Expectations of health benefits in plant materials in Southeast Asia based on Washoku (Japanese cuisine) study focusing on Kyo-yasai (heirloom vegetables in Kyoto)

Y. Nakamura1*, A. Sasaki1, E.Y. Park1, N. Kubo1, T. Nakamura1, Y. Kubo1, S. Okamoto2
1 Faculty of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan
2 Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan

Abstract
An assumption “plant-derived chemicals having strong flavors and tastes can contribute for human health in carcinogenesis” was built up based on studies of cancer chemoprevention and chemotherapy with Kyo-yasai (heirloom vegetables in Kyoto) in Washoku (Japanese cuisine). Since we have studied phytochemicals in Kyo-yasai, most phytochemicals revealed to accompany their biological activity with strong flavors and/or tastes. Three biological activities involved in cancer prevention and therapy (desmutagenicity, bioantimutagenicity, and differentiation-inducing effect) have been detected in Washoku materials, and some active ingredients in Kyo-yasai can be identified. In Katsura-uri (Japanese pickling melon), 3-methylthioacetic acid ethyl ester possessed all three activities and apricot fragrance. Phenethyl isothiocyanate in Mizu-na (potherb mustard), and 4-methylthio-3-butenyl isothiocyanate in Sabaga-daikon (Japanese white radish) possess the bioantimutagenicity, and anticarcinogenic effects in animal model, and also accompanied strong flavors and tastes. In plant materials in South East Asia, spearmint has distinctive flavor, and the ingredient piperitenone oxide possesses the differentiation-inducing effect. Sweet basil also showed the differentiation-inducing effect in its oleophilic fraction. Because various varieties in vegetables, fruits, and herbs are cultivated in South East Asia, and many of them possess strong flavors and tastes, the expectations to reveal health benefits in food materials in South East Asia will be expanded in future.

Keyword: Cancer, Differentiation, Antimutagen, Vegetable, Spearmint, Basil

INTRODUCTION

In 1997 and 2007, The World Cancer Research Fund and American Institute for Cancer Research released “Food, Nutrition and the Prevention of Cancer: a global perspective” that did a comprehensive review of published research studies from around the world, recommended to establish a predominantly plant-based diet that includes eating 400-800 g/day of a variety of vegetables and fruits, and 600-800 g/day of a variety of grains, legumes and root crops1,2. The amount of food materials should vary by each population, and 350 g of vegetables a day is recommended by the Ministry of Health, Labor and Welfare in Japan to reduce the risk of cancer3.

In the traditional dietary habit in Japan, many varieties of vegetables have been used in Japanese cuisine that is termed “Washoku”. From 1960s to 1990s, the consumer demands by the Japanese population resulted in the breeding of new varieties with milder flavors and tastes against traditional vegetables4. Breeding has been successfully produced present Japanese conventional vegetables with a milder flavor. In contrast of the conventional vegetables, the seeds of heirloom vegetables in Kyoto that is termed “Kyo-yasai” have been preserved by traditional cultivation methods, and typically offer a more distinctive flavor and taste than conventional vegetables. The original characters of Kyo-yasai, including their flavors and tastes,
have been maintained by plant breeders for more than 300 years.

Phytochemicals, which is a term essentially used for chemicals derived from plant, but has been actually used for plant-derived chemicals contributed for human health. In human sense of taste and smell, most phytochemicals contribute strong flavors and tastes such as pungency of isothiocyanates, astringency of flavonoids, and unpleasant odors of sulfur or carbonyl compounds; and some of them can be also beneficial to human health\textsuperscript{5-12}. Since 1996, we have studied phytochemicals in Kyo-yasai, greater health benefit involved in cancer prevention and therapy than the conventional counterpart as determined by decreased mutagenic rates and/or increased differentiation-inducing rates in three assays with oxygen radical absorbance capacity, ultraviolet irradiated-\textit{Escherichia coli} B/r WP2 and RCM-1 human colon cancer cells\textsuperscript{13-15}. As the resultant data has accumulated, most phytochemicals found in Kyo-yasai revealed to accompany the biological activity with strong flavors and tastes. Thus, an assumption “plant-derived chemicals having strong flavors and tastes can contribute for human health in carcino-genesis” was built up based on the studies of cancer prevention and therapy with Kyo-yasai in Washoku. We are therefore expanding the research field from Japan to South East Asia, because more varieties in vegetables, fruits, and herbs are cultivated in South East Asia, and many of them possesses strong flavors and tastes.

