Review

A Mainstay of Functional Food Science in Japan—History, Present Status, and Future Outlook

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The development of food science in the near future probably depends on the advance in functional food science, the concept of which was proposed first in Japan nearly 15 years ago. The new science has been internationally distributed and accepted as conceptually being beyond nutrition. In Japan, however, it traced a unique path of progress in the form of a product-driven rather than concept-driven science. Actually, a number of substances and products with potential for disease risk reduction rather than simply for health maintenance have been investigated for their body-modulating functions. Some of them have been applied in practice to the industrialization of functional foods in terms of “foods for specified health uses” legally defined by new legislation. A variety of sophisticated methods have been introduced as well, including the so-called “XYZ” evaluation system, database construction for assessment of the function, and even the DNA microarray technique. The Ministry of Agriculture, Forestry, and Fisheries (MAFF) and the Ministry of Health and Welfare (MHW) also commenced their scientific as well as political activity, with its spread to industries which almost simultaneously began to vigorously investigate functional food products for enhancement of the food market. With all of this as a background, the Japan Liaison of the International Union of Food Science and Technology (IUFoST) hold a function food science symposium on behalf of related scientific bodies including the Japan Section of the International Life Science Institute (ILSI). This paper is an overview compiled from 12 presentations made in the symposium, with the aim of internationally publicizing the activity of functional food science in Japan.

Key words: functional foods; food factors; foods for specified health uses; health claim

Neither a terminology nor a concept had been available for “functional food” until 15 years ago, when an ad hoc research group in Japan started a relevant study as a national project. It seems that the initiation of this functional food science may reflect an underlying thought in the minds of Japanese people through a long history. The thought has come from the ancient Chinese saying, “Medicine and food are isogenic”.

However, the advent of modern science in Japan made its food research proceed toward a nutritional rather than medical science. Especially during the time throughout the pre-, mid-, and post-World War-II periods, the nutritive values of foods were of greatest academic as well as social concern. Accordingly, nutrients in foods were the major research target of food science. The best example may be offered by oryzanin (vitamin B1) which the outstanding scientist Umetaro Suzuki discovered in rice bran.1

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Meanwhile, the nutritional problem of food shortage was over and a new period characterized by high economic growth came. It prevailed in the time encompassing the 1960s, when the social climate focussed more on food preference. Such a trend of hedonism motivated academic studies on tastes and smells of foods. Epoch-making advances in instrumental analysis assisted the studies. Japan, among a good many of other countries of the world, thus established and developed two mainstreams of food science—one on nutrition and the other on hedonics.

In the 1980s, when the aging society began to manifest itself, growing public awareness was observed of the need to prevent what were called life style-related diseases through improved dietary practice in our daily life. This gave a very strong impetus to food science in Japan. In 1984, the first national project on functional foods commenced under the sponsorship of its Ministry of Education, Science, and Culture (MESC), which was entitled “Systematic Analysis and Development of Food Functions”. Here the food value criteria were defined by three categories: primary function identified as the function of ordinary nutrients in the body, secondary function referring to the function of tasteants and/or odors toward sensory organs and even toward the brain, and a tertiary function newly defined as the body-modulating function of non-nutrients that is directly or indirectly related to disease prevention. Incidentally, Clydesdale has recently commented that Arai’s concept on the tertiary function is a most interesting notion and certainly has merit, since it is much broader in aspect and obviously based on science. Practically, the concept provides a basis for giving rise to functional foods, which may be defined as new foods designed to be effective in reducing the risk of life style-related diseases.

The first project (1984–1987) was followed by a second one (1988–1991) entitled “Analysis of Body-modulating (i.e., Tertiary) Functions of Foods”. Shortly after, the last project in a series of the MESC “functional food” projects was realised in 1992. It focussed on “Analysis and Molecular Design of Functional Food”, with the subthemes: body-regulating food factors, body-defending food factors, and development of a technological basis specific to designing functional foods at the molecular level. A total of 59 academic members participated, most of whom were from medical as well as food science fields.

Political events followed. In 1991, the MHW established the world’s first policy of legally permitting the commercialization of some functional foods in terms of “foods for specified health uses” (FOSHU) defined by new legislation. The policy is characterized by approving the presentation of a health claim for each FOSHU product. In 1993, the first FOSHU product was approved, which is a hypoallergenic rice developed after detailed immunological studies in the above-mentioned MESC project and industrialized after extensive clinical intervention tests in many medical centers. In the meantime, the MAFF commenced functional food studies from the aspect of preharvest as well as postharvest science. Almost simultaneously, a great many of food and pharmaceutical industries began to investigate functional foods for the development of FOSHU products. All these events and activities were internationally publicized first by the Nature’s news report with the headline “Japan explores the boundary between food and medicine”. It seems that the old Chinese saying “Medicine and food are isogenic” is being scientifically, politically, and industrially fixed to the Japanese soil.

