Review

Research and Development for Bioavailable Functional Foods in Food Science

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The tertiary functions, health benefits, and promoting functions of food have been well researched, and this knowledge has facilitated the development of supplements. Marketable supplements must include components that give users the bodily sensation that they are effective when taken. These components include highly bioavailable chemicals that are incorporated in their original form without undergoing modification and resistant to the catabolic decompositions in the body. These components can be prepared from rich sources other than food. Chemicals in food are limited by their intake amounts, and their incorporation also interfere with food ingredients such as dietary fiber. Supplements can supply large amounts of chemicals in a single dose, but also have the potential to produce chronic side effects. Research on tertiary food functions can provide consumers with valuable information, but how this knowledge can be applied to the development of supplements remains unclear.

Keywords: tertiary function, supplement, flavonoid, light irradiation, fucoxanthin, food processing

Introduction

The metabolic functions of food ingredients have been studied by food and nutrition scientists. Around the last decade of the 20th century, various studies on the nutritional and taste functions of carbohydrates, proteins, lipids, vitamins, and minerals were completed. On the other hand, several researchers noticed that everyday foods contained other minor ingredients that exhibited different functions from their nutritional and taste functions. A paper on the anti-mutagenic activity of herb components (Natake et al., 1989) was one of the first reports of these different functions. The active components were identified as flavonoids (Kanazawa, 1995; Samejima et al., 1998) and their anti-mutagenicity mechanism was reported (Kanazawa et al., 1998; Kanazawa et al., 1999; Ashida et al., 2000).

Food components like flavonoids are non-nutrients that do not produce the energy chemical ATP, are not involved in energy-producing metabolism, and do not undergo catabolic decomposition. Thus, these non-nutrients largely remain in their original form after being incorporated into the body, and as such exhibit their functions. There are around 8000 species of phenolics including phenylpropanoids, flavonoids, and anthraquinones; about 25,000 terpenoids including terpenes, carotenoids, xanthophylls, and iridoids; 12,000 alkaloids; and several sulfate-containing chemicals such as isothiocyanates.

The non-nutrients were found to exhibit tertiary functions that produce beneficial and promoting effects on human health, besides their primary function (nutritional function) and secondary function (taste function), and have been investigated in agricultural products (Kanazawa, 2013).

With progress in the investigation of tertiary functions, preparatory methods for active components have been developed, and the extracted and purified components have been applied for commercial uses such as in supplements. Recently, preparations of pure chemicals have been easily obtained from other chemical-rich sources beside foodstuffs.

Food is a mixed source of chemicals such as carbohydrates, proteins, lipids, vitamins, minerals, and others. These can produce energy for our working and supply the materials to reform our body cells. Supplements composed of extracted and purified non-nutrients that express tertiary functions are different from everyday

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foods in that they lack the components necessary to produce energy. Research on the functions of dietary non-nutrients is an important issue, and provides additional information to consumers. However, the development of supplements using extracted and concentrated non-nutrients is still not well understood.

In the present review, research on tertiary food functions and the development of commercial applications will be discussed using four of our studies as example data.

The use of highly bioavailable chemicals for functional food Among non-nutrients, phenolics mostly undergo conjugating reactions and are converted to an inactive form during the absorption process in intestinal epithelial cells, after which they are transported to the intestinal lumen site (Murota and Terao, 2003). Dietary phenolics are detected at plasma concentrations of less than 1 μmol/L and are the inactive form after being conjugated in the human body (Manach et al., 2005; Williamson and Manach, 2005). Generally, dietary phenolics exhibit low bioavailability.

Among phenolics, prenyl, a geranyl chemical, is resistant to conjugation reactions by restricting the approach of conjugation enzymes. Artepillin C in Brazilian propolis, which honeybees collect from Baccharis dracunculifolia, possesses two prenyl groups adjacent to the target hydroxyl for conjugation enzymes, as shown in Figure 1. Further investigation revealed that artepillin C was largely absorbed without undergoing conjugation (Shimizu et al., 2004). Following incorporation into the body, artepillin C induced G0/G1 arrest through stimulation of Cip1/p21 expression in human colon cancer cells, and suppressed formation of aberrant crypt foci in the colon of carcinogen-challenged mice (Shimizu et al., 2005).

