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Current Topics in Food and Biodynamic Chemistry Research

Teruo MIYAZAWA*, Yuki KAWAKAMI, Toshiyuki KIMURA, Yoshihiro SUZUKI, Shinji YAMASHITA, Renpei ABE, Daigo IBUSUKI, Phumon SOOKWONG, Tsuyoshi TSUZUKI, Fumiko KIMURA and Kiyotaka NAKAGAWA

Food & Biodynamic Chemistry Laboratory (FBCL), Graduate School of Agricultural Science (GSAS), Tohoku University, Sendai 981-8555, Japan

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Summary

Studies on chemical structures, metabolisms and physiological significances of food and bioactive natural products are currently being addressed in our laboratory to explain their health effects in humans. Some novel molecules and functions of food and nutrients were discovered, and new foodstuffs and products were developed. The researches were carried out on stable authentic phosphatidylcholine hydroperoxide; membrane lipid glycation and its inhibitors; cancer growth suppression by conjugated triene fatty acids; antiangiogenicity of rice bran tocotrienol; glucosidase inhibition by mulberry 1-deoxynojirimycin; and high quality broccoli products regarding hepatoprotective sulforaphane contents.

Key words: lipid hydroperoxide, lipid glycation, conjugated fatty acid, rice bran tocotrienol, mulberry 1-deoxynojirimycin, broccoli sulforaphane

Abbreviations: CAM, chick embryo chorioallantoic membrane; CDHA, conjugated docosahexaenoic acid; CEPA, conjugated eicosapentaenoic acid; CL, chemiluminescence; CLA, conjugated linoleic acid; DAS, dorsal air sac; DNJ, 1-deoxynojirimycin; ELSD, evaporative light scattering detection; ESA, eleostearic acid; HILIC, hydrophilic interaction chromatography; HPLC, high performance liquid chromatography; HUVEC, human umbilical vein endothelial cells; MS, mass spectrometry; MxP, 2-methoxypropene; PCOOH, phosphatidylcholine hydroperoxide; PE, phosphatidylethanolamine; QTRAP, hybrid quadrupole/

*Corresponding author. Tel.: +81-22-717-8904; fax: +81-22-717-8905.
E-mail address: miyazawa@biochem.tohoku.ac.jp
(Complete mailing address of the corresponding author for office use)
Teruo Miyazawa
Professor
Food & Biodynamic Chemistry Laboratory
Graduate School of Agricultural Science, Tohoku University
1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan

linear ion trap mass spectrometer ; RBC, red blood cells ; Toc, tocopherol ; T3, tocotrienol ; VEGF, vascular endothelial growth factor

1. Stable authentic phosphatidylcholine hydroperoxide

Peroxidative stress on lipid molecules not only invites diminution of the nutritive value of foods, but also takes part in several biochemical consequences, aging and the pathophysiology of diseases (Witztum and Steinberg, 1991). It is important to measure lipid hydroperoxides as a primary oxidation product in order to determine the degree of lipid oxidized in foods and in the biomembranes of the body, and also to maintain health and the wholesomeness of foods. In 1987, Miyazawa et al. established a new methodology called chemiluminescence detection-high performance liquid chromatography (CL-HPLC), which is sensitive and selective enough to determine the lipid-class hydroperoxide levels in foods and human blood plasma (Miyazawa, 1989). The unique post-column chemiluminescence reagent consists of cytochrome *c* and luminol in an alkaline borate buffered solution. Using CL-HPLC, bis-hydroperoxides formation other than mono-hydroperoxides was confirmed in the initial stage of triacylglycerol oxidation in food oils (Miyazawa et al., 1995). Long term fish oil consumption in animals invites a high risk of membrane lipid peroxidation together with an increased need for α-tocopherol as membrane antioxidant (Song and Miyazawa, 2001). The principal cause of cellular lipid peroxidation is aging (senescence) of the animal body (Miyazawa et al., 1993). Membrane lipid peroxidation is essential to the pathogenesis of atherosclerosis (Kinoshita et al., 2000), diabetes (Nagashima et al., 2002) and some other diseases (Moriya et al., 2001).

In a case of the determination of lipid hydroperoxides, researchers often suffer from lack of suitable pure hydroperoxide standards. Lipid hydroperoxides, which are prepared from photo- or free radical-oxidized lipids, are generally used as standard compounds. However, the purity of the hydroperoxides in such standards is not so high. Impurities in the standard can be attributed to a number of analytical errors. Therefore, Miyazawa et al. recently established a synthetic method for a pure reference of lipid hydroperoxide by using a vinylether molecule (2-methoxypropene, MxP) (Fig. 1). The procedures include protecting hydroperoxide group as perketal by using MxP. By using HPLC the perketal was separated, and then the pure hydroperoxide was regenerated from the perketal. The obtained lipid hydroperoxide (e.g., phosphatidylcholine hydroperoxide ; PCOOH) was essentially pure ($>99\%$ purity), which can be preserved for several weeks at $-20^{\circ}C$ (Fig. 2). The prepared PCOOH is pure and stable enough to be used as the standard for the determination studies, as well as cell culture studies for evaluation of PCOOH pathogenicity.

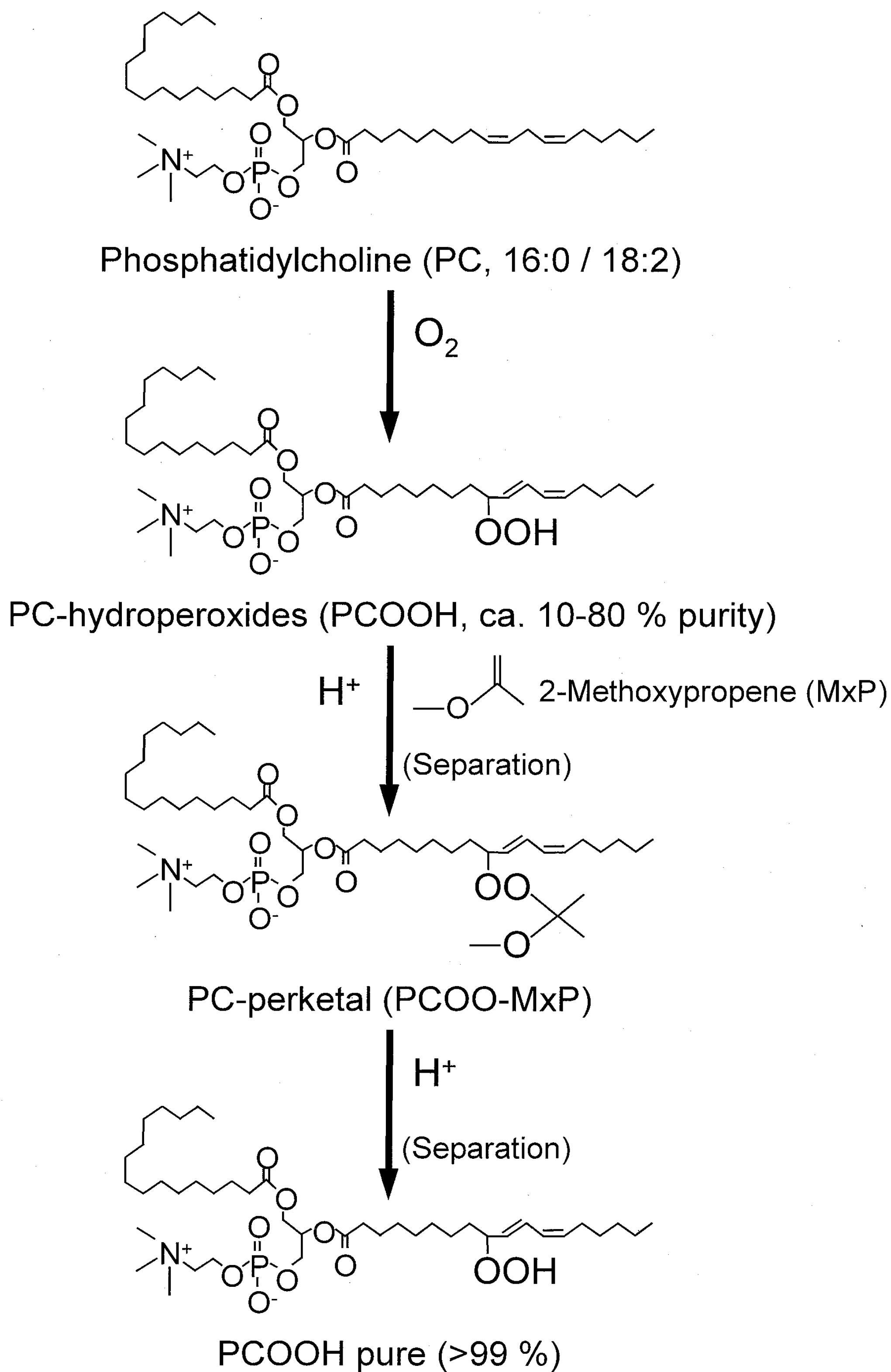


FIG. 1. Synthetic method for a pure phosphatidylcholine hydroperoxide (PCOOH).