Recently, European countries have begun to vigorously study the functional food science with its own concept. The science in Japan has been progressing more greatly to occupy a central position of modern bioscience. It is characterized especially by the strength in a product-driven rather than concept-driven way of study. Examples are offered by the studies on antioxidative polyphenols, anticarcinogenic phytochemicals, and food protein-derived oligopeptides with body-modulating functions, as well as on food allergies and their countermeasures. Recent progress in this new science also involve the exploitation of sophisticated methods to assess the oxidative stress due to food factors (“XYZ” evaluation system), how to compile data on the structure-function relationships of non-nutrients (database construction), and even how to make overall evaluations of desirable or undesirable functions of individual food factors at the molecular level (DNA microarray technique).

Thus, not only academic but also governmental and industrial studies on functional foods are successfully progressing in Japan. Here we overview the state of the art, with the expectation of further development of this potential food science in the 21st century.

**Analysis of Functional Food Factors**

*Antioxidative factors.* Oxidative stress may cause free radical reactions to produce deleterious modifications in membranes, proteins, enzymes, and DNA. Age-related diseases such as cancer, atherosclerosis, and diabetes are supposed to be correlated with oxidative stress although the detailed mechanisms are still unclear. Our research group has been involved in developing novel ELISA methods by application of immunochemistry. We have developed many types of monoclonal and polyclonal antibodies, which are specific to oxidatively damaged DNA bases such as 8-OH-deoxyguanosine and lipid peroxidation products
including lipidhydroperoxides, malondialdehyde, 4-hydroxynonenal, and acrolein. By monitoring these oxidatively damaged products as biomarkers, we have been screening many different types of dietary antioxidants. From our hypothesis that endogenous antioxidants in plants must play an important role for antioxidative defense systems against oxidative stress, an intensive search for novel types of natural antioxidants has been done.

Recently, we found strong antioxidative lignans, e.g., sesaminol glucosides (SG), in sesame seeds and we evaluated their antiatherogenic activity by feeding SG to Watanabe Heritable Hyperlipidemia (WHHL) rabbits. The percentage area of aorta covered with plaque in the SG-treated rabbits was reduced compared to the control, it was shown that lipid peroxide was decreased significantly, and there was also a significant increase in the activity of glutathione peroxidase and glutathione S-transferase in tissues including liver and aorta.

In the course of our investigation to find novel types of antioxidative substances in plant materials, most natural lipid-soluble antioxidants can be classified into two different types: phenolic and β-diketone type antioxidants. However, there have not been found any antioxidative substances that have both functional groups; phenolic and β-diketone groups in the same molecule. In order to develop a new type of antioxidative compound that has both phenolic and β-diketone moieties in the same molecule, the authors focused on curcumin, the main yellow pigments of Curcum a longa (turmeric). Curcumin has been used widely and for a long time in the treatment of sprain and inflammation in indigenous medicine; however, there is some limitation on the use of curcumin for food and medicinal purposes because of its yellow color. Recently, we succeeded in obtaining a strong lipid-soluble antioxidant, tetrahydrocurcumin (THC), by hydrogenation using Pd-C (or Raney-nickel) as the catalyst. Recently, we tested many biological activities of THC using a wide variety of evaluation systems. THC was found to be produced from curcumin during absorption from the intestines, and is a more potent antioxidant than curcumin. Recently, we found that THC is a more promising chemopreventive agent than curcumin in the 1,2-dimethyl-hydrazine (DMH) induced mouse colon carcinogenesis model.

Cancer-preventive factors. A large body of data from epidemiologic and rodent studies has demonstrated that ingestion of vegetables and fruits is occasionally beneficial for reduction of cancer risks in humans. Thus, chemoprevention with food phytochemicals including minor dietary ingredients (food factors) is currently regarded as one of the new important scientific fields. The author and colleagues have hitherto done an in vitro screening study of dietary plants for their anti-tumor promoting properties, and chemical analyses of the active constituents. Cancer preventive effects in animal models as well as the modes of their action have also been investigated.

In an extensive screening study covering common vegetables and fruits in Japan and southeast Asian countries, anti-tumor promoting potential, detected by the inhibitory rate of tumor promoter-induced Epstein-Barr virus (EBV) activation, was generally higher in the plants from tropical zones than those from Japan. Moreover, the plants in the families of Zingiberaceae, Rutaceae, Labiatae, Cruciferae, and Umbelliferae, which are used for purposes other than their nutritive value (i.e., flavors, condiments, and occasionally traditional medicines, were shown to contain potent anti-tumor promoters at high rates.