Based on this evidence, an intervention study was performed with frequent colorectal adenoma polyp patients (Ishikawa et al., 2012). Artepillin C is a folk medicine, and Japanese regular users generally take around 500 mg/day/capita (Ishikawa et al., 2012). In a human trial, smaller amounts, 18.36 mg/day/patient, were dosed for 3 months. The formation of 8-oxo-2'-deoxyguanosine, an oxidative product of the DNA base guanosine, showed a clear tendency to be suppressed in the group given artepillin C. However, a surprising side effect was found after biochemical analysis of subjects’ blood. After 3 months of artepillin C, creatine phosphokinase activity was slightly, but significantly, higher than in the placebo group. In determination of the ratio of isozymes in creatine phosphokinases, the myocardial band form significantly increased in the propolis group, from a basal level of 1.4 ± 0.3 to a final level of 1.6 ± 0.4 (P value = 0.044).

Leakage of the myocardial band form of creatine phosphokinase could be found in a human study that was able to collect sufficient blood. These findings indicate that the routine use of artepillin C may cause myocardial disease, revealing that highly bioavailable chemicals have the potential to produce adverse side effects.

Utilization of agricultural waste products for functional food Among phenolics, the flavonol quercetin has been extensively investigated from the early stages of functional studies (Hertog et al., 1993). Dietary quercetin undergoes conjugation during the incorporation process, but exhibits partial activity, such as antioxidant potency, and contributes to human health promotion (Moon et al., 2000). In addition, the conjugated quercetin can be converted to the active de-conjugated form by β-glucuronidase, which is secreted around inflamed tissues, such as carcinogenic cells, in animal experiments (Oi et al., 2008). Also, chronic intake of quercetin has been reported to improve parameters related to metabolic syndrome (Kobori et al., 2011). Thus, dietary quercetin shows moderate bioavailability.

For use as a functional food, quercetin is obtained from the edible part of onion bulbs (Allium cepa L.). However, the bulb is a high-cost product because it is one of the most popular and familiar vegetables. On the other hand, the bulb is encased by a hard skin, which contains the greatest amount of quercetin among the agricultural products shown in Table 1 (Sakakibara et al., 2003). The skin discards as a large amount of waste and makes cultivation difficult, because the skin is strongly resistant to decay when composted and requires large amounts of heavy oil when incinerated. Thus, the use of the skin for quercetin preparation may help to alleviate cultivation difficulties.

After development of a preparation method for onion bulb skin, the extract was found to be composed of 0.82 g quercetin, 0.65 g quercetin glycosides, 0.42 g protocatechuic acid, 2.5 g ash, 25 g water, and other polysaccharides. Sulfur-containing chemicals were below the detection limit in 100 g of the extract, and a patent was acquired (no. 4344913). However, it was difficult to rid the...
Research and Development for Bioavailable Functional Foods

Skin of agricultural chemicals because the onion bulb is grown in soil containing various chemicals. Washing with a solvent decreased the amount of chemical residues to a level below the residue standards, but complete removal was not possible.

On the other hand, the skin extract exhibited marked bacteriostatic effects on multiple-drug resistant microorganisms (Table 2) as well as microorganisms associated with acne and tooth decay (Fig. 2). The extract acquired an INCI name and was developed for cosmetics and toiletries (patent application no. 2012-29036).

Table 1. Quercetin contents in agricultural products.

<table>
<thead>
<tr>
<th>Agriculture products</th>
<th>Amount of quercetin in mg/100 g of fresh weight*</th>
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<tbody>
<tr>
<td>Onion (Allium cepa) edible part of bulb</td>
<td>53</td>
</tr>
<tr>
<td>Onion bulb skin</td>
<td>820</td>
</tr>
<tr>
<td>Green tea leaf (Camellia sinensis)</td>
<td>270</td>
</tr>
<tr>
<td>Asparagus (Asparagus officinalis)</td>
<td>28</td>
</tr>
<tr>
<td>Garden pea (Pisum stivum)</td>
<td>19</td>
</tr>
<tr>
<td>Coriander (Coriandrum sativum)</td>
<td>14</td>
</tr>
<tr>
<td>Green bell pepper (Capsicum grossum)</td>
<td>6.9</td>
</tr>
<tr>
<td>Mizuna (Elatostema umbellatum var. majus)</td>
<td>4.0</td>
</tr>
<tr>
<td>Apple (Malus pumila)</td>
<td>3.9</td>
</tr>
<tr>
<td>Sweet cherry (Prunus avium)</td>
<td>3.9</td>
</tr>
<tr>
<td>Lemon (Citrus limon)</td>
<td>1.4</td>
</tr>
<tr>
<td>Peach (Prunus persica)</td>
<td>1.2</td>
</tr>
<tr>
<td>Tomato (Lycoperscion esculentum)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Values are for aglycone in quercetin glycosides, and do not include rutinoside form of quercetin (rutin).