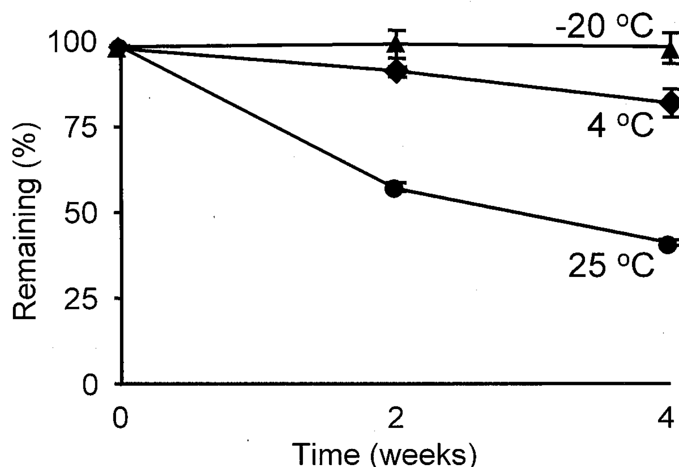


FIG. 2. Stability of authentic linoleic acid hydroperoxide; (LAOOH). LAOOH was synthesized (according to the scheme of Fig. 1), and dissolved in methanol. The LAOOH was stored for 4 weeks at −20, 4, and 25°C. LAOOH content was checked by CL-HPLC.

2. Membrane lipid glycation and its inhibitors

As described above, to determine lipid hydroperoxides as a primary oxidation product, CL-HPLC method has been established (Miyazawa, 1989). Using the method, it was confirmed that plasma PCOOH abnormally increases in hyperlipidemic and type 2 diabetic patients (Kinoshita et al., 2000; Nagashima et al., 2002). From the results, it was hypothesized that PCOOH-mediated cytotoxity closely involves in the pathophysiology of these diseases.

Recently, in the investigation why PCOOH increases in diabetic plasma, it was found that diabetic plasma contained an abnormally high amount of glycated lipid. The glycated lipid was identified as Amadori product of phosphatidylethanolamine (deoxy-D-fructosyl phosphatidylethanolamine, namely Amadori-PE; Figs. 3, 4) by HPLC on-line with hybrid quadrupole/linear ion trap mass spectrometer (QTRAP HPLC/MS/MS) (Miyazawa et al., 2005; Nakagawa et al., 2005). Amadori-PE generates reactive oxygen species and thereby triggers lipid peroxidation (Oak et al., 2000). It is therefore likely that phosphatidylethanolamine (PE) is exposed to glycation under hyperglycemic condition, yielding Amadori-PE in vivo. Amadori-PE causes oxidative stress (i.e., PCOOH), leading to disorders of cellular integrity (i.e., angiogenesis stimulation) (Oak et al., 2003). Amadori-PE and PCOOH could therefore play a role in the development of diabetes. In foods having a high amount of Amadori-PE (i.e., infant formula and chocolate) (Oak et al., 2002), lipid glycation could impair their flavor, color and nutritive value.

Despite potential significance of Amadori-PE in pathological signaling and food deterioration, lipid glycation inhibitors have never been discovered yet.

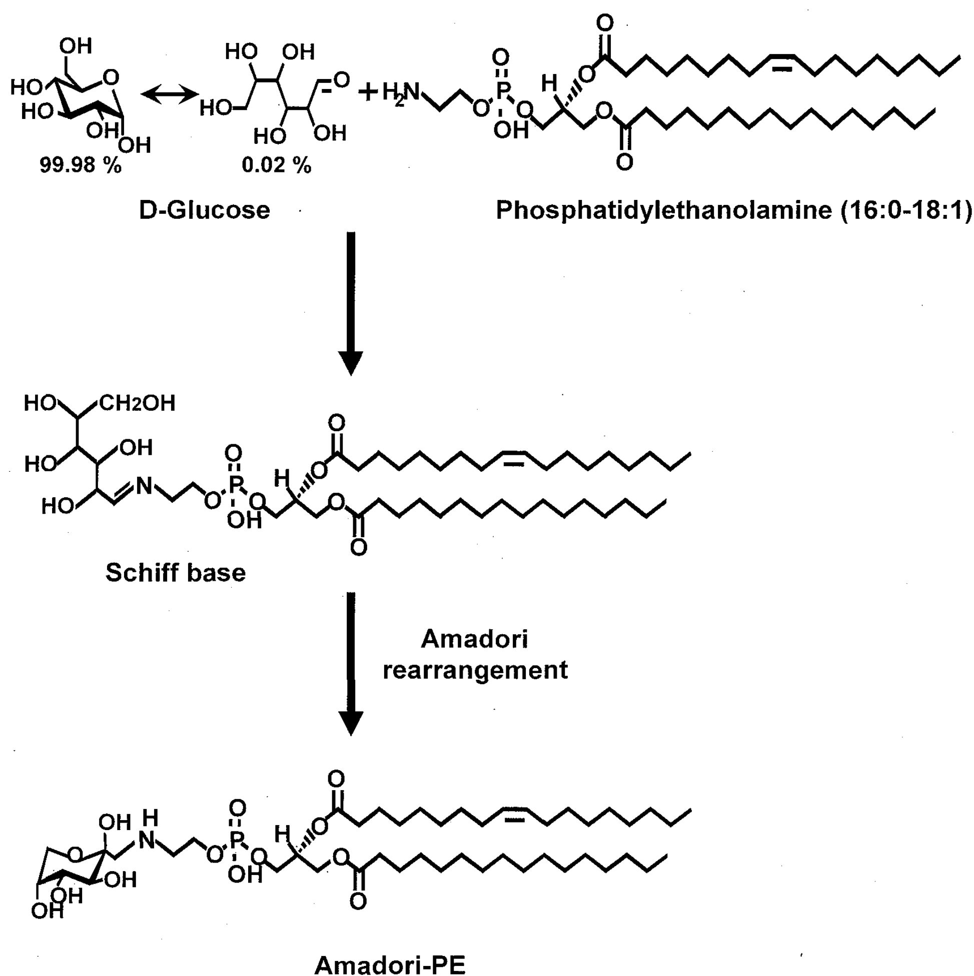


FIG. 3. Scheme for the glycation of phosphatidylethanolamine (PE). Glucose reacts with the amino group of PE to form an unstable Schiff base, which undergoes an Amadori rearrangement to yield the stable PE-linked Amadori product (Amadori-PE ; deoxy-D-fructosyl phosphatidylethanolamine).

This is because of lack of a lipid glycation model study useful for inhibitor screening. Higuchi et al. therefore optimized and developed a lipid glycation model considering various reaction conditions (glucose concentration, temperature, buffer type and pH) between PE and glucose (Higuchi et al., 2006). Using the developed model, various protein glycation inhibitors (aminoguanidine, pyridoxamine, aspirin and carnosine), antioxidants (ascorbic acid, α-tocopherol, quercetin and rutin) and some interesting food compounds (L-lysine, L-cysteine, pyridoxine, pyridoxal and pyridoxal 5′-phosphate) were evaluated for their anti-glycative property. As results, pyridoxal 5′-phosphate and pyridoxal (vitamin B6 derivatives) were the most effective anti-glycative compounds. These pyridoxals could easily be condensed with PE before glucose/PE reaction (Fig. 5). Since it was found that PE-pyridoxal 5′-phosphate adduct was detectable in