Of more than 20 in vitro anti-tumor promoters so far identified, two case-studies on 1'-acetoxychavicol acetate (ACA) and auraptene are highlighted in the following. ACA was isolated from Languas galanga (Zingiberaeaceae), a plant used as a ginger substitute and a stomachic medicine in Thailand. In a variety of animal model experiments, significant inhibitory effects of ACA on tumor promoter TPA-induced skin tumor promotion in mice, 4-NQO-induced oral and AOM-induced colon carcinogenesis in rats and endogenous rat liver carcinogenesis were found. Recently, the inhibitory effects on NMBA-induced rat esophageal tumorigenesis and BOP-initiated Syrian hamster cholangiocarcinogenesis have also been found. As for the in vitro physiological characteristics of ACA, inhibitory activities toward TPA-induced O2− generation in DMSO-differentiated HL-60 cells and NO generation in murine macrophage RAW 264 cells have so far been found. ACA also had a suppressive effect on TPA-induced oxidative stress in mouse skin. Thus, radical generation inhibitory action may be one of the mechanisms of ACA for cancer inhibition. Auraptene (7-geranyloxycoumarin), widely occurring in citrus fruits, basically had similar in vivo and in vitro effects to those of ACA. For practical application (human intervention trials) of these promising preventive compounds, studies on the chemical stability of each compound, its bioavailability and metabolism in biological systems etc. are required. We have also obtained preliminary results on the metabolism and absorption of auraptene.

Food protein-derived peptides. Since the isolation of the opioid peptide β-casomorphin from casein peptone by Brantl et al., many kinds of bioactive peptides have been isolated from enzymatic digests of food proteins (Table 1). These are classified as follows: ligands for receptors, enzyme inhibitors, peptides regulating intestinal absorption, antimicrobial peptides, antioxidative peptides, and others. Most endogenous bioactive peptides are in-
effective after oral administration inspite of their specific activities being high. On the other hand, some bioactive peptides derived from food proteins are active after oral administration even though their specific activities are not very high. This is partly because the molecular sizes of bioactive peptides derived from food proteins are usually smaller than endogenous ones, and some of them are resistant to peptidases. Such peptides are expected to be effective in preventing lifestyle-related diseases such as hypertension, hyperlipidemia, atherosclerosis, and osteoporosis. FOSHU products containing such bioactive peptides are commercially available now.

We have shown that \( \delta \)-selective opioid peptides derived from wheat gluten and leaf protein improved learning performance after oral administration in mice. After oral administration in rats, an immunostimulating peptide soymetide, which was derived from soybean \( \beta \)-conglycinin, prevented hairloss induced by a cancer chemotherapy agent. We have also shown that biological activities of peptides derived from food proteins could be strengthened by 100 times after replacement of a single amino acid residue. This means that we can create genetically modified crops containing proteins of which the function to prevent lifestyle-related diseases is much improved.

### Development of Immunologically Functional Foods

**Immune system modulators.** Probiotics are living microorganisms that favorably influence the health of the host by improving the indigenous microflora.\(^{23}\) There is mounting evidence being generated confirming the medical benefits of selected probiotic microorganisms. Especially, we should note the ability of probiotic microorganisms in stimulating the immune defense system of the host. Recent progress in studies on the gastrointestinal immune system (GI) has contributed to further understanding of the details of the mechanisms involved in the interaction between probiotics and the GI.\(^{24,25}\)

Prebiotics are materials, such as oligosaccharides, that favorably influence the indigenous intestinal microflora in terms of microbial balance and improve our health. We also searched for probiotics that had an inhibitory effect on allergic reactions.\(^{26}\) *Lactobacillus casei* is a non-pathogenic Gram-positive bacterium widely used in dairy products and this organism has been shown to improve the cellular immunity of the host. The effects of these bacteria on IgE production and T helper phenotype development (Th1 or Th2) were examined, because the Th1/Th2 balance strongly influences the generation of allergy. When Th2 > Th1, an allergic reaction is easily generated. *L. casei* induced IFN-\( \gamma \), but inhibited IL-4 and IL-5 secretion, and markedly suppressed total and antigen-specific IgE secretion. Augmented IL-12 production was also observed in cell cultures containing *L. casei*. IL-12 is known to contribute to induction of Th1-type T cells that can inhibit IgE production. Therefore, *L. casei* can inhibit antigen-induced IgE production through induction of IL-12 secretion. These findings suggest that this organism is potentially useful for preventing IgE-mediated allergy.

We found that bifidobacteria have potent immunopotentiating activity and show strong mitogenic activity.\(^{27}\) In addition, this activity was increased after disruption of the cells, indicating the existence of

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Table 1. Bioactive Peptides Derived from Food Proteins*\(^{26}\)