Table 2. Bacteriostatic effects of the extract from onion bulb skin.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Minimum inhibitory concentrations*</th>
</tr>
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<tbody>
<tr>
<td>Escherichia coli</td>
<td>&gt;2.5 mg/mL</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;2.5 mg/mL</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.63 mg/mL</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.32 mg/mL</td>
</tr>
<tr>
<td>Staphylococcus epidermis N-8</td>
<td>0.63 mg/mL</td>
</tr>
<tr>
<td>Staphylococcus epidermis T-8</td>
<td>0.32 mg/mL</td>
</tr>
</tbody>
</table>

*Microorganisms were in microplates in LB medium containing the extract from onion bulb skin and cultured shaking at 37°C for 18 h.

Fig. 2. The bacteriostatic activity of an extract from onion bulb skin compared to the activity of the anti-microorganism chemical thymol. Microorganisms were cultured in medium containing the skin extract (●) or thymol (□) at 37°C for 18 h, and the concentration of microorganisms was determined by the absorbance at a 660 nm wavelength. (A) Propionibacterium acnes NBRC 107605 in GAM medium. (B) Streptococcus mutans NBRC 13955 in BHI medium.
These cosmetics and toiletries have been commercialized and marketed; however, the goal of the study, to assist agriculture through utilization of the waste skin, has not yet been achieved. Notably, the amount of onion skin is sufficient for extraction because it contains high concentrations of quercetin, and the market for organic cosmetics and toiletries is moderate. Also, quercetin destined for the production of supplements is obtained from roadside trees called Chinese scholar trees (Sophora family) as another quercetin-rich material. As a result, it was not possible to facilitate the agricultural production of onion skin as a supplement material to reduce agricultural waste and alleviate cultivation problems.

Development of easily edible functional food Kombu (Saccharina japonica, formerly named Laminaria japonica Areschoug) is sold as a dried food; however, its consumption has been decreasing annually. Kombu is rarely eaten in its dried form because it is hard; instead, it is usually used for making broth (dashi) at home or is marketed after processing as boiled kombu (tsukudani). Cooking dashi is time consuming, and the processed kombu is often eaten together with a large amount of rice because of its salty taste and black color. These features do not suit the recent consumers trends and are considered to be the reason for the decrease in kombu consumption.

On the other hand, kombu includes two bioavailable and functional components, fucoxanthin and fiber. Dietary fucoxanthin is partly incorporated into the human body after its metabolism to a more active form, de-acetylated fucoxanthinol, and is easily excreted via the urine without accumulating in the body, and thus does not produce any side effects (Hashimoto et al., 2009 and 2012). Fucoxanthin plays protective roles in colon cancer by inducing cell-cycle arrest at the G$_2$/M phase with up-regulation of p21(WAF1/Cip1) (Das et al., 2006 and 2008), and has ameliorating effects on metabolic syndrome by inducing uncoupling of protein 3 in white adipose tissue (Maeda et al., 2006 and 2015).

Among the various dietary fiber polysaccharides in kumbo, one is composed of fucose and sulfated fucose and is called F-fucoidan, which is part of a family of fucoidans such as G-fucoidan in wakame (Undaria pinnatifida Suringar) and U-fucoian in mozuku (Nemacystis decipiens Kuckuck). Dietary F-fucoidan passes through the intestinal lumen and is not incorporated into the body, thereby producing no side effects. In the intestinal lumen, F-fucoidan acts on NADPH oxidase 1 and dual oxidase 2 in the epithelium. The enzymes produce a small amount of hydrogen peroxide and stimulate secretion of eicosanoid prostacyclin, which induces a dis-aggregation of aggregated platelets in capillary vessels (Ren et al., 2013). Also, dietary F-fucoidan acts on the epithelium to secrete galectin-9, which plays a role in suppressing type I allergies (Tanino et al., 2016). Thus, the two components in kombu exhibit various health benefits, including cancer preventing (Das et al., 2006; Das et al., 2008), metabolic syndrome suppressing (Woo et al., 2009; Maeda, 2015), anti-inflammatory (Choi et al., 2016; G.-López et al., 2016), anti-thrombosis (Ren et al., 2013), and anti-allergic (Tanino et al., 2016) effects.

