). PEITC was previously identified as antimutagenic by our group [19], while the remaining two compounds, BUlITC and PEN are new discoveries. The IC_{50} of BUlITC, PEN, and PEITC were 53.6, 1,230, and 21.3 nmol/plate, respectively. Typical IC_{50} values for this assay ranged from 0.02 to 10 mg/plate [15] and indicated that BUlITC (0.06 mg/plate) and PEITC (0.04 mg/plate) were on the lowest end of this range, but PEN (0.16 mg/plate) on the lower end of this range, thus stronger antimutagenic potential.

Some of the antimutagenic compounds identified from the vegetables in this assay were *S*-methyl methanethiosulfonate from cauliflower [20, 21], 4-methylthio-3-butenyl isothiocyanate from daikon [22], and 3-methylthiopropionic acid ethyl ester from *Katsura-uri*, a pickling melon of *Kyo-yasai* [23]. These compounds exhibited anticarcinogenic activities in in vivo experiments [24–32] or on human colon cancer cells [33]. PEITC also exhibited anticarcinogenic activities [34–36]. Thus, these results should be investigated further for the anticarcinogenic properties of BUlITC and PEN in in vivo experiments.

**Changes in the amount of BUlITC, PEN, and PEITC produced by mizuna over time for 1 year**
BUlITC, PEN, and PEITC each had unique fragrances as determined by human sense of smell. BUlITC has mizuna-like odor, and PEN has cinnamon-like odor that sometimes smells like mizuna harvested in winter season. PEITC has a principal odor ingredient of watercress (*Nasturtium officinale*). Individually, we found that BUlITC and PEN are mainly responsible for a specific mizuna-like fragrance. For high added value of fragrance and antimutagenicity, BUlITC, PEN, and PEITC must be guaranteed in mizuna in a fixed amount. However, mizuna sometimes showed individual difference in the proportion of the three chemicals produced due to changes in production over time for one year. Among all mizuna...
samples, the most abundant amount was BUITC, followed by PEITC and then PEN (Fig. 7). The maximum value produced in 100 g of mizuna was 23.1 mg for the most abundant BUITC, 1.34 mg for PEITC, and 0.39 mg for PEN.

The amount of BUITC (specific mizuna-like fragrance) produced in 100 g of mizuna purchased at local supermarket (cultivated in greenhouse throughout the year) ranged from 0.08 (less than detection limit) to 3.71 mg produced (Fig. 7A). In contrast, this ranged from 0.37 to 23.1 mg/100 g for open-field cultivation harvested from early November to late December, but the maximum value was 1.91 mg/100 g for the greenhouse cultivation during the same period. The highest amount (3.71 mg/100 g) of BUITC in greenhouse cultivation was plotted in July, and this value was exceeded in the open-field cultivation harvested from late November to late December and dropped to the same level as the greenhouse cultivation in early February. Late November to late January is winter season in Kyoto city, Japan, and the air temperature ranges from 5 to −5 °C in lowest. Mizuna was originally harvested as well-branched forms as shown in Fig. 1A in the winter season in the past. It has also been called ‘Syun’ season (in Japanese pronunciation), meaning the best harvest season for taste and flavor. The inside of the greenhouse usually has a higher temperature than the outside air temperature, and it promotes mizuna growth. In the future, it should be conducted which factors, i.e., low temperature and its exposure time, or short-day length etc., could facilitate BUITC production in mizuna.

The amount of PEN produced in mizuna ranged from 0.03 (less than detection limit) to 0.26 mg/100 g for cultivation in greenhouse throughout the year (Fig. 7B). In contrast, it ranged from 0.03 (less than detection limit) to 0.39 mg/100 g for open-field cultivation harvested from late November to early February. The amount of PEN produced was relatively higher in open-field cultivation than in greenhouse cultivation during the same period (from late November to early February). Some individuals increased the amount of PEN seen in greenhouse cultivation in February and March.

The amount of PEITC produced in mizuna ranged from 0.02 (less than detection limit) to 0.43 mg/100 g for cultivation in greenhouse throughout the year (Fig. 7C). In contrast, it ranged from 0.03 (less than quantification limit) to 1.34 mg/100 g for open-field cultivation harvested from late November to early February. The amount of PEITC was also relatively higher in the open-field cultivation than in the greenhouse cultivation during the same period. Some individuals increased the amount of PEITC seen in cultivation in greenhouse from December to February.