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Origin</th>
<th>Physiological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioid peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-casomorphin (YPFPQPI)</td>
<td>Casein</td>
<td>Suppression of intestinal motility</td>
</tr>
<tr>
<td>Gluten exorphin A (GYYPT)</td>
<td>Gluken</td>
<td>Improvement of learning</td>
</tr>
<tr>
<td>Rubiscoil (YPDLD)</td>
<td>Rubisco</td>
<td>Improvement of learning</td>
</tr>
<tr>
<td>Vasorelaxing peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovokinin (2-7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunostimulating peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soymetide (MITLAPIVNKPGPR)</td>
<td>Glycinin</td>
<td>Immunostimulation, protection from hair loss</td>
</tr>
<tr>
<td>Inhibitor for ACE(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFVAPFPEVFGK</td>
<td>Casein</td>
<td>Anti-hypertension</td>
</tr>
<tr>
<td>IPP, VPP</td>
<td>Casein</td>
<td>Anti-hypertension</td>
</tr>
<tr>
<td>LKPNM</td>
<td>Fish protein</td>
<td>Anti-hypertension</td>
</tr>
<tr>
<td>Modulator for intestinal absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein phosphopeptide</td>
<td>Casein</td>
<td>Improvement of calcium absorption</td>
</tr>
<tr>
<td>Soybean HMF</td>
<td>Soybean protein</td>
<td>Hypcholesterolemic effect</td>
</tr>
<tr>
<td>Antimicrobial peptide</td>
<td>Lactoferrin</td>
<td>Anti-infection</td>
</tr>
<tr>
<td>Lactoferricin</td>
<td>Lactoferrin</td>
<td>Anti-infection</td>
</tr>
<tr>
<td>Antioxidative peptide</td>
<td>Soybean protein</td>
<td>Anti-oxidation</td>
</tr>
<tr>
<td>LLPHH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Constituent amino acids shown in the one-letter symbols.
\(^{b}\) Angiotensin I-converting enzyme.
an intracellular soluble immunopotentiator. A purified soluble polysaccharide fraction was shown to have immunopotentiating activity for Peyer's patch cells. Furthermore, the immunopotentiating fraction was found to contain galactofuranosyl residues as characteristic constituents.

Oral tolerance is an unresponsive state of the immune system induced by oral administration of an antigen. It has been reported that bacterial lipopolysaccharide affects oral tolerance. Recent reports have shown that the intestinal bacterial flora plays a crucial role in induction of oral tolerance. Since the breakdown of oral tolerance results in the generation of allergy, it is evident that the intestinal bacterial flora contributes to the suppression of food allergic reactions.

Raffinose (RAF) is a trisaccharide consisting of D-galactose, D-glucose, and D-fructose, and is a typical example of a prebiotic. Excluding sucrose, RAF is the most widely distributed oligosaccharide, and it is found in beets, sugarcane, and many other plants. It has been shown that the ingestion of RAF results in a significant increase in the abundance of fecal bifidobacteria and a decrease in fecal levels of putrefactive products. Recently, oral administration of RAF was shown to improve atopic dermatitis in children. We have examined the action of RAF on the GI, because it influences the generation of allergy. We found that the amount of IL-12 production by Peyer's patch cells was significantly higher in the case of animals fed a RAF diet than that in control animals. Antigen-presenting cells from the Peyer's patches of animals fed the RAF diet induced a higher amount of IFN-γ production in CD4+ T cells. These results suggest that oral intake of RAF induces Th1-type T cells which suppress allergic reactions in the Peyer's patch, through changes in the intestinal microflora.

It has been reported that dietary nucleotides improve Th1-type T cell activities. We have measured the effects of dietary nucleotides on the GI. Ovalbumin-specific T-cell receptor transgenic mice, which show allergy-like symptoms, were fed a diet supplemented with dietary nucleotides. The level of production of interferon-γ by spleen cells was significantly higher in mice fed the nucleotide-supplemented diet than that in mice fed the control diet, and the level of IgE production was decreased. These results show that dietary nucleotides up-regulate the antigen-specific Th1 immune response through the increase of IL-12 production and suppress the antigen-specific IgE response. We have investigated the effects of dietary nucleotides on subsets of intraepithelial lymphocytes (IELs) and their production of cytokines. The proportion of TCRγδ+ T cells from mice fed a diet supplemented with nucleotides (NT (+) diet) was significantly higher than that in the case of mice fed the control diet. Experiments examining the relationship between this alteration in T cell phenotype and IgE suppression, described above, are now in progress.

**Hypoallergenic wheat products.** The number of patients with allergies seems to be increasing in recent years, particularly in developed countries. Food allergy is the hypersensitivity caused by consuming foods or, more exactly, by ingesting their particular constituents called allergens. In most cases, these allergens are proteins. Wheat allergy is a great problem in many countries where the custom of consuming wheat products has been fixed throughout a long history. The first objective of this work is to clarify the structural characteristics of wheat allergens for development of a method for production of hypoallergenic flour. The second is to make wheat items from the hypoallergenic flour that has lost its original dough-forming properties.

A major allergen in wheat flour is a low molecular-weight glutenin in gluten, and it has a Gln-Gln-Gln-Pro-Pro motif identified as an IgE-binding epitope. Some glycoproteins and a polysaccharide as well were found to be allergens. By enzymatic decomposition of these allergens, hypoallergenic wheat flour was produced. Wheat flour was mixed with a cellulase solution and the mixture was incubated at 50°C for 1 h to hydrolyze carbohydrate allergens. The hydrolysate was further incubated with actinase at 40°C for 1 h with mild stirring to decompose proteaceous allergens. The product was evaluated for allergenicity by enzyme-linked immunosorbent assay, the results of which suggested negative allergenicity in most cases. The product changed to a batter state that was difficult to process by usual methods. Gelatinization of the starch in the product and the addition of a surfactant were beneficial for food processing. Food items selected were cup cakes, pasta, pizza, cookies, wafers, and a puffed item. The optimal ingredient compositions and procedures for the items were proposed.