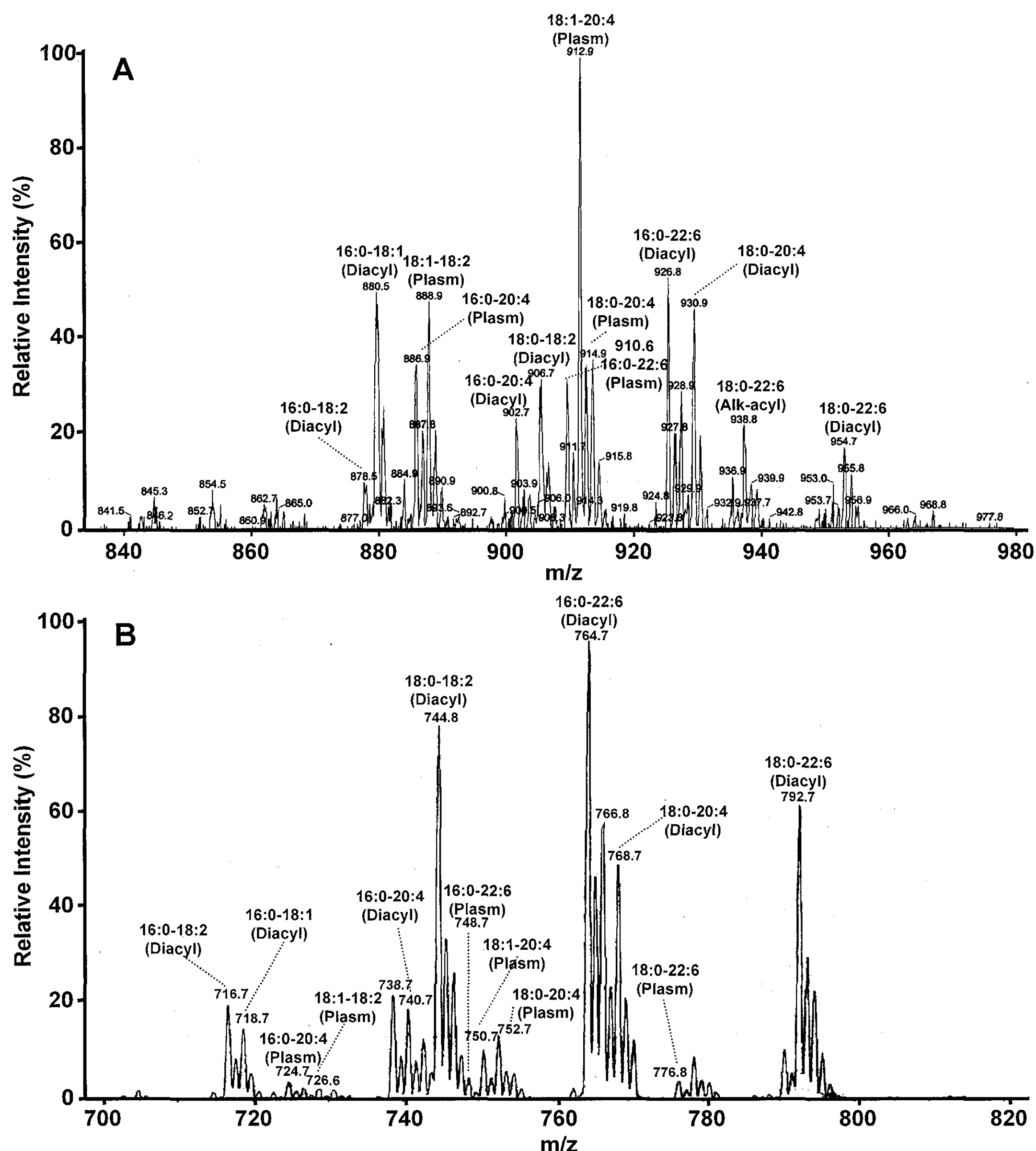


FIG. 4. QTRAP analysis (neutral loss scan) of the plasma extract of a diabetic patient. A, Neutral loss of 303 Da showing the specific detection of plasma Amadori-PE species. B, Neutral loss of 141 Da for nonglycated PE detection. Total lipids were extracted from plasma of a diabetic patient (female, 72 years old; fasting blood glucose, 185 mg/dl; hemoglobin A1c, 7.2). The lipid extract was dissolved in 100 μl of methanol-water (99 : 1, v/v; containing 5 mM ammonium acetate) and analyzed by QTRAP.

human red blood cells (RBC) (Fig. 6) and that elevated plasma Amadori-PE concentration in streptozotocin-induced diabetic rats was decreased by dietary supplementation of pyridoxal 5′-phosphate, it is likely that pyridoxal 5′-phosphate acts as lipid glycation inhibitor in vivo, which possibly contributes to diabetes prevention. Further investigation are necessary to determine the effects

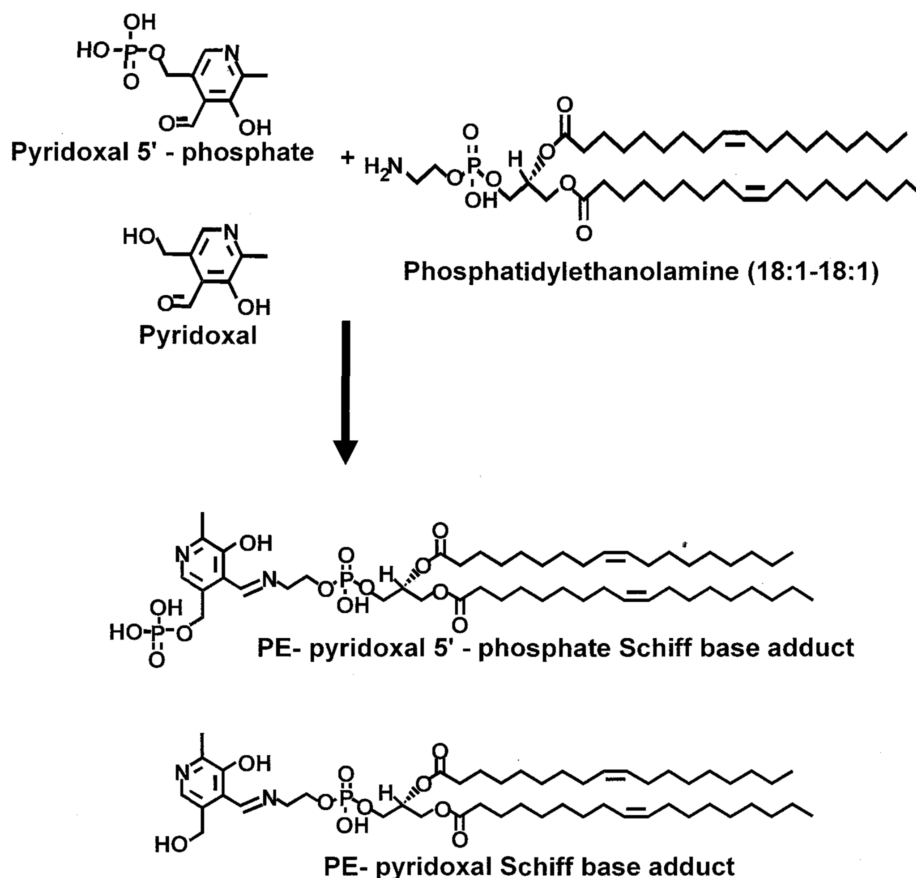


FIG. 5. Scheme for the condensation of pyridoxal 5′-phosphate or pyridoxal with phosphatidylethanolamine (PE). Pyridoxal 5′-phosphate or pyridoxal reacts with amino group of PE to form Schiff base adduct.

of lipid glycation inhibitors (i.e., pyridoxal 5′-phosphate) on lipid glycation, lipid peroxidation (i.e., PCOOH formation), oxidative stress, pathophysiology of human diseases as well as food deterioration.

3. Cancer growth suppression by conjugated triene fatty acids

Conjugated fatty acid is a generic term used for fatty acids with conjugated double bond systems, as exemplified by conjugated linoleic acid (CLA) (Fig. 7) (Ha et al., 1987). Several CLA isomers exist due to positional and geometrical isomerism of conjugated double bonds. The major naturally-existing CLA isomer is 9Z11E-18 : 2. CLA was first reported to have an anticarcinogenic effect, and subsequently various physiological effects (i.e., antiarteriosclerotic effect and lipid metabolism regulation). CLA is naturally found and, especially, present in ruminant fats such as beef tallow and milk fat. However, CLA level in these foodstuffs is around 1%, and it is not allowed to use natural fats as a CLA

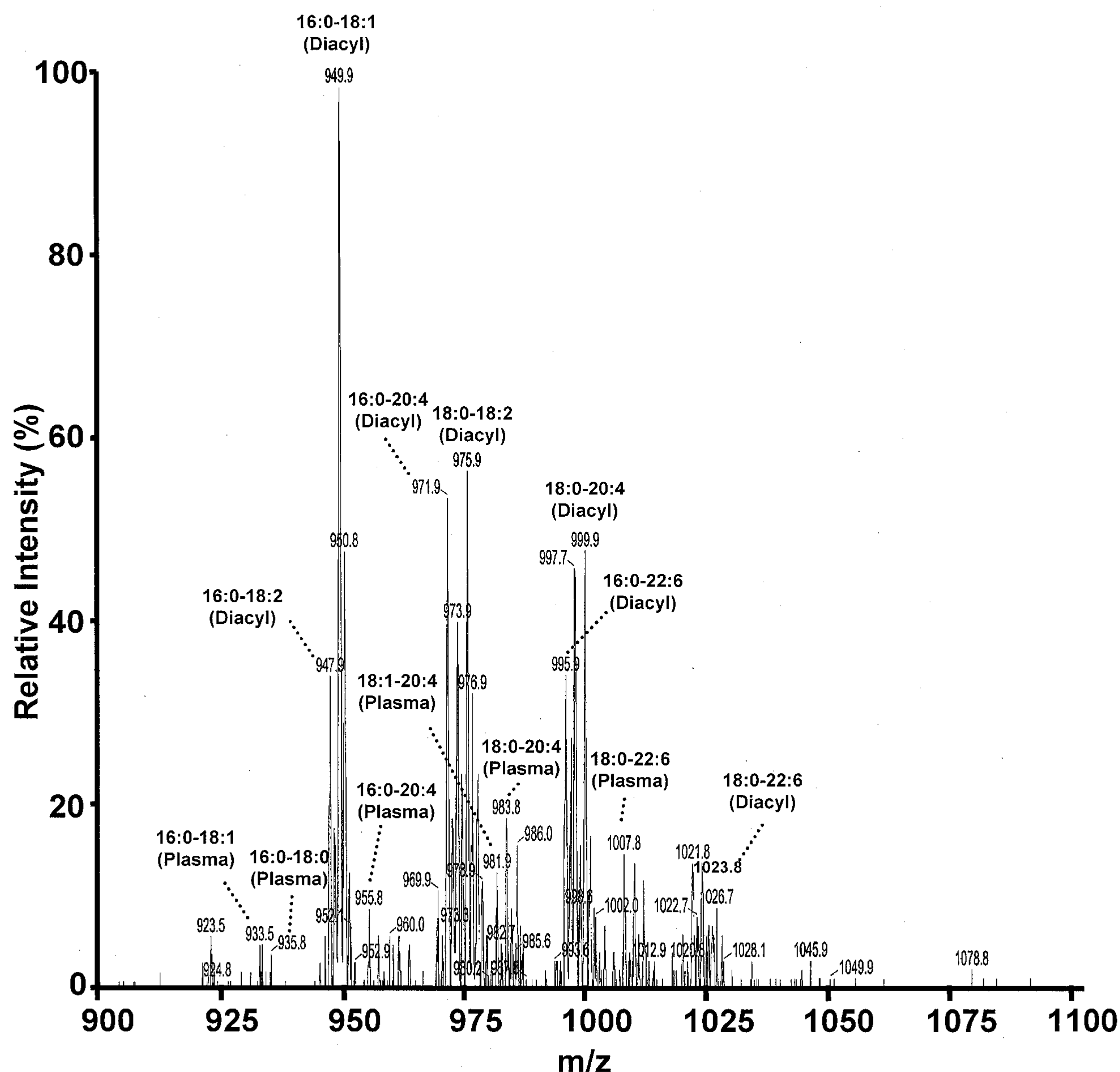


FIG. 6. QTRAP analysis (neutral loss scan) of human red blood cells (RBC) extract. Neutral loss of 372 Da showing the specific detection of molecular species of PE-pyridoxal 5′-phosphate adduct (reduced form) in RBC. Total lipids were extracted from human RBC (packed cells). RBC lipid extract was treated with $NaBH_4$, and analyzed by QTRAP.

health-promoting food. Therefore, at present, oils that include CLA are prepared from alkali-isomerization of vegetable oils and the products are commercialized as health foods (Larsen et al., 2003).