Contribution of BUITC, PEN, and PEITC to antimutagenicity and factors that may increase their amount
To calculate the antimutagenicity of BUITC, PEN, and PEITC in mizuna extract is important to know the comprehensive antimutagenic potency of mizuna. It can be calculated by a criterion, “yield/IC$_{50}$” value that allowed the comparison between the comprehensive antimutagenic potency and the quantity of the extract (e.g., sample with higher antimutagenic potency shows higher...
yield and lower IC$_{50}$) [15, 37]. Based on this criterion, the yields (mg)/IC$_{50}$ (mg/plate) of BUlTC, PEN, and PEITC in mizuna harvested on December 28th in open field culture were 315 (18.9/0.06), 1.6 (0.26/0.16), and 33.5 (1.34/0.04), respectively. In contrast, the yield/IC$_{50}$ of that in greenhouse cultivation was 31.7 (1.9/0.06), 0.02 (0.0026/0.16), and 10.0 (0.4/0.04), respectively. Thus, the value of yield (mg)/IC$_{50}$ (mg/plate) of mizuna can be compared to the comprehensive antimutagenicity of both antimutagenic activity and yield in mizuna harvested in open field culture and that in greenhouse cultivation. The open field culture is superior to cultivation in greenhouse based on the contribution of the three antimutagens.

Isothiocyanate (ITC) is known as a pungent component of *Brassica* vegetables, some of which are anticarcinogenic. ITC is produced by the degradation of the precursor glucosinolate by myrosinase enzymes when the plants are cooked after cutting or grating. Nitrile is also produced as a by-product depending on the conditions. Mizuna also contains ITC, containing mainly BUlTC and PEITC and producing PEN as the by-product. These three compounds are significant in affecting the taste and flavor of mizuna. Several studies have been conducted on the relationship between the glucosinolate content of *Brassica* vegetables and environmental factors. For example, Yamato-mana (*Brassica rapa* L. Oleifera Group) is high in isothiocyanates and glucosinolates, when grown in Japanese winter season [38], and kale (*Brassica oleracea* var. *acephala*) has increased glucosinolate content at chilling and freezing temperature stress [39]. Therefore, it is possible that low-temperature exposure in open-field cultivation in Japanese winter season is involved in the increase in BUlTC, PEITC, and PEN in mizuna. These are challenges that determine factors of BUlTC, PEN, and PEITC production that guarantee high added value of taste, fragrance, and antimutagenicity in mizuna in the future.

**Conclusion**

Through hearing survey with former employees in Kyoto Prefecture office involved in the nationwide distribution of mizuna in Japan, we were able to clarify the administrative measures from 1980 to 1990s to demonstrate the paradigm shift in the new use of mizuna for salad due to increased demand of the vegetable in contemporary dishes. We also discussed that breeding programs of the young form ironically resulted in reduction the flavor and the antimutagenicity of this vegetable. It was also found that the current mizuna is of heirloom vegetables, but its shape is different from the traditional mizuna depicted in the ancient paintings. Traditional open-field cultivation of mizuna needs a long period to harvest and the dishes are limited, but the fragrant compounds, BUlTC, PEITC, and PEN of mizuna are higher in amount at the best harvest season ‘Syun’. BUlTC, PEITC, and PEN have been found to be antimutagenic revealing the significance of the traditional habit of eating mizuna at ‘Syun’. Therefore, it is highly possible that low-temperature exposure at open-field cultivation in Japanese winter is involved in increase in BUlTC, PEITC, and PEN of mizuna, and it is important to clarify this factor while distributing healthy vegetables in the future.

Most mizuna is currently cultivated at greenhouse. The advantage of greenhouse cultivation was that the crop could be distributed all year round, and farmers could earn more stable income than in open-field cultivation. However, one of the disadvantages of year-round cultivation was that the awareness about the best harvest season ‘Syun’ was not strong. From this research, we obtained the result that we can determine the best seasonal taste of mizuna even in open-field cultivation. As mentioned above, greenhouse cultivation has undeniable merits, but traditional open-field cultivation might be suitable if wishing for the best seasonal mizuna flavor.

**Abbreviations**

BUlTC: 3-Butenyl isothiocyanate; GC–MS: Gas chromatography–mass spectrometry; PEITC: Phenylethyl isothiocyanate; PEN: 3-Phenylpropionitrile; RMA: Relative mutagenic activity.

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**Author contributions**

TN, AS, SO, and YN designed the experiments. TN, AN, MW, KU, TM, SM, TY, MH, AK, KS, AS and YN performed the experiments. The manuscript was drafted by TN and revised by YN. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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