**Hypoallergenic soybean products.** Soybeans and soybean products are known as one of the major allergenic foodstuffs for Japanese, while soybean have been recognized as an important protein source for Japanese to maintain the traditional food culture. In recent years, soybean protein isolate (SPI) prepared from defatted soybean meal has been used in various processed foods for reasons of the nutritional quality, processing functionality, and economical basis, so that it becomes difficult for soybean-allergic patients to find allergen-free products in processed foods. At the present time, a strict elimination of offending foodstuffs from the diet is generally adopted as a conventional and effective therapy for food allergies. The elimination of nutritionally-fundamental or essential foods for extended periods of the
treatment, however, may lead to malnutrition. There is, therefore, an urgent demand for food scientists to identify the protein components responsible for causing allergic manifestations upon the ingestion of foods and to reduce the allergenicity for the healthy use of soybean products by soybean-allergic patients.

Before the development of the hypoallergenic products, we demonstrated the occurrence of about 15 protein components recognized by IgE antibodies in sera of soybean-allergic patients. Among them, three protein species named Gly m Bd 60K (α subunit of β-conglycinin), Gly m Bd 30K (oil-body-associated protein, homologous to the mite allergen Der p(f), and Gly m Bd 28K (vicilin-like protein) were identified as major allergens with the high incidence of 23.5%, 65%, and 23.5% among the patients, respectively.

Many approaches such as physicochemical procedures including heat denaturation or selective precipitation of allergenic proteins, enzymatic destruction or chemical modification of allergenic sites (epitopes), i.e., introduction of sugar moiety, molecular breeding (selection of allergen-deficient variety), gene engineering, and fractionation and fabrication of non-allergenic protein components, have been proposed to reduce the allergenicity. Furthermore, at the same time, selective and sensitive methods for an evaluation of allergenicity of soybean products through the course of development have been established. We developed, first, a convenient method to detect and measure the major allergens by the immunoblot and enzyme-like immunosorbent assay (ELISA or sandwich ELISA) using allergen-specific monoclonal antibodies. This report describes a strategy for reducing soybean allergenicity by selective combination of these techniques.

A new soybean line (Glycine max Tohoku 124) lacking α subunit of β-conglycinin was induced by irradiation with 20 kr (1.0 kr/hr) γ-rays of a variety of Kariki 434 which was characterized by a marked decrease of the level of the α, α’ and β subunits of β-conglycinin. This variety also lacks Gly m Bd 28K. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot, and sandwich ELISA using monoclonal antibodies were done according to the usual procedures. The major soybean allergen, Gly m Bd 30K, was removed from the soymilk prepared from the defatted Tohoku 124 meal by treatment with 1 mM Na2SO4 at pH 4.5, and by centrifugation. For enzymatic destruction of allergens, soybeans soaked in water overnight were autoclaved at 120°C for 20 min, then treated with a Bacillus proteinase sterilized in advance by ultrafiltration, and incubated at 37°C for 20 hr with gentle shaking.

A mutant line of Tohoku 124 that lacks one of major allergens, α subunit of β-conglycinine, was established by a molecular breeding technique. Lack of both α and α’ subunits of β-conglycinin together with another major allergen, Gly m Bd 28K, was clearly shown in the SDS-PAGE and immunoblotted patterns of its protein fraction (Fig. 1). No mutant lacking Gly m Bd 30K could be found by a screening of even about 5,000 soybean varieties available in the National Agricultural Experimental Stations. This fact indicates that an application of Tohoku 124 to soybean processing is effective for developing hypoallergenic soybean products because of lacking the major two allergens in advance. The physicochemical treatment of the defatted soymilk (Tohoku 124) as described above could produce substantially complete removal of three major allergens from SPI with processing functionality (Fig. 1) and this soymilk was shown to be usable in soybean curd (tofu) processing. Another hypoallergenic process was also done by the enzymatic treatment of whole soybean seeds using a proteinase from a Bacillus sp. at 37°C for 20 hr, which are the same conditions as for the natto fermentation with Bacillus natto. When the allergenicity was examined in vitro by the immunoblot and ELISA (or ELISA inhibition), the products showed no immunological reactivity against monoclonal antibodies and patient’s sera. We succeed in the reduction of soybean allergenicity by a combination of different techniques, such as molecular breeding of soybean varieties, physicochemical treatment of soymilk (or SPI), and enzymatic digestion of soybean grains. All other minor allergens as well as major allergens could be completely destroyed by the enzymatic digestion of whole soybean seeds. It was also shown, by in vivo clinical evaluation, that about 80% of soybean-allergic patients could take these products without any typical manifestations of food allergies.