\textbf{A concept of three biological activities involved in cancer chemoprevention and chemotherapy: desmutagenicity, bioanti-mutagenicity, and differentiation-inducing effect}

Compounds that decrease gene mutations are generically called antimutagens. Antimutagens can be divided into desmutagens and bioanti-mutagens depending on their action of mechanisms\textsuperscript{16}. Desmutagens can suppress mutations by decreasing levels of DNA lesions through various mechanisms such as preventing or decreasing the conversion of a pre-mutagen to a mutagen (Figure 1A), or by chemically degrading a mutagen (Figure 1B), or by inducing enzymes that will detoxify the mutagen before it reaches the cell’s DNA (Figure 1C)\textsuperscript{4}. When pre-mutagen is unsaturated lipid, compounds possessing oxygen radical absorbance capacity (ORAC) can work to suppress a change of the lipid to mutagenic peroxide, and the compounds are considered to be one of the desmutagens. Desmutagens showing ORAC would be expected for human health promotion, because some diseases are believed to be derived from endogenous peroxides causing in human body.

In contrast, bioantimutagens can suppress the effects of preexisting DNA lesions by increasing the level of error-free DNA repair (Figure 1D), or increasing the opportunity for DNA repair by delaying DNA replication and mutation fixation (Figure 1E). After DNA lesion is induced by mutagens, in the presence of bioantimutagen, the damaged cell can be restored to normal cell. When we consider antimutagen in plant materials, the bioantimutagen, which can act even after the cell has acquired a DNA lesion, is as important as desmutagen. After mutant cell is produced in human body, it is not still cancer cells. DNA lesion is further induced by mutagens, and accumulation of mutation into the mutant cells, cancer cell might be produced from the mutant cells. Desmutagen and bioantimutagen also have chance to work in this duration to disturb to change from a mutant cell to a cancer cell.

Once, cancer cell is produced, it has changed to be an undifferentiated cells, and obtained an unlimited life time. The obtained unlimited life time of cancer cells would be a significant nature to kill human. In the presence of differentiation-inducing chemicals, it can change from cancer cell to a differentiated cell, and again obtain limited life time, and the cell would be no longer cancer cell (Fig. 1F). Thus, differentiation-inducing chemicals is considered to be chemopreventive compounds for cancer cell just appeared (<50 µm) and chemotherapeutic compounds for tumor (>1 mm). When we considered to cancer prevention and therapy with administration of plant materials, differentiation-inducing chemicals, which can act even after human body has acquired cancer cell or tumor, is as important as desmutagen and bioantimutagen.
Figure 1. A concept of cancer prevention and cancer therapy with antimutagens (desmutagen and bioantimutagen) and differentiation-inducing chemicals. The action of mechanisms of desmutagen (A-C): suppression of mutation before mutagens reach the cell's DNA either by preventing or decreasing the conversion of a pre-mutagen to a mutagen (A), by chemically degrading a mutagen (B), by inducing detoxification enzymes for a mutagen (C). The action of mechanisms of bioantimutagen (D, E): suppression of mutation either by increasing the level of error-free DNA repair for preexisting DNA lesions (D), by increasing the opportunity for DNA repair by delaying DNA replication and mutation fixation (E). The action of mechanisms of differentiation-inducing chemical (F): change cancer cell (undifferentiated cell having unlimited life time) to differentiated cell (having limited life time) by alteration of some hyper- or hypo-expressing genes.