Conjugated fatty acids other than CLA exist in nature, but there are only a few studies on their physiological functions. Some certain seed oils contain conjugated triene fatty acids such as α-eleostearic acid (α-ESA; 9Z11E13E-18:3; Fig. 7) at levels of 60–80%. Since the seed oils having α-ESA is particularly interested, the tumor growth suppressive effect of α-ESA was compared with that of CLA by using nude mice transplanted with DLD-1 human colon cancer cells

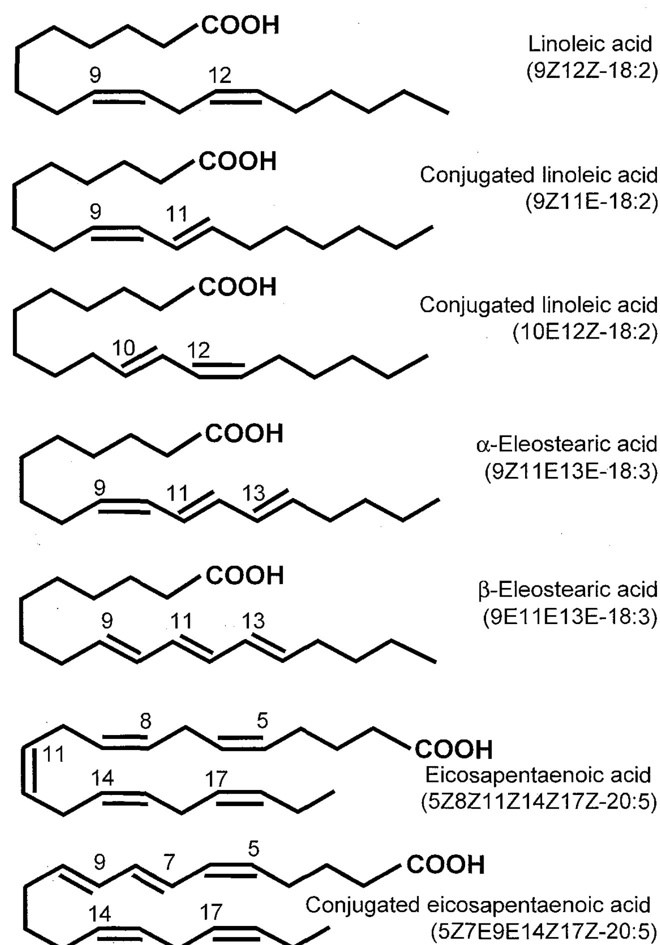


FIG. 7. Chemical structures of linoleic acid, conjugated linoleic acids (CLA), eleostearic acids (ESA), eicosapentaenoic acid (EPA, 5Z8Z11Z14Z17Z-20 : 5), and conjugated eicosapentaenoic acid (CEPA, 5Z7E9E14Z17Z-20 : 5).

(Tsuzuki et al., 2004a). The results showed that α-ESA had a stronger antitumor effect than CLA (Fig. 8). DNA fragmentation and lipid peroxidation were enhanced in tumor tissues of α-ESA-fed mice, suggesting that α-ESA induced apoptosis via lipid peroxidation. To further ascertain the effect and mechanism of α-ESA, cell culture studies were conducted, and confirmed that α-ESA had a stronger antitumor effect than CLA (Fig. 9). The induction of apoptosis by α-ESA was consistent with enhanced DNA fragmentation and increased caspase mRNA expression and caspase activity. An addition of α-tocopherol suppressed the α-ESA-induced oxidative stress and apoptosis, suggesting the effects of α-ESA associating with lipid peroxidation.

Seaweeds such as red and green algae contain more highly unsaturated conjugated fatty acids (i.e., conjugated eicosapentaenoic acid, CEPA; 5Z7E9E14Z17Z-20 : 5; Fig. 7). It was hypothesized that CEPA may produce a strong bioactivity. Therefore, CEPA from alkaline treatment of EPA was

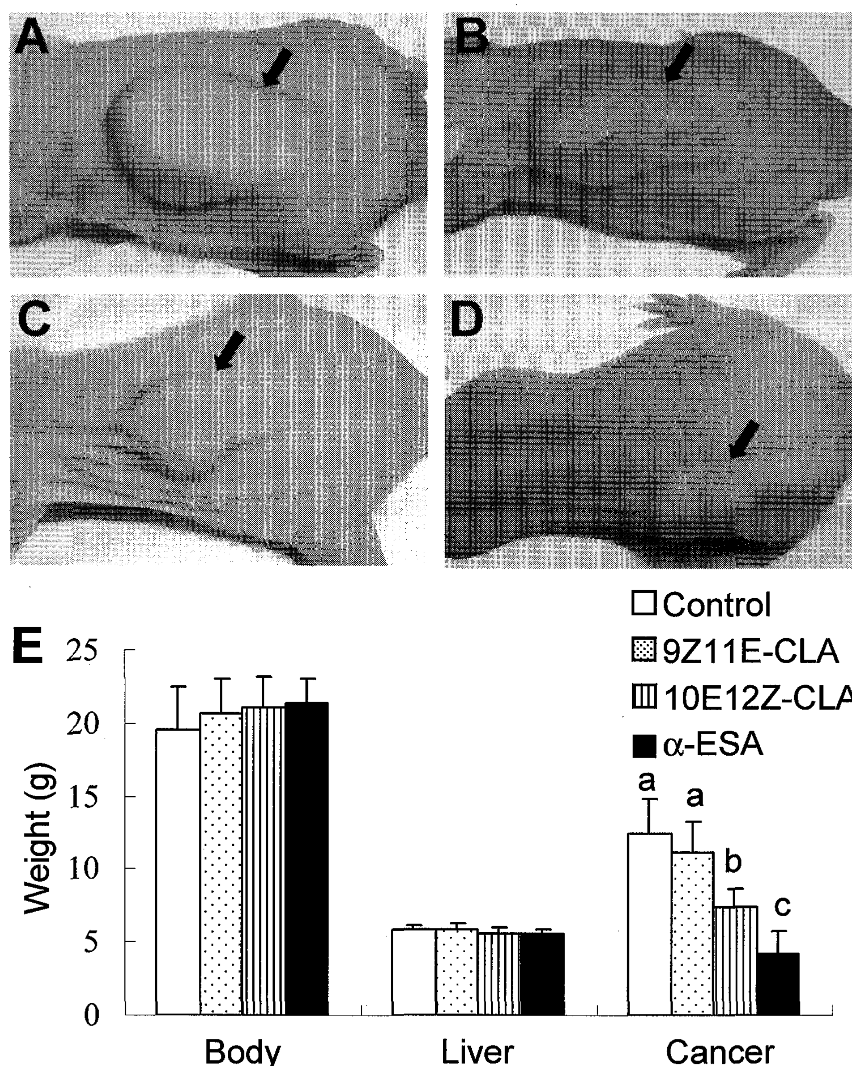


FIG. 8. The backs of nude mice transplanted with DLD-1 cells that received forcible fatty acid medication for 32 days. A, Transplanted mice fed the control (safflower oil fatty acid) diet. B, Transplanted mice fed the 9Z11E-CLA diet. C, Transplanted mice fed the 10E12Z-CLA diet. D, Transplanted mice fed the tung (tung oil α-ESA) diet. E, Tumor weights, body weights and liver weights of mice transplanted with DLD-1 cells that were fed a control, 9Z11E-CLA, 10E12Z-CLA or tung diet.

prepared, and it was found that CEPA induced a strong and selective apoptosis in cultured human tumor cells, with the mechanism proceeding via lipid peroxidation (Igarashi and Miyazawa, 2000a; 2000b). To verify the antitumor effect of CEPA in vivo, nude mice transplanted DLD-1 tumor cells were prepared, and compared the tumor growth between CEPA-fed mice and CLA- and EPA-fed mice. As results, tumor growth in CEPA-fed mice was markedly suppressed, compared with that in CLA- and EPA-fed mice (Fig. 10). CEPA-fed mice showed significant DNA fragmentation (apoptosis induction) and lipid peroxida-