![Fig. 1. SDS-PAGE and Immunoblotted Patterns of Defatted Soymilk Centrifuged in 1 M Na2SO4 at pH 4.5.](image-url)
Evaluation Technologies for Functional Foods

The use of “XYZ” evaluation system. In the process of study on soybean saponins such as DDMP saponins, we have discovered a phenomenon photon emission in the visible region. A certain substance [Z] is essential for this photon emission in the presence of reactive oxygen species [X] and hydrogen donor [Y]. The photon emission in the XYZ system is thought to arise from electron translation or hydrogen abstractions between X, Y, and Z, because properties of Y are that of a radical scavenger and an electron donor like phenolic compounds. The upshot is X scavenging photon emission. In addition, we find the synergistic X scavenging photon emission between Zs, and the amputative effect of a trace of metal ion. Experimental evidence based on the XYZ system suggests that this phenomenon is a fundamental in our life and environment. The Z conception is indicated to protect all living things from the oxygen pressure environment, and should be introduced into the field of functional foods as the new concept (Fig. 2).

Database construction. Occurrence of various lifestyle related diseases, such as cancer, cerebrovascular diseases, and diabetes mellitus, are closely influenced by dietary habits. Tea polyphenols and phytoestrogens are good examples of the potent food factors for the prevention of diseases. Excess intake of these substances, however, may have a toxic effect. Measurement of their absorption rates, biological effects and their mechanisms, metabolic rate, and excretion rate is necessary to discover the safe and effective dose to avoid toxic effects. Integrated health effects of these food factors could be estimated by calculation from an appropriate food content table.

To make such a database, a group study has started under the support of the Science and Technology Agency. The subjects to be studied are standardization of evaluation systems for functional food factors, isolation and identification of functional food factors, and epidemiological evaluation and database development. The study focuses on polyphenols such as flavones, iso-flavones, and catechins, terpenoids and carotenoids, sulfur-containing food factors and volatile compounds, and functional peptides. It also covers nearly 80% foods that are daily consumed. A relational database shall be developed after peer review of these functions.

Application for health related research: interaction of phytochemicals and biologically active molecules inside the body has not been studied yet. Antioxidant activities of phytochemicals should have an interaction in oxidation-reduction reaction inside the body. Continuous intake of these foods should maintain a high plasma level, and contribute to prevention of life-style related diseases. Several large population-based prospective studies are going on in Japan, so the health effects can be confirmed by measuring stored plasma and calculated amounts from the planned database. This study can provide a solid scientific bases for functional foods.

DNA microarray technique. It has been speculated that various food factors play important roles to maintain the homeostasis of metabolism in our bodies. However, systematic estimation for their possible functions has not been done. To approach to this problem, DNA microarray may be helpful because of its high throughput potency to assess the effects of food factors on the expression profile of genes.

For example, to analyze the action mechanism of anti-carcinogenic carotenoids, we have carried out the estimation one by one for the changes in expression of genes, such as ornithine decarboxylase and c-myc gene, and/or activity of promoter region of genes, including RB gene and Waf 1 gene. Thus, a great deal of time and effort has been invested. Recently, DNA macroarrays and microarrays have been developed, and now are commercially available. We have tried to analyze the action mechanism of β-cryptoxanthin using these new methods, and found that the expression of a lot of genes was affected by β-cryptoxanthin (Table 2). Then, we expanded the analysis of the action mechanism of various natural carotenoids by this new method, and found that the patterns of the influences of carotenoids on the gene expression profile were different one from another.

Table 2. Degrees of Gene Induction after Treatment with β-Cryptoxanthin

<table>
<thead>
<tr>
<th>Name of gene</th>
<th>1 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>p73</td>
<td>3.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p16-INK4a</td>
<td>1.8</td>
<td>2.8</td>
<td>—</td>
</tr>
<tr>
<td>CDC42GAP</td>
<td>—</td>
<td>—</td>
<td>9.0</td>
</tr>
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</table>

* Values in arbitrary units.
In addition to the application of the DNA array technique for the analysis of gene expression profile, it is also very important that the DNA array may be useful for the detection of single nucleotide polymorphism (SNP). SNPs are the most abundant type of DNA sequence variation in genomes. A SNP is a site on the DNA in which a single base-pair varies from person to person, and may influence and/or regulate the responses of genes for food factors. In this context, we are now engaging in a survey of new SNPs, and examine their association with individual variation in responses to food factors. These efforts should be useful for the development of custom-made designer foods. By the way, various methods for SNPs detection are proposed, besides the DNA array method, and thus it is important to select an ideal method in the field of food sciences. In any case, DNA array technology seems to be applicable in a wide variety of food sciences.