Cancer chemopreventive and chemotherapeutic effects of fragrant compounds involved in a Washoku (Japanese cuisine) material, Katsura-uri (Japanese pickling melon)

Katsura-uri, Japanese pickling melon (Cucumis melo var. conomon) is Kyo-yasai, an heirloom vegetable in Kyoto, Japan. The fruit is 40-90 cm in length and 2-5 kg in weight and not sweet in any of its ripening stages. In traditional Washoku (Japanese cuisine), the midripened fruit is primarily used as pickles “Kasu-zuke” that is made from the fruit dipped with lees of “Sake” (Japanese liquor) and “Mirin” (Japanese sweet seasoning liquor) for several month. When the fruit reaches fully ripened stage, it starts to produce a strong muskmelon-like odor, which should demonstrate health benefits. The oleophilic (n-hexane soluble) fraction of fully ripened Katsura-uri fruit exhibited bioantimutagenicity assessed by UV-induced mutation assays using E. coli B/r WP2, and differentiation-inducing effect in a RCM-1 human colon cancer cell line. Interestingly, the sub-fraction from the oleophilic fraction (obtained with 2.5% acetone contained n-hexane eluted from silica-gel column) possesses differentiation-inducing effect accompanying with a strong muskmelon-like odor constructed with six fragrant compounds (Figure 2). Desmutagenic (antioxidative), bioantimutagenic, and differentiation-inducing effects of the six ingredients are shown in Figure 2: ingredient having more number of “+” means higher activities possessed. Except benzyl acetate
(BA), at least one activity was observed in five compounds. 3-Methylthioacetic acid ethyl ester (MTAE) possesses all three activities; and 3-methylthiopropionic acid ethyl ester (MTPE) and acetic acid 3-methylthio propyl ester (AMTP) possess two activities; and acetic acid 3-methylthio ethyl ester (AMTE) and eugenol have antioxidative effect.

<table>
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<tr>
<th>Ingredient</th>
<th>Chemical structure</th>
<th>Desmutagenic</th>
<th>Bioantimutagenic</th>
<th>Differentiation-inducing</th>
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<td>4. AMTP</td>
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<td>–</td>
</tr>
<tr>
<td>5. BA</td>
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<td>6. Eugenol</td>
<td><img src="image" alt="Eugenol structure" /></td>
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**Figure 2.** Desmutagenic (antioxidative), bioantimutagenic, differentiation-inducing effects of six fragrant ingredients in Katsura-uri. Six ingredients were sorted in the sequence from low to high in boiling temperature: methylthioacetic acid ethyl ester (MTAE), acetic acid 2-methylthio ethyl ester (AMTE), 3-methylthiopropionic acid ethyl ester (MTPE), acetic acid 3-methylthio propyl ester (AMTP), benzyl acetate (BA), and eugenol.

**Some phytochemicals of Washoku materials in plant-origin contribute both strong flavors and health benefits in carcinogenesis**

Some bioantimutagenic compounds, identified from **Kyo-yasai** detected in *E.coli* B/r WP2, can exhibit anticarcinogenic activities in animal experiments. Phenethyl isothiocyanate in Mizu-na (potherb mustard, *Brassica campestris* var. *japonica*), and 4-methylthio-3-butenyl isothiocyanate in Sabaga-daikon (Japanese white radish, *Raphanus sativus*) have been reported to be anticarcinogenic in breast, and pancreas cancer in animal model, respectively. Daikon is also reported to be a factor to reduce esophageal squamous carcinoma in human epidemiological study of Japanese population.

Some differentiation-inducing compounds (e.g. analogue chemicals of 3-methylthio acetic acid ethyl ester in Katsura-uri) identified in RCM-1 bioassay is also being revealed their anticarcinogenic activities by mouse skin two-stage carcinogenesis models. Four sulfur-containing esters in Katsura-uri possess muskmelon, apricot, flowery, and fruity fragrance (Figure 3. A-D). Two isothiocyanates in cruciferous vegetables possess pungent taste, and cresson or radish fragrance (Figure 3. E, F). Two carbonyl compounds in sweet pepper and cucumber possess off-flavor of lipid and grass fragrance (Figure 3. G, H). All of the chemicals possess distinct flavors and/or tastes. Thus these results gave expectations of further investigation into the anticarcinogenic properties of plant-derived chemicals possessing strong flavors and tastes.
Cancer chemopreventive and chemotherapeutic effects of fragrant compound in herbal plant material, spearmint (Mentha spicata)