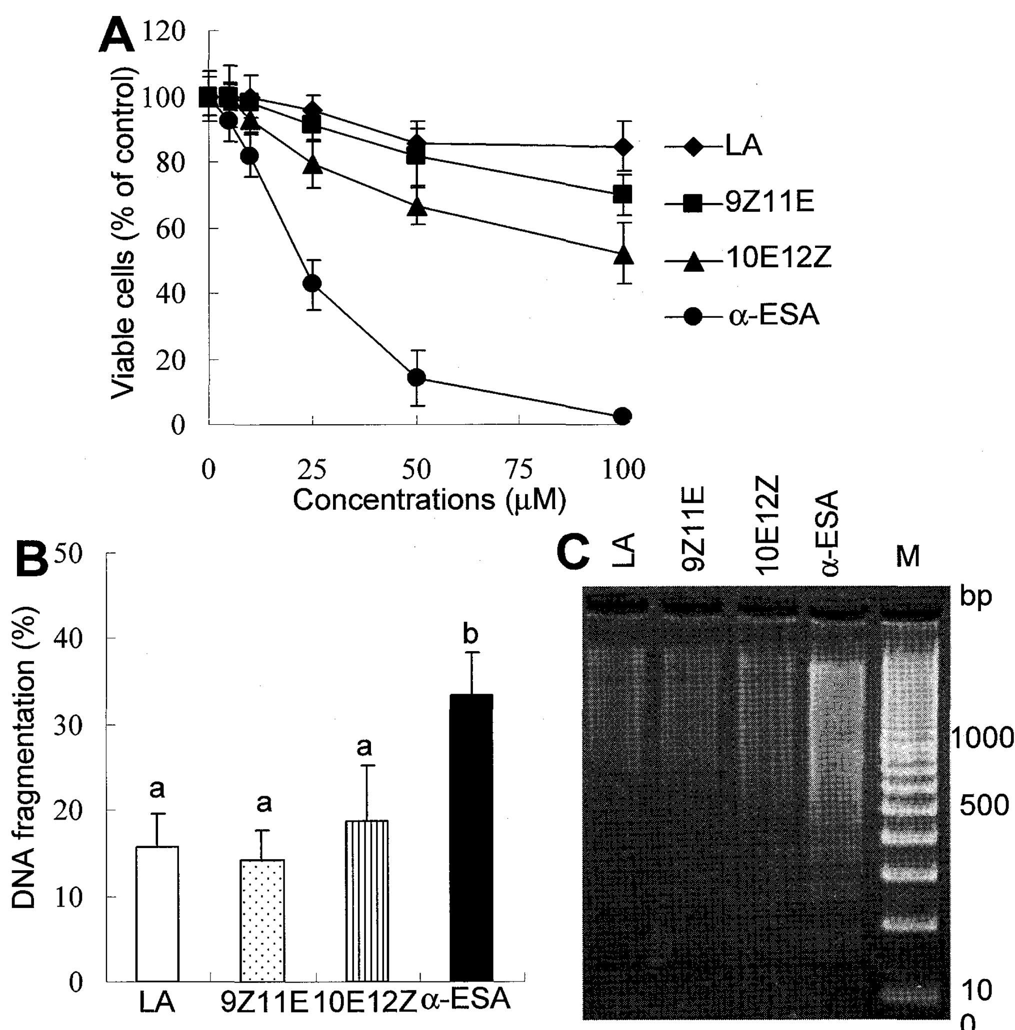


FIG. 9. Comparison of cytotoxicity and DNA fragmentation of linoleic acid (LA), 9Z11E-CLA (9Z11E), 10E12Z-CLA (10E12Z) and α-eleostearic acid (α-ESA) in DLD-1 cells. A, DLD-1 cells were incubated for 12 h after treatment with each fatty acid. B, DLD-1 cells were incubated for 12 h after treatment with 20 μM LA, 9Z11E-CLA, 10E12Z-CLA, and α-ESA. C, Agarose gel electrophoresis of low-molecular-weight DNA extracted from DLD-1 cells. DLD-1 cells were exposed to 20 μM LA, 9Z11E-CLA, 10E12Z-CLA, and α-ESA for 12 h. M, molecular weight markers.

tion in tumor tissues, compared with CLA- and EPA-fed mice. Therefore, it was verified that CEPA has a strong in vivo antitumor effect, and that CEPA acts through induction of apoptosis via lipid peroxidation (Tsuzuki et al., 2004b).

Besides antitumor effect, physiological functions, metabolism, analysis and oxidative stability of highly unsaturated conjugated fatty acids (i.e., α-ESA, CEPA and conjugated docosahexaenoic acid (CDHA)) have been reported (Tsuzuki et al., 2004c; 2005; 2006). The use of these conjugated fatty acids in food and medicine is likely to have great promise after their safety is confirmed.

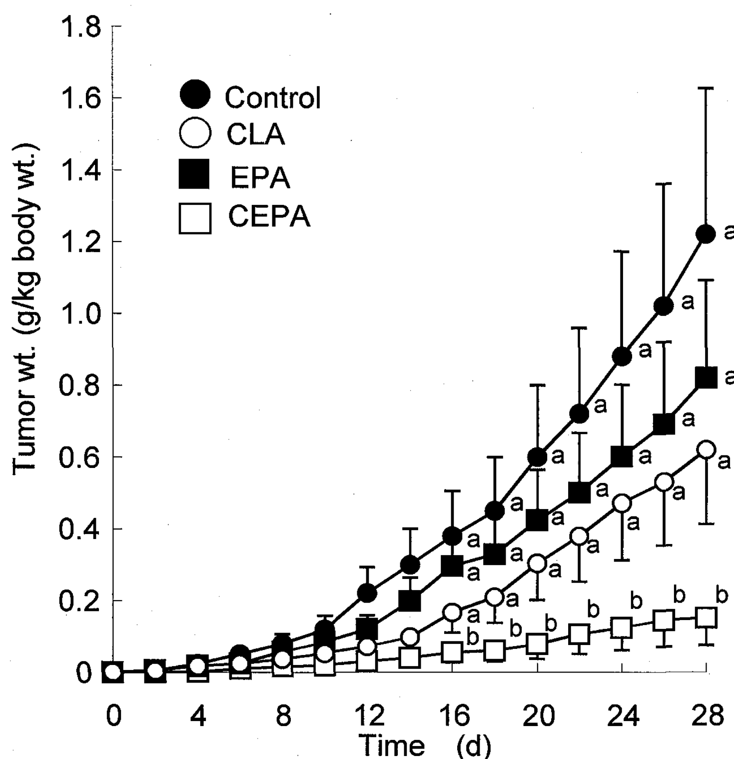
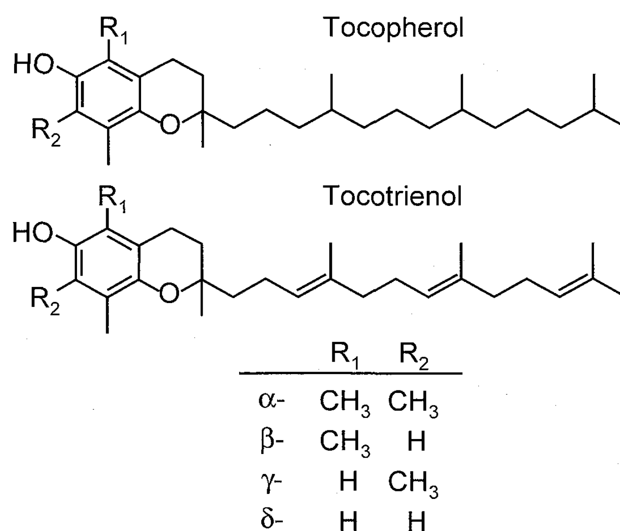


FIG. 10. Changes in the tumor weights in mice transplanted with DLD-1 cells that were fed the control, CLA, EPA, or CEPA diet for 4 wk.

4. Antiangiogenicity of rice bran tocotrienol

Angiogenesis, the formation of new blood vessels from a preexisting vascular bed, is of fundamental importance in several pathological states such as tumor growth, diabetic retinopathy and rheumatic arthritis (Kim et al., 1993). Angiogenesis normally involves a series of steps including endothelial cell activation and breakdown of the basement membrane, followed by proliferation, migration and tube formation of the endothelial cells. If the progression of these abnormal processes is minimized, the responsible food constituents can be used as therapeutic agents for the prevention of various angiogenesis-mediated disorders. Therefore, food ingredients bearing antiangiogenic property have been investigated by in vitro culture experiments (Inokuchi et al., 2003 ; Miyazawa et al., 2004a). As a result, vitamin E was screened out as one of the most attractive angiogenesis inhibitor.

Vitamin E occurs in nature as at least eight different isoforms that include α-, β-, γ-, and δ-isomers of both tocopherol (Toc) and tocotrienol (T3) (Fig. 11). T3 is a minor plant constituent, especially abundant in rice bran. T3 has an isoprenoid structure that differs from Toc bearing saturated phytyl side chain. Interestingly, in previous in vitro studies (Inokuchi et al., 2003 ; Miyazawa et al.,



	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

FIG. 11. Chemical structures of vitamin E.

2004a), T3 showed a stronger antiangiogenic effect than Toc (Fig. 12). In a subsequent in vivo study (Miyazawa et al., 2004b), it was found that T3 inhibited the new blood vessel formation on the growing chick embryo chorioallantoic membrane (CAM assay for in vivo angiogenesis). Orally administered T3 suppressed the tumor cell-induced angiogenesis in the mouse dorsal air sac (DAS) assay. In contrast, Toc showed very weak inhibition effect. Based on DNA microarray analysis, antiangiogenic effect of T3 was attributable in part to regulation of intracellular vascular endothelial growth factor (VEGF) signaling (phospholipase C-γ and protein kinase C) (Nakagawa et al., 2004 ; Mizushina et al., 2006). Our findings suggest that rice bran T3 has potential as a therapeutic dietary supplement for preventing angiogenic disorders. This possibility is now being investigated in clinical studies.

5. Glucosidase inhibition by mulberry 1-deoxynojirimycin

1-Deoxynojirimycin (DNJ) is naturally-occurred alkaloid with promising physiological activities in vivo (i.e., inhibition of intestinal α-glucosidase) (Watson et al., 2001). The compound is a D-glucose analogue, in which the pyranose ring oxygen is replaced by nitrogen (Fig. 13). In 1976, DNJ was isolated from mulberry tree (*Morus alba*) for the first time. This DNJ isolation prompted the concept that dietary mulberry DNJ might be beneficial for the suppression of abnormally high blood glucose levels, thereby preventing diabetes.

