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Original Paper

Naturally grown rucola, *Eruca sativa*, contains more α-linolenic acid than conventionally grown rucola

Kousaku Ohta[1], Tsuyoshi Takeshita[2], Masatoshi Funabashi[3], Shoji Oda[1,*]

[1] Laboratory of Genome Stability, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba 277–8562, Japan; [2] Laboratory of Plant Life System, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba 277–8562, Japan; [3] Sony Computer Science Laboratories, Inc., Shinagawa, Tokyo 141–0022, Japan
* E-mail: odasho@k.u-tokyo.ac.jp Tel: +81-4-7136-3671 Fax: +81-4-7136-3669

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Abstract The ratio of dietary n-6/n-3 polyunsaturated fatty acids is higher in the modern Western meal when compared with that of the Paleolithic era. Evidences have been accumulating that the extremely high ratio of dietary n-6/n-3 polyunsaturated fatty acids increases the risk of disease and the deterioration of physical conditions among humans. In this study, the ratio of linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3) of rucola, *Eruca sativa*, was compared between naturally grown samples and conventional farming products ($n=3$ for each). We found that the naturally grown rucola contained significantly higher amount of α-linolenic acid ($p=0.026$), resulting in the lower ratio of linoleic acid and α-linolenic acid than the conventional ($p=0.016$). This finding suggests that vegetables cultured in conventional farming method could decrease the health promotion effects that the vegetables originally possess in natural environment.

Key words: α-linolenic acid, *Eruca sativa*, naturally grown vegetables, n-6/n-3 ratio.

The ratio of dietary n-6/n-3 polyunsaturated fatty acids has been one of the major topics in nutrition research. The ancient humankind in the Paleolithic era thrived on wild plants and animals, which contained a rich amount of n-3 polyunsaturated fatty acids. It is estimated that the meal in the Paleolithic era had a ratio of dietary n-6/n-3 polyunsaturated fatty acids of approximately 1 : 1. This ratio can be interpreted as the default value for a sane functioning of human metabolism. Compared to that, the modern Western meal has extremely high ratio of dietary n-6/n-3 polyunsaturated fatty acids and Simopoulos has estimated it to be 15 : 1 (Simopoulos 2002).

Naturally grown diets and artificially grown diets can be distinguished with their nutrient composition (Funabashi 2015). Fatty acid composition in the beef meat fed with grass has low ratio of n-6/n-3 polyunsaturated fatty acids, when compared with the ones produced with grain feed (Daley et al. 2010). Analogously in plants, ratio of dietary n-6/n-3 polyunsaturated fatty acids is reported to be lower in wild plants than cultivated crops (Vardavas et al. 2006). This suggests that plants metabolism is altered under cultivated conditions from its original profile, according to the change in ecological condition.

In this study, we compared the ratio of linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3) extracted from conventionally and naturally grown rucola, *Eruca sativa*, and found that naturally grown rucola has significantly lower ratio of n-6/n-3 fatty acids.

Materials and methods

We prepared a plot for the production of naturally grown vegetables with synecoculture in Kashiwa campus of The University of Tokyo (Kashiwa-no-ha, Kashiwa, Chiba) in June 2014. Synecoculture is a non-fertilizer, non-tillage, non-pesticides/herbicides cultivation system, in which vegetables and weeds compete and grow with each other (Funabashi 2011). For simplicity, we call in this paper the culture condition of synecoculture as "natural" condition. Commercially available seeds of rucola, *Eruca sativa*, were planted to grow in natural condition (Figure 1). Rucola cultivated in conventional farming were purchased from supermarkets around Kashiwa city. The difference between conventional cultivation and natural condition lies in the application of tillage, fertilizer, and chemicals with reduced biodiversity in monocultural environment (Funabashi 2015). The leaves were thoroughly dried up at room temperature, then stored with sealed bags in dark. After mincing, lipids components were extracted with methyl tertiary-butyl ether and analyzed with the gas chromatograph mass spectrometry (GC-MS; QP-2010Plus, Shimadzu, Kyoto) equipped with Supelco SP-2380 column ($30\,\text{m}\times0.25\,\text{mm}\times0.2\,\mu\text{m}$) (Sigma-Aldrich). These preparation and measurement are according to Takeshita et al. (Takeshita et

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al. 2014).