**Political and Industrial Activities of Functional Foods**

**MAFF research.** At present, our daily diet is becoming satisfactory in terms of quantity. However, with changes of food habits, food-related diseases such as cancer, allergy, obesity, and geriatric diseases are increasing in Japan. Furthermore, the aged population is expected to reach a maximum in the next century. From these social backgrounds, the demands of consumer and food industries are rapidly rising toward the high quality of foods providing well-balanced nutrients, rich tastes, and health promotion. In order to respond to the needs, research groups in the MAFF had and have done research projects on the discovery of physiological functions of agricultural products, the development of the evaluation technology of functionalities, and the creation of new agricultural products with high contents of functional components. Through this research, many physiological functions of the components of agricultural products have been clarified.

Epidemiological studies have indicated that consumption of citrus fruits is associated with a reduced risk of cancer. The researches on anti-cancer effects of components of vegetables and fruits demonstrate that glycoproteins, polyphenols, and phloretin have anti-mutagenic effects, killing or apoptosis-inducing effects on mouse melanoma and human leukemia cells, and inducing effect on differentiation of human leukemia cells to monocytes or macrophages. From citrus fruits, 3 kinds of new cancer-preventive components of beta-cryptoxanthin, auraptene, and nobiletin are found to prevent the N-methyl- nitrosourea-induced colon carcinogenesis in F344 rats. New citrus hybrids rich in those cancer-preventive components are now produced by means of citrus genetic resources.

Dietary phospholipids of various sources (soybean, egg yolk, and safflower seeds) as well as soybean protein lowers hepatic fatty acid synthesis. Polyunsaturated fatty acids of n-6 and n-3 series, particularly the latter, increase hepatic fatty acid oxidation while decrease fatty acid synthesis.\(^{52}\) Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation. In addition, this lignan lowers hepatic fatty acid synthesis. These metabolic changes should decrease synthesis, assembly and secretion of lipoproteins by the liver and thus account for the serum lipid-lowering effect of these food components. It is also demonstrated that the feeding of powder of a brown seaweed, Undaria pinnatifida (Wakame) in rats reduces the levels of triacylglycerol in their serum and liver.\(^{53}\) Inhibitory activity of edible plants on arachidonic acid metabolism: arachidonic acid metabolites are involved in many kinds of diseases including allergy, arteriosclerosis, and cancer. Strong inhibitory activities (IC\(_{50}\) under 0.01 mg/ml) on the metabolizing enzymes, cyclooxygenase, 12-lipoxygenase, and 5-lipoxygenase are found in perilla, olive, tea leaf, spinach, and onion.\(^{54}\) Active constituents such as luteolin and hydroxytyrosol are identified from perilla and olive, respectively.\(^{55}\)

Two catechin derivatives of (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3'Me) and (−)-epigallocatechin-3-O-(4-O-methyl) gallate (EGCG4'Me) with potent anti-allergic activity is isolated from "Benihomare" green tea or "Tong ting" oolong tea by HPLC techniques.\(^{56}\) Oral administration of EGCG3'Me and EGCG4'Me significantly and dose-dependently (5–50 mg/kg) inhibit type I allergic (anaphylactic) reactions in mice sensitized with ovoalbumin and Freund's incomplete adjuvant. The two catechins strongly inhibit mast cell activation through the prevention of tyrosine phosphorylation of cellular protein after Fc epsilon RI cross-linking.

To measure red cell deformability, leukocyte deformability and adhesiveness, and platelet aggregability, which are factors critically affecting the capillary blood flow rate, microchannel arrays are created as model capillaries using photolithography and etching.\(^{57}\) By using these arrays, the blood rheology is found to be improved by *in vitro* addition and oral administration of various food such as tea, fermented vinegar, and extract of plum.

Sweet potato juice with a high content of anthocyanin is found to prevent carbon tetrachloride-induced liver injury in rats. It is also demonstrated that by oral administration of the juice to the the subjects with hepatic diseases on for 44 days, the levels of γ-GTP, GOT, and GPT and the levels of serum LDH and total billirubin are lowered.\(^{58}\)

As described above, agricultural products have been found to contain many functional components which have the potentiality of improvement of health and prevention of diseases. In the future, it is neces-
necessary to discover the mode of action of functional components in vivo and the bio-regulating functions based on the mutual interaction of functional components or foods.

**MHW policy.** Under the Nutrition Improvement Law, there are five categories of “Foods for Special Dietary Uses”. They are foods for the ill, milk powder for pregnant or lactating woman, formulated milk powder for infants, foods for the aged with difficulty in masticating or swallowing, and FOSHUs. The “FOSHU” system was introduced in 1991, and currently it is the only such system through which government permission for health claims pertaining to food is granted in Japan. According to the Nutrition Improvement Law, FOSHUs are defined as, “foods in the case of which specified effects contributing to maintain health can be expected based on the available data concerning the relationship between the foods/food’s contents and health, as well as foods with permitted labelling which indicates the consumer can expect certain health effects upon intake of these particular foods”. The MHW is responsible for granting the permit.