Various mulberry products (i.e., tea, powder and tablet) have been now commercially available in Japan and many countries. These products appeal the bioavailability of DNJ, but their DNJ contents are not specified because of lack

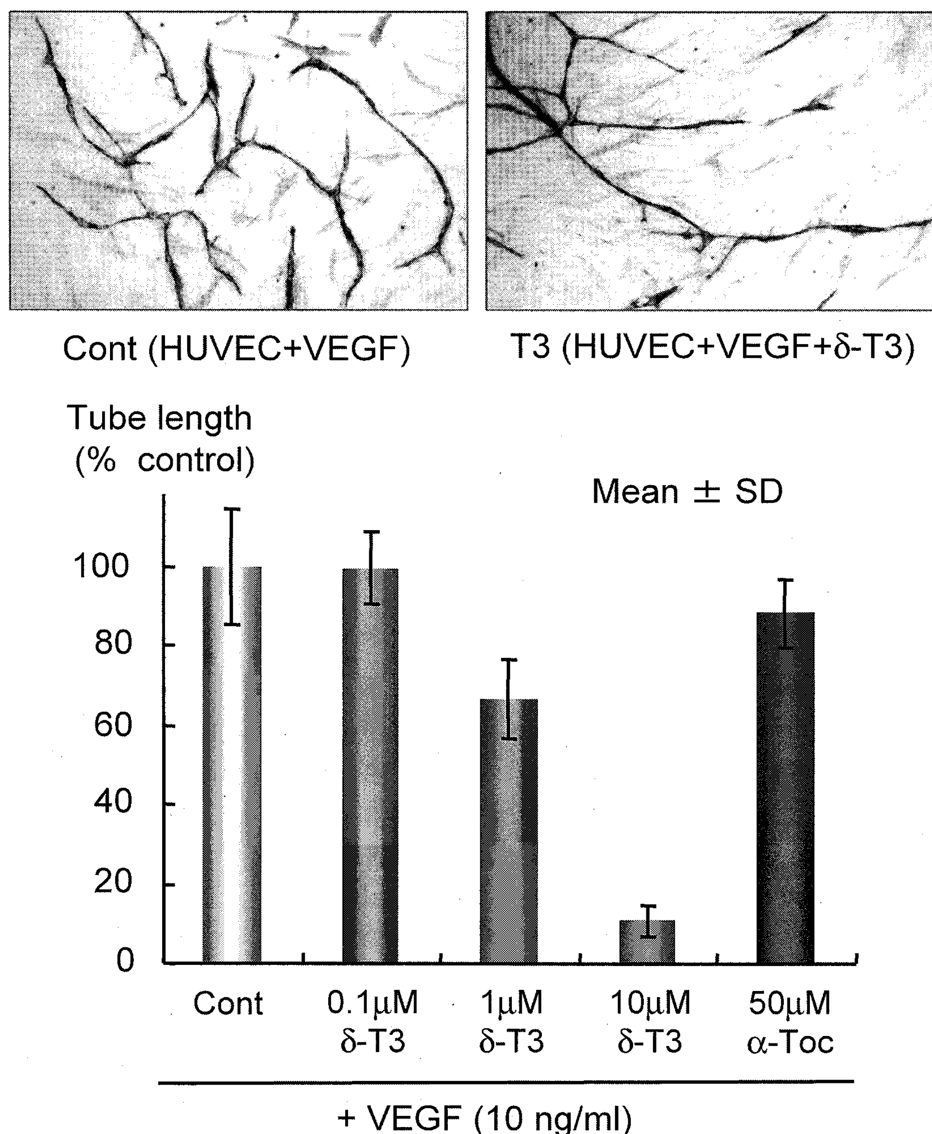


FIG. 12. Effects of vitamin E on the vascular endothelial growth factor (VEGF)-stimulated tube formation of human umbilical vein endothelial cells (HUVEC). HUVEC co-cultured with fibroblasts were incubated in the medium containing VEGF (10 ng/ml) with several concentrations of tocotrienol (T3) and tocopherol (Toc). After 11 days, the cells were stained and photographed, and the lengths of the tubes were determined.

of method to measure DNJ. Kimura et al. have therefore previously developed a method for determining DNJ by hydrophilic interaction chromatography with evaporative light scattering detection (HILIC-ELSD) (Kimura et al., 2004a; 2004b) (Fig. 14). Using HILIC-ELSD, it was confirmed that DNJ contents in mulberry products are relatively low as about 0.1% (100 mg/100 g dry product), and, surprisingly, there are some products with a trace amount of DNJ (less than 0.05%) (Table 1). Development of DNJ-enriched product is therefore desired.

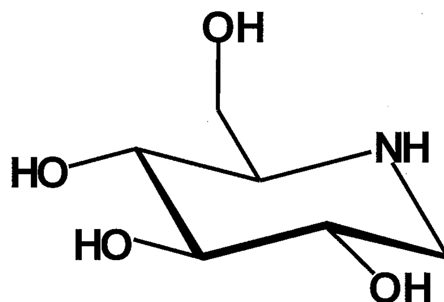


FIG. 13. Chemical strucuture of 1-deoxynojirimycin (DNJ).

Table 1. 1-Deoxynojirimycin (DNJ) concentrations in mulberry leaf cultivars and mulberry leaf products on the market

Cultivar	DNJ conc. (%)
Kinuyutaka (*Morus alba*)	0.14±0.0029
Kairyonezumikaeshi (*Morus alba*)	0.13±0.0026
Shinkenmochi (*Morus bombysis*)	0.10±0.0020
Product (form)	**DNJ conc. (%)**
A (tea leaf)	0.23±0.0036
B (tea leaf)	0.23±0.0019
C (tea leaf)	0.21±0.0039
D (tea leaf)	0.17±0.0028
E (tea leaf)	0.17±0.0017
F (tea leaf)	0.13±0.0019
G (tea leaf)	<0.05
H (powder)	0.20±0.0087
I (powder)	0.20±0.0013
J (tablet)	0.48±0.0062
K (tablet)	0.28±0.0068

Values are means±SD, $n=3$.

Pharmacological administration of DNJ-enriched product may be a promising therapeutic approach for diabetes prevention.

In order to develop DNJ-enriched product, DNJ concentrations in mulberry leaves obtained from different cultivars, harvest seasons and harvest regions were determined and compared by HILIC-ELSD, and mulberry leaves containing the higher level of DNJ were selected. As results, it was found that young mulberry leaves from top part of branch contain relatively high amount of DNJ (0.2–0.5%). After optimization of harvesting, drying and extraction processes of mulberry leaves, food-grade mulberry powder enriched with DNJ (1.0%) could be successfully produced. Human studies indicated that the developed DNJ-enriched