Geometric isomers of linoleic acid (C18:2) were separated with SP-2380 column. The surface area of the corresponding 2 peaks (geometric isomer) were summed up to obtain the total dose of linoleic acid (C18:2). In each chromatograms of GC-MS analysis, 7 or 8 peaks of fatty acids were detected and identified as palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and 2 peaks remained unidentified. The sum of linoleic acid (C18:2) and linolenic acid (C18:3) peaks occupied more than 70% of the total peak area of the top 10 peaks in all of the GC chromatograms. This indicates that linoleic acid (C18:2) and linolenic acid (C18:3) were the major fatty acids in the rucola examined. Area of both peaks of linoleic acid and linolenic acid were normalized with the area of internal standard (72332, Sigma-Aldrich). According to Food Composition Database (http://fooddb.mext.go.jp/) constructed by Ministry of Education, Culture, Sports, Science and Technology in Japan, linolenic acid contained in rucola is α-linolenic acid. Vardavas and his colleagues reported that α-linolenic acid occupies 44.3% of the total fatty acids in rucola, while γ-linolenic acid occupied only 0.4% of the total (Vardavas et al. 2006). These references strongly suggest that almost all of linolenic acid detected in this study was not γ- but α-linolenic acid, although the GC column used in this study was not able to separate α- and γ-linolenic acid.

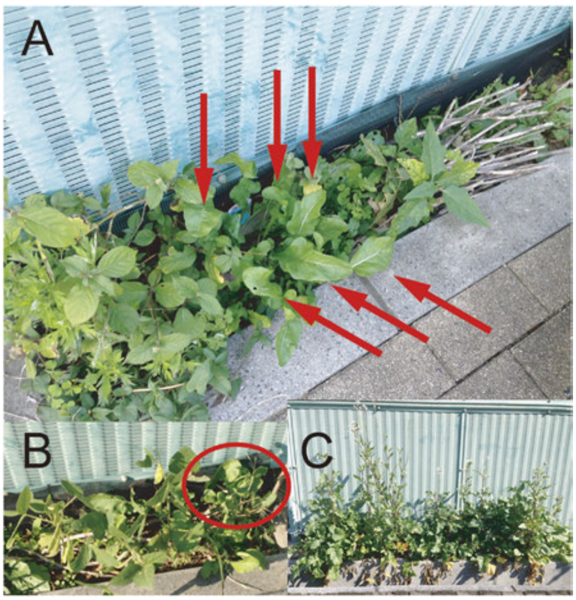

Figure 1. Rucola naturally grown with weeds in the synecoculture garden in Kashiwa campus of The University of Tokyo. In A, leaves of rucola are indicated with arrows. In B, rucola are circled. Plants shown in C are all rucola.

Results

We analyzed 3 samples of rucola for each category between 2014 and 2015 (SYN 2014, SYN 2015-1, SYN 2015-2 for natural samples, and CON 2014, CON 2015-1, CON 2015-2 for conventional samples in Table 1). Averaged area of linoleic acid (C18:2) and α-linolenic acid (C18:3) peaks were 40.7±4.66% and 33.2±1.91% in the naturally grown rucola and 59.2±6.97% and 18.5±4.71% in the conventional products, respectively

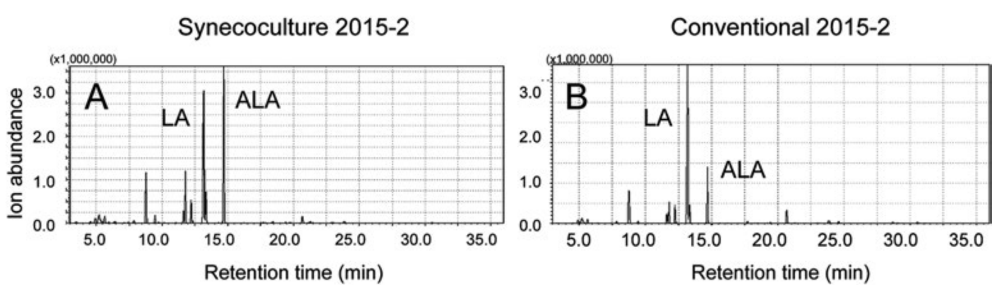

Figure 2. Typical GC-MS chromatograms of fatty acids extracted from naturally grown (A) and conventionally cultivated (B) rucola, *Eruca sativa*. LA: linoleic acid, ALA: α-linolenic acid. The conventionally cultivated rucola was produced in Chiba and Ibaraki Prefecture, Japan.

Table 1. linoleic acid and α-linolenic acid compositions in naturally and conventionally cultivated rucola.