Basically, in filing for approval of a FOSHU or permission to market such a product, the applicant must supply a sample of the product and the initial documentation requested by the appropriate authority. The MHW criteria for obtaining the permission/approval are as follows: The food should be expected to contribute to the improvement of one’s diet and the maintenance/improvement of health; The health benefits of the food or its constituents should have a clear medical nutritional basis; Based on medical and nutritional knowledge, appropriate amounts of daily intake should be definable for the food or its constituents; Judged from experience, the food or its constituents should be safe to eat; The constituents of the food should be well-defined in terms of physicochemical properties and qualitative/quantitative analytic measurement; There should be no significant loss of nutritive constituents of this food in comparison with the same ones normally present in similar types of foods; The food should be of a form normally consumed in daily dietary patterns, rather than consumed only occasionally. The product should be in the form of a usual food, not in another form, such as pills or capsules. Also, the food and its constituents should not be those exclusively used as a medicine.

After review, foods which are so recognized are permitted to bear on the product label an approved statement indicating the specified health benefit. The mark indicative of the MHW permission/approval must also be shown on the label of such FOSHU (Fig. 3). Health claims for 192 food products as FOSHU have been permitted to date. These foods belong to the following categories: food which promotes an increase in beneficial bacteria in the intestinal microflora and helps to maintain a healthy intestinal environment; food helpful for people with a high blood cholesterol level; food for mineral (calcium or iron) supplementation, with high absorbability; food of low cardiogenicity; food helpful for people with mild hypertension; food helpful for people who are concerned about their blood glucose level; food helpful for people who are concerned about their blood triglyceride level. These foods have effective ingredients such as dietary fiber, sugar alcohols, oligosaccharides, proteins, peptides, polyphenols, lactobacilli or bifidobacteria, chitosan, and others. Thus, FOSHUs are foods which will contribute to improving the quality of life. However, at present, the number and kinds of such foods available are still limited and it is expected that something new will be developed.

**ILSI-Japan activity.** Most food industries paid attention to the first introduction of the concept of functional foods when the MESC research project started in 1984, as mentioned already. However, in the first five years after the regulation of FOSHU was implemented in 1991, not many of them showed an interest in it because, compared with a large volume of scientific data required, the permitted claims were limited to a fairly small number of indirect and unattractive expressions. Following with deregulation gradually carried out in recent years, several existing...
products of popular brands have entered the market. Together with them, development of such new products as moderating blood pressure and suppressing fat accumulation has been contributing to the expansion of FOSHU market. From the standpoint of food industries, elaborate efforts on research and development should be rewarded only if the scientific results are exactly and adequately reflected in the health claims leading to correct understanding and support of the product by consumers. With those backgrounds, a committee on functional foods was organized in ILSI Japan in October 1996. The main purpose was to study and sort out the history and current situation of functional foods from the viewpoints of science, regulation and the market. 26 members from 23 companies, about one third of all member companies in ILSI Japan, actively participated in the committee.

The members were allocated into four groups; criteria for evaluation of scientific data, regulations on health claims, market situations, and scientific research. After actively working for about one year with close collaboration among the groups, the functional food committee issued the first report entitled “The Status quo of Functional Foods and The Subjects to be Discussed” in July, 1998. It is composed of 8 chapters including a brief history of functional foods, regulations and the market of FOSHU, trends in research and development, and current problems. Copies of this report both in Japanese and in English were distributed to the administration, the academic society, industries belonging to ILSI Japan, and all overseas ILSI branches. Since then members of the committee have often cited the content in domestic and overseas conferences.

When getting into the second stage, the committee reinforced its activity both by reorganizing it into two groups; scientific substantiation of health claims, and regulatory systems with 34 members from 30 companies and by consulting from time to time Professor S. Arai, the first author of this article, as an advisor of the committee. The committee issued the second report (in Japanese) entitled “Health Claims on Functional Foods—Proposals on Scientific Substantiation and Regulatory Systems” in December 1999 and the English version is going to be issued in August 2000. This report puts emphasis on proposals about what should be taken into account to conduct a clinical trial and to make full use of health claims. As a basis of these proposals, domestic and overseas information is introduced on recent progress of health claims from a regulatory aspect and on recent development of functional foods from the commercial aspect.

In April 2000 the committee started its activities of the third stage with 46 members from 29 companies. It aims at reviewing scientific literature detailing the efficacy and safety of specific food ingredients chosen from a viewpoint of Japanese features in research. The third report will be issued in March 2001 and it might be referred to at the international symposium on functional foods to be held in Paris in October 2001. ILSI Japan is strengthening relationships with the academic societies and this is crucial to achieve its goal. Accordingly, the committee of functional foods is collaborating with leading scientists through the International Union of Food Science and Technology (IUFoST). As deregulation is going on, the new category of Nutrient Function Foods will be established in a year. The committee’s activity may contribute to giving a scientific basis for the legislation. In the very near future, such a voluntary system of health claims as the code of practice in the UK should be studied in Japan as well, and ILSI Japan is expected to play the role of a bridge among the administration, the academic societies, food-related industries, and consumers.

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