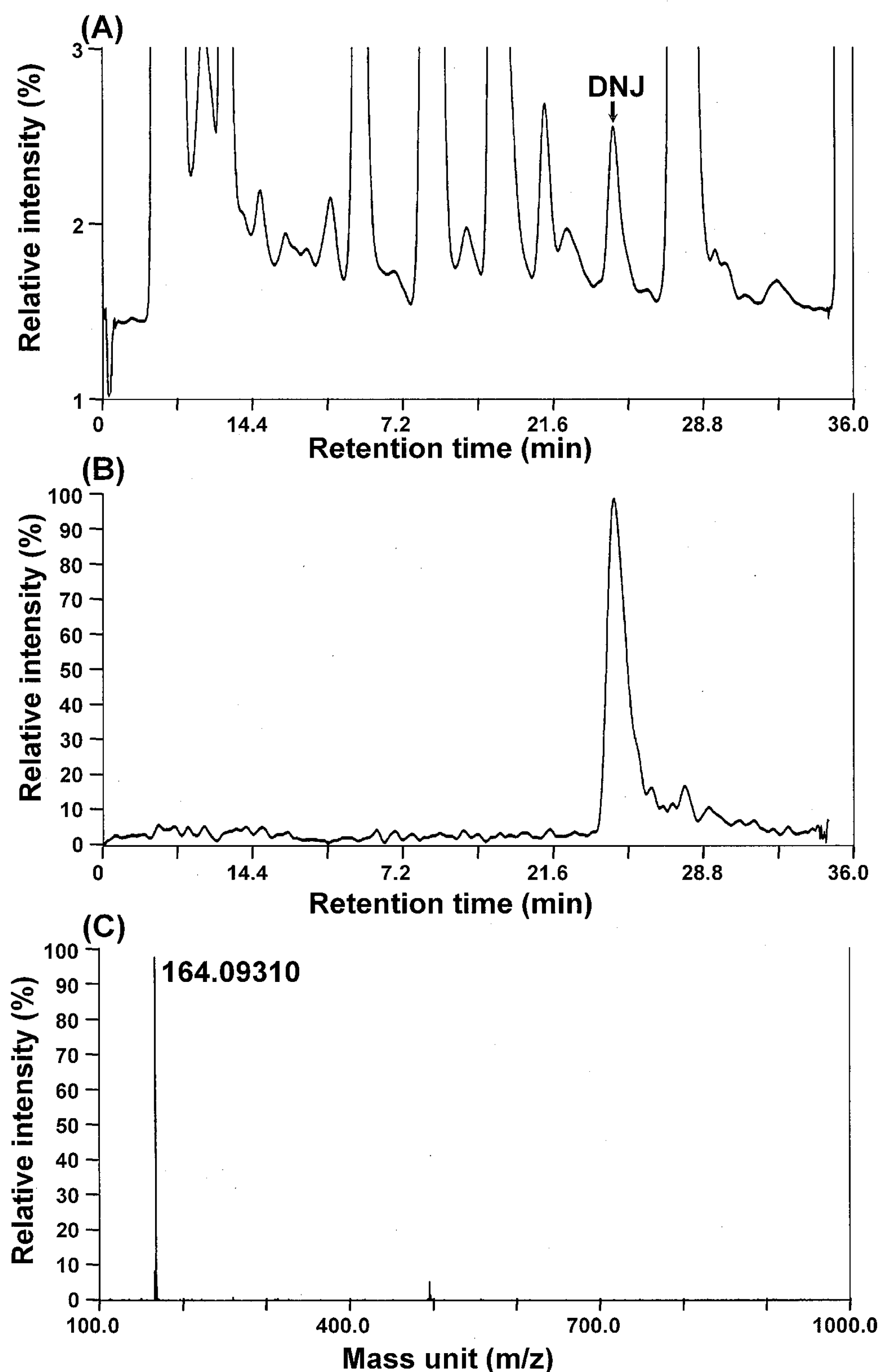


FIG. 14. HILIC-ELSD/MS analysis of 1-deoxynojirimycin (DNJ) present in mulberry leaves. A, ELSD chromatogram. B, Single ion plot of the mass corresponding to the $[M+H]^+$ ion of DNJ (m/z 164.1). C, MS spectrum of the peak detected at 24.5 min in the chromatogram B. Leaf extract from Kairyonezumikaeshi, a popular mulberry cultivar in Japan, was analyzed by HILIC-ELSD/MS following optimal conditions; column TSKgel Amide-80 (4.6×250 mm); mobile phase, a mixture of acetonitrile and distilled water (81 : 19; containing 6.5 mM ammonium acetate; pH 5.5); flow rate, 1 mL/min; column temperature, 40°C.

powder has an effect of lowering blood-sugar level. Therefore, the DNJ-enriched product may be used therapeutically in the oral treatment of the non-insulin-dependent diabetes mellitus (type 2 diabetes). This possibility is now being investigated in clinical studies, to obtain approval of "Food for Specified Health Use (FOSHU)" status in Japan.

6. High quality broccoli products regarding hepatoprotective sulforaphane contents

Sulforaphane (4-methylsulfinylbutyl isothiocyanate) is a naturally occurring sulfur-containing isothiocyanate (Fig. 15). Sulforaphane is abundant in cruciferous vegetables such as broccoli, cauliflower, cabbage and kale, of which the highest concentration is in broccoli. Sulforaphane is formed from its glucosinolate (glucoraphanin) by myrosinase, when broccoli tissue is crushed or chewed.

In 1992, Zhang et al. initially identified broccoli sulforaphane as an inducer of quinone reductase, a phase II detoxification enzyme. Subsequently, these authors reported that sulforaphane administration prevented dimethylbenz(a)-

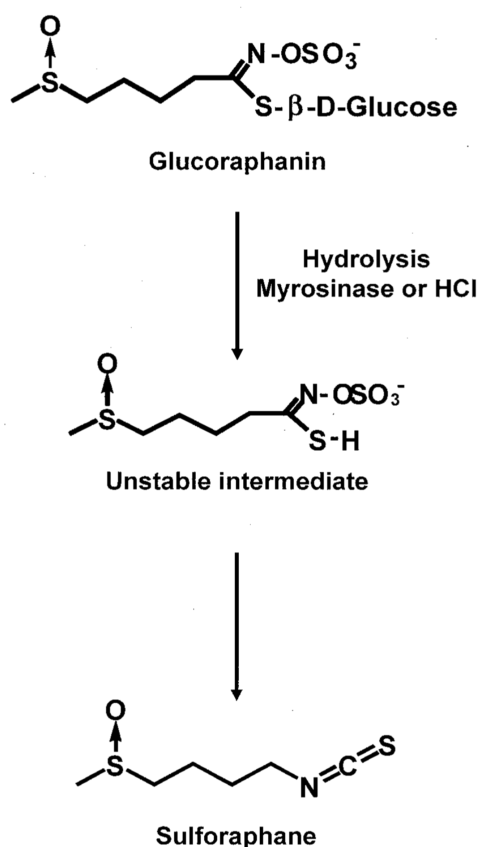


FIG. 15. Sulforaphane is the aglycone breakdown product of the glucosinolate glucoraphanin.

induced mammary tumors in rats. Other recent studies revealed that sulforaphane induced apoptosis in several cancer cell lines (Gamet-Payrastre et al., 2000). These findings suggest broccoli sulforaphane potentially beneficial for cancer prevention.

Various broccoli products (i.e., powder and tablets) are now commercially supplied. Their labels show the bioavailability of sulforaphane, but the majority of the products do not display the exact amounts of sulforaphane. This is because of lack of a suitable analysis method for sulforaphane. In general, HPLC with UV detection (201 nm) is used for sulforaphane analysis. However, the HPLC-UV method is insensitive, because sulforaphane has no UV chromophore. Nakagawa et al. therefore developed a method for determining sulforaphane by using HPLC coupled with ELSD (Nakagawa et al., 2006) (Fig. 16). Briefly, sulforaphane was extracted from acid-hydrolyzed broccoli samples, followed by solid-phase extraction and reversed-phase HPLC. Sulforaphane was detected by ELSD and concurrently identified by electrospray ionization time-of-flight mass spectrometry (MS). The recovery rate of sulforaphane from the extraction method is above 95%. The detection limit is 0.5 μg. The method is sensitive enough to determine sulforaphane in mature broccoli, broccoli sprouts and commercial broccoli products. Sulforaphane concentration in broccoli sprout (1,200 mg/100 g dry weight) was about 10 times higher than that of mature broccoli (40–170 mg/100 g dry weight) (Table 2). Therefore, the broccoli sprout is recommended as a source of sulforaphane-rich products. In contrast, sulforaphane could not be detected in most of broccoli products, suggesting present commercial broccoli products having low quality. By using this method as

Table 2. Sulforaphane concentrations in mature broccoli, broccoli sprouts and commercial broccoli products

Samples	Part or form	Sulforaphane conc. (%)
Mature broccoli A	Floret	0.17±0.0047
	Stem	0.09±0.0028
Mature broccoli B	Floret	0.07±0.0045
	Stem	0.04±0.0003
Broccoli sprouts	Whole	1.15±0.0327
Broccoli product A	Tablet	<0.001
Broccoli product B	Powder	<0.001
Broccoli product C	Tablet	0.02±0.0032
Broccoli product D	Powder	<0.001
Broccoli product E	Tablet	<0.001
Broccoli product F	Tablet	<0.001
Broccoli product G	Tablet	<0.001

Values are means±SD, $n=3$.

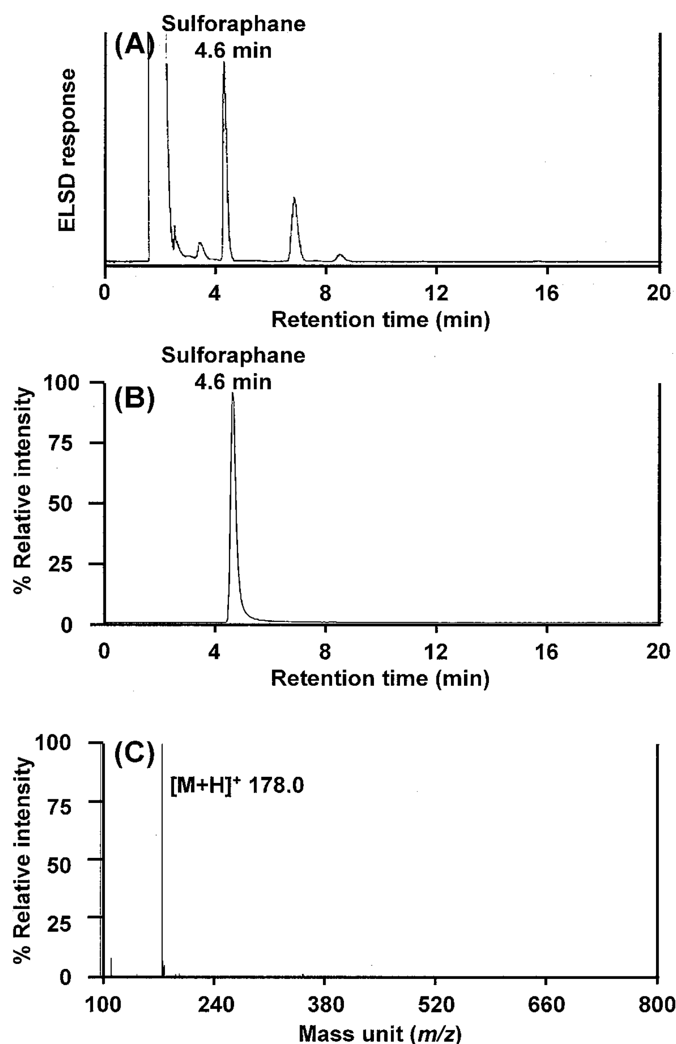


FIG. 16. HPLC-ELSD/MS analysis of broccoli sulforaphane. A, ELSD chromatogram. B, Single ion plot of the mass corresponding to the $[M+H]^+$ ion of sulforaphane (*m/z* 178.0). C, MS spectrum of the peak detected at 4.6 min in the chromatogram A and B. Sulforaphane was extracted from mature broccoli (floret), and analyzed by HPLC-ELSD/MS.

reference, it is possible to develop sulforaphane-rich products, which is now being studied as further objective.