We are focusing on plant materials possessing strong flavors and tastes to identify phytochemicals showing differentiation-inducing effect. On the criteria, vegetables, fruits and herbs cultivated in South East Asia would be expected other than Kyō-yasai in Japan. Initially, we chose spearmint (Mentha spicata) leaves that have strong flavor, and successfully identified piperitenone oxide from its n-hexane extract with purification via a bioassay-guided fractionation based on the induction of differentiation in RCM-1 cells. Piperitenone oxide has been considered as an odor producing compound in spearmint cultivars, and is chemically categorized into monoterpene such as carvone and menthol, which are also familiar ingredients in Mentha genus plants. Carvone is principally found in some species of spearmint, and menthol is mainly contained in peppermint (Mentha piperita L.), American wild mint (Mentha arvensis) and Japanese mint (Mentha canadensis). However, both carvone and menthol do not possess the differentiation-inducing effect in RCM-1 bioassay, and therefore, not all monoterpenes have the ability. The active specificity of piperitenone oxide is interesting and should be investigated to understand its anticarcinogenic properties in future. In addition, the effective dose of piperitenone oxide was lower than that of any differentiation-inducers found in the RCM-1 bioassay. The regional difference in spearmint should be also studied in South East Asia to compare the amount of piperitenone oxide and the ability of differentiation-inducing effect in future.
Expectations of health benefits in food materials in Thailand

When we focus on Thai food materials to possess health benefit with strong flavors, one candidate might be basil, because the variety is spread and used in the world, particularly Thai people well use each basil variety into each dish properly. Sweet basil (horapha, *Ocimum basilicum*), hairy basil (maenglak, *Ocimum citriodorum*), and holy basil (kaphrao, *Ocimum tenuiflorum*) are commonly used for different Thai dishes: sweet basil as fresh and for soups (sometimes for steam with mussels, and for fry with seafood), hairy basil as fresh and for soups, holy basil for fry with meat (but not as fresh). The fragrance of basil can be extracted with chloroform, and the smells of the resultant extracts were quite different among three varieties of the basils. In Figure 4, chloroform extract of the three varieties were analyzed with gas chromatography (GC), and peaks having earlier retention time on GC-chromatogram were usually compounds having lower boiling point. The difference of the fragrance ingredients was revealed with the GC analysis, and the variation of boiling points was surprisingly correlated to the cooking temperature for dishes and the ingredients for smells in human sense (mainly use sweet basil and hairy basil as fresh and soups; holy basil as fry)\(^2\). We have just confirmed the differentiation-inducing effect of the chloroform extract of sweet basil in RCM-1 cells, and would identify the active compound, and also check the activity of other basil varieties. In addition, Genovese basil (sometimes called as Italian basil) showed different GC-chromatogram from Thai basil, and its one fragrant ingredient was linalool that shows no effect in the differentiation-inducing effect (Figure 4). From the example in basil, Thai people have used the variety of basil properly to keep some fragrant ingredients in dishes for long time, and this dietary habit might contribute to health promotion. In addition, health benefit of hairy basil (often called as hoary basil) was summarized in antioxidative effect and amount of some phytochemicals (apigenin, kaempferol, and luteolin)\(^2\).

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**Figure 4.** Profile of gas chromatography of chloroform extract of basil. Chloroform extract of four varieties of basil (A: sweet basil, B: hairy basil, C: holy basil, D: Genovese basil) and authentic monoterpane (E: linalool) were analyzed with a Hewlett-Packard 6890 gas chromatograph). The capillary column was a Rtx-5MS (0.25 mm ID×30 m). The column oven temperature was held at 60 °C for 5min and then was increased to 250 °C at a rate of 5 °C/min.
CONCLUSIONS

On the basis of Washoku study focusing on Kyo-yasai, we built up an assumption “plant-derived chemicals having strong flavors and tastes can contribute for human health in carcinogenesis”. According to the assumption, we are now undertaking to identify novel phytochemicals in plant materials cultivated in Southeast Asia. Focusing on herbs cultivated in Thailand and Vietnam, we have identified piperitenone oxide from spearmint as showing differentiation-inducing effect in human colon cancer cells, and also confirmed the effect in sweet basil extract. Because the number of varieties of vegetables, fruits, and herbs cultivated in South East Asia is much more than in that in Japan, and many of them possesses strong flavors and tastes. Therefore, the aforementioned facts let us expect the earlier identification of novel phytochemicals in South East Asian plant materials, which contribute to human health.

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