ID	SYN2015-1	SYN2015-2	SYN2014	SYN2014A	CON2015-1	CON2015-2	CON2014
Synecoculture/Conventional	S	S	S	S	C	C	C
LA area	9.6	8.7	12.4	10.5	9.5	13.7	24.5
ALA area	9.2	7.2	8.5	5.8	4.4	3.1	7.0
Total fatty acids area	26.3	21.7	27.2	21.6	18.6	21.9	38.6
LA area ratio (%)	36.4	40.0	45.6	48.6	51.2	62.8	63.6
ALA area ratio (%)	35.0	33.3	31.2	26.8	23.5	14.1	18.0
ALA/LA (%)	96.3	83.3	68.4	55.1	45.9	22.4	28.3
n-6/n-3 ratio	1.04	1.20	1.46	1.81	2.18	4.46	3.53

Averaged area of linoleic acid (C18:2) and α-linolenic acid (C18:3) peaks were 40.7±4.66% and 33.2±1.91% in the naturally grown rucola and 59.2±6.97% and 18.5±4.71% in the conventionally cultivated rucola, respectively. Student's *t*-test indicates that the ratio of n-6/n-3 in the naturally grown rucola was lower than that in the conventionally cultivated rucola ($p<0.05$). SYN2014A was re-prepared from the same sample as SYN2014 after 10 months storage. LA: linoleic acid, ALA: α-linolenic acid.

(Figure 2, Table 1). This is translated as the significant increase of α-linolenic acid in natural samples with Student's *t*-test ($p=0.026$). The ratio of n-6/n-3 in the naturally grown rucola was lower than in the conventional ($p=0.016$).

Additionally, SYN 2014 was analyzed again to examine the temporal decay after 10 months storage (SYN 2014 A in Table 1). Area of linoleic acid peak decreased 15.3% (12.4→10.5), and α-linolenic acid decreased 31.8% (8.5→5.8) during the storage, suggesting that α-linolenic acid (C18:3) with 3 double bonds is less stable than linoleic acid (C18:2) with 2 double bonds. The ratio of dietary n-6/n-3 polyunsaturated fatty acids increased to 1.81 : 1 after storage (1.46 : 1 before storage), also confirming that α-linolenic acid (C18:3) is less stable. However, the ratio after the 10 months storage (SYN 2014 A:1.81) were still lower than the ratio in conventionally cultivated rucola (CON 2015-1 : 2.18, CON 2015-2 : 4.46, CON 2014 : 3.53). This supports the assumption that the significance of high ratio of dietary n-6/n-3 polyunsaturated fatty acids in the conventionally cultivated rucola did not attribute to the storage period during commercial distribution, usually within a few days. In general, unsaturated fatty acids are easily oxidized, while α-linolenic acid can be resistant to oxidization in glycolipid of chloroplast (Yamaguchi et al. 2012). This is consistent with our observation that the amount of α-linolenic acid was stably maintained in the naturally grown rucola during the 10 months storage. These results strongly suggest that the ratio of dietary n-6/n-3 polyunsaturated fatty acids in naturally grown rucola is lower than the cultivated rucola with conventional farming, regardless of the possible loss of α-linolenic acid during commercial distribution.

Discussion

α-Linolenic acid is one of the essential fatty acids that animals cannot produce by themselves. It is a precursor of the other n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is widely accepted that α-linolenic acid can promote human health, especially reducing the risk of cardiovascular diseases (de Lorgeril et al. 1994). It is considered to relate with the fluidity and physiological functions of cell membrane, signal transduction and gene expressions in living cells (Stark et al. 2008). Moreover, it is also known that the n-6/n-3 ratio of diets is more essential than total intake of each fatty acid, since the signaling cascades of n-6 and n-3 feedback to each other to maintain homeostasis. It is reported that wild plants have lower n-6/n-3 ratios compared with cultivated vegetables (Vardavas et al. 2006). The results of this study suggest that the characteristics of the synecoculture products are qualitatively similar to those grown in wild environment in terms of the polyunsaturated fatty acid composition, and can be significantly distinguished from those of conventionally cultivated ones.

What is the cause of the low n-6/n-3 ratio of polyunsaturated fatty acids in the naturally grown rucola? α-Linolenic acids are highly unsaturated, though it cannot be oxidized easily in water circumstance (Miyashita et al. 1993). It is also highly stable in glyceroglycolipids (Yamaguchi et al. 2012). For all living organisms, oxidation of their cell is harmful for survival, therefore they must keep cellular environment in a reduced condition in order to sustain their lives. The low n-6/n-3 ratio may represent better oxidation-reduction condition for metabolic functionality. This general conjecture applies both for the organism of plant and human dietary intake. With this view, our results suggest the importance of natural condition for the metabolic homeostasis of plants and human through food production. As is the case with conventional agriculture, when plants are cultivated out of natural condition with intensive application of fertilizer, pesticides, and tillage, their n-6/n-3 ratio could increase by metabolic disturbance and might consequently generate negative impact on human health.

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