Prospects

In this paper, recent activities and on-going studies in our laboratory were introduced. More information is referred to our website (http://www.agri.tohoku.ac.jp/kinoubunshi/index-j.html). Now, we are welcome for persons who are interesting in our activities especially in Doctoral course. If you are interesting to become a member of our research team, please feel free to contact Prof. Teruo

Miyazawa via e-mail (miyazawa@biochem.tohoku.ac.jp).

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References

Gamet-Payrastre, L., Li, P., Lumeau, S., Cassar, G., Dupont, M.A., Chevolleau, S., Gasc, N., Tulliez, J., and Terce, F., Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res.*, **60**, 1426-1433 (2000)

Ha, Y.L., Grimm, N.K., and Pariza, M.W., Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis*, **8**, 881-1887 (1987)

Higuchi, O., Nakagawa, K., Tsuzuki, T., Suzuki, T., Oikawa, S., and Miyazawa, T., Aminophospholipid glycation and its inhibitor screening system: a new role of pyridoxal 5′-phosphate as the inhibitor. *J. Lipid Res.*, **47**, 964-974 (2006)

Igarashi, M., and Miyazawa, T., Do conjugated eicosapentaenoic acid and conjugated docosahexaenoic acid induce apoptosis via lipid peroxidation in cultured human tumor cells? *Biochem. Biophys. Res. Commun.*, **270**, 649-656 (2000a)

Igarashi, M., and Miyazawa, T., Newly recognized cytotoxic effect of conjugated trienoic fatty acids on cultured human tumor cells. *Cancer Lett.*, **148**, 173-179 (2000b)

Inokuchi, H., Hirokane, H., Tsuzuki, T., Nakagawa, K., Igarashi, M., and Miyazawa, T., Anti-angiogenic activity of tocotrienol. *Biosci. Biotechnol. Biochem.*, **67**, 1623-1627 (2003)

Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., and Ferrara, N., Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo. *Nature*, **362**, 841-844 (1993)

Kimura, T., Nakagawa, K., Saito, Y., Yamagishi, K., Suzuki, M., Yamaki, K., Shinmoto, H., and Miyazawa, T., Determination of 1-deoxynojirimycin in mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. *J. Agric. Food Chem.*, **52**, 1415-1418 (2004a)

Kimura, T., Nakagawa, K., Saito, Y., Yamagishi, K., Suzuki, M., Yamaki, K., Shinmoto, H., and Miyazawa, T., Simple and rapid determination of 1-deoxynojirimycin in mulberry leaves. *Biofactors*, **22**, 341-345 (2004b)

Kinoshita, M., Oikawa, S., Hayasaka, K., Sekikawa, A., Nagashima, T., Toyota, T., and Miyazawa, T., Age-related increase in plasma phosphatidylcholine hydroperoxide concentrations in normal subjects and patients with hyperlipidemia. *Clin. Chem.*, **46**, 822-828 (2000)

Larsen, T.M., Toubro, S., and Astrup, A., Efficacy and safety of dietary supplements containing CLA for the treatment of obesity : evidence from animal and human studies. *J. Lipid Res.*, **44**, 2234-2241 (2003)

Miyazawa, T., Determination of phospholipid hydroperoxides in human blood plasma by a chemiluminescence-HPLC assay. *Free Radic. Biol. Med.*, **7**, 209-217 (1989)

Miyazawa, T., Suzuki, T., and Fujimoto, K., Age-dependent accumulation of phosphatidylcholine hydroperoxide in the brain and liver of the rat. *Lipids*, **28**, 789-793 (1993)

Miyazawa, T., Kunika, H., Fujimoto, K., Endo, Y., and Kaneda, T., Chemiluminescence detection of mono-, bis-, and tris-hydroperoxy triacylglycerols present in vegetable oils. *Lipids*, **30**, 1001-1006 (1995)

Miyazawa, T., Inokuchi, H., Hirokane, H., Tsuzuki, T., Nakagawa, K., and Igarashi, M., Anti-angiogenic potential of tocotrienol in vitro. *Biochemistry* (Moscow), **69**, 67-69 (2004a)

Miyazawa, T., Tsuzuki, T., Nakagawa, K., and Igarashi, M., Antiangiogenic potency of vitamin E. *Ann. N.Y. Acad. Sci.*, **1031**, 401-404 (2004b)

Miyazawa, T., Oak, J.H., and Nakagawa, K., Tandem mass spectrometric analysis of Amadori-glycated phosphatidylethanolamine in human plasma. *Ann. N.Y. Acad. Sci.*, **1043**, 280-283 (2005)

Mizushina, Y., Nakagawa, K., Shibata, A., Awata, Y., Kuriyama, I., Shimazaki, N., Koiwai, O., Uchiyama, Y., Sakaguchi, K., Miyazawa, T., and Yoshida, H., Inhibitory effect of tocotrienol on eukaryotic DNA polymerase lambda and angiogenesis. *Biochem. Biophys. Res. Commun.*, **339**, 949-955 (2006)

Moriya, K., Nakagawa, K., Santa, T., Shintani, Y., Fujie, H., Miyoshi, H., Tsutsumi, T., Miyazawa, T., Ishibashi, K., Horie, T., Imai, K., Todoroki, T., Kimura, S., and Koike, K., Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res.*, **61**, 4365-470 (2001)

Nagashima, T., Oikawa, S., Hirayama, Y., Tokita, Y., Sekikawa, A., Ishigaki, Y., Yamada, R., and Miyazawa, T., Increase of serum phosphatidylcholine hydroperoxide dependent on glycemic control in type 2 diabetic patients. *Diabetes Res. Clin. Pract.*, **56**, 19-25 (2002)

Nakagawa, K., Eitsuka, T., Inokuchi, H., and Miyazawa, T., DNA chip analysis of comprehensive food function : inhibition of angiogenesis and telomerase activity with unsaturated vitamin E, tocotrienol. *Biofactors*, **21**, 5-10 (2004)

Nakagawa, K., Oak, J.H., Higuchi, O., Tsuzuki, T., Oikawa, S., Otani, H., Mune, M., Cai, H., and Miyazawa, T., Ion-trap tandem mass spectrometric analysis of Amadori-glycated phosphatidylethanolamine in human plasma with or without diabetes. *J. Lipid Res.*, **46**, 2514-2524 (2005)

Nakagawa, K., Umeda, T., Higuchi, O., Tsuzuki, T., Suzuki, T., and Miyazawa, T., Evaporative light-scattering analysis of sulforaphane in broccoli samples : quality of broccoli products regarding sulforaphane con-

tents. *J. Agric. Food Chem.*, **54**, 2479-2483 (2006)

Oak, J.H., Nakagawa, K., and Miyazawa, T., Synthetically prepared Amadori-glycated phosphatidylethanolamine can trigger lipid peroxidation via free radical reactions. *FEBS Lett.*, **481**, 26-30 (2000)

Oak, J.H., Nakagawa, K., and Miyazawa, T., UV analysis of Amadori-glycated phosphatidylethanolamine in foods and biological samples. *J. Lipid Res.*, **43**, 523-529 (2002)

Oak, J.H., Nakagawa, K., Oikawa, S., and Miyazawa, T., Amadori-glycated phosphatidylethanolamine induces angiogenic differentiations in cultured human unbilical vein endotherial cells. *FEBS Lett.*, **555**, 419-423 (2003)

Song, J.H., and Miyazawa, T., Enhanced level of n-3 fatty acid in membrane phospholipids induces lipid peroxidation in rats fed dietary docosahexaenoic acid oil. *Atherosclerosis*, **155**, 9-18 (2001)

Tsuzuki, T., Tokuyama, Y., Igarashi, M., and Miyazawa, T., Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation. *Carcinogenesis*, **25**, 1417-1425 (2004a)

Tsuzuki, T., Igarashi, M., and Miyazawa, T., Conjugated eicosapentanoic acid inhibits transplanted tumor growth via membrane lipid peroxidation in nude mice. *J. Nutr.*, **134**, 1162-1166 (2004b)

Tsuzuki, T., Tokuyama, Y., Igarashi, M., Nakagawa, K., Ohsaki, Y., Komai, M., and Miyazawa, T., α-Eleostearic acid (9Z11E13E-18 : 3) is quickly converted to conjugated linoleic acid (9Z11E-18 : 2) in rats. *J. Nutr.*, **134**, 2634-2639 (2004c)

Tsuzuki, T., Kawakami, Y., Suzuki, Y., Abe, R., Nakagawa, K., and Miyazawa, T., Intake of conjugated eicosapentaenoic acid suppresses lipid accumulation of liver and epididymal adipose tissue in rats. *Lipids*, **40**, 1117-1123 (2005)

Tsuzuki, T., Kawakami, Y., Nakagawa, K., and Miyazawa, T., Conjugated docosahexaenoic acid inhibits lipid accumulation in rats. *J. Nutr. Biochem.*, **17**, 518-524 (2006)

Watson, A.A., Fleet, G.W.J., Asano, N., Molyneux, R.J., and Nash, R.J., Polyhydroxylated alkaloids-natural occurrence and therapeutic applications. *Phytochemistry*, **56**, 265-295 (2001)

Witztum, J.L., and Steinberg, D., Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.*, **88**, 1785-1792 (1991)

Zhang, Y., Talalay, P., Cho, C.G., and Posner, G.H., A major inducer of anticarcinogenic protective enzymes from broccoli : Isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 2399-2403 (1992)