It is expected that a novel processing method for kombu that is capable of extracting fucoxanthin and F-fucoidan from raw kombu while removing unfavorable factors will be popular with modern consumers. An extraction method for fucoxanthin has been developed on a commercial scale (Kanazawa et al., 2008), and crude F-fucoidan has been prepared from the extracted residues, in which unfavorable components are removed. The functional components in kombu were reconstructed by subsuming fucoxanthin with F-fucoidan and making a fine powder, as shown in Figure 3. The powder allowed the production of various easily eaten foods, while the processes created only a small amount waste salts and ashes.

The novel processed kombu has an advantage over preserved kombu. Specifically, the fucoxanthin forms a tight complex with chlorophylls in algae and is difficult to prepare (Lepetit et al., 2010). After its extraction from kombu, fucoxanthin remains partly in this tight complex and is sensitive to oxidation under light. Thus, to examine methods for optimizing fucoxanthin stability, the following two kinds of powder were reconstructed using the extracted and purified fucoxanthin and the kombu fibers prepared from extracted residues, composed of F-fucoidan and other dietary fibers such as alginate, laminaran, and alcohol-soluble carbohydrates. The one type was composed of fucoxanthin dissolved in a small amount of alcohol and then mixed with the kombu fibers. The other type was prepared by dissolving fucoxanthin in a small amount of a mixture of cyclodextrin and medium-chain fatty-acids, and then subsumed with the kombu fibers. Scanning electron microscopy (Fig. 4) revealed that the subsumed powder swelled up following absorption of the fucoxanthin solution (Fig. 4B), but the mixed powder of fucoxanthin and fibers did not (Fig. 4A). Measurement of the stability of fucoxanthin in these reconstructed powders revealed that the fucoxanthin content in the mixed powder decreased to almost 0% at 40°C and 73% at 5°C after 12 months of storage (Fig. 4C), while the fucoxanthin in the subsumed powder remained at 70% at 40°C and 95% at 5°C after 12 months of storage (Fig. 4D). The subsumed powder permitted approximately one year for distribution to market, and thus was viable for commercial use.

The subsumed powder containing fucoxanthin and F-fucoidan enabled us to make highly edible foods. However, its use did not spread because of several difficulties accompanying its industrial preparation. The transportation of raw kombu from the culture site to the production facility is costly, because raw kombu contains large amounts of water, and is thus heavy to transport. Alternatively, it is relatively easy to extract fucoxanthin from another alga, wakame, and it is possible to prepare this at the culture site. However, the production of wakame is limited, and the use of wakame for fucoxanthin preparation is likely to disturb the wakame market. Other brown algae, such as akamoku (Sargassum
horneri (Turn.) Ag.) or hondawara (Sargassum fulvellum (Turn.) Ag.), also contain abundant fucoxanthin; however, harvesting of these wild algae is unstable. Kombu can be increased during cultivation and remains the best source of fucoxanthin, despite the high transportation costs.

These studies have demonstrated the health benefits of fucoxanthin. Subsequently, fucoxanthin has been obtained from sources other than foods, such as microorganisms (Wakahama et al., 2012; Xia et al., 2013).

Development of a preservation method for fresh vegetables under storing in a home refrigerator This author suggests that the daily intake of fresh produce is better for consumers than functional chemicals. Consumers purchase vegetables and fruits at the market and store them in a home refrigerator for a few days. Fresh produce gradually loses their nutrients during storage, even under refrigeration. The development of a storage method to prevent the loss of nutrients from vegetables and fruits will likely be popular among consumers. Interestingly, every plant cell possesses the ability to re-form its whole body, similar to stem cells in animals; thus, a suitable stimulus may control the cell’s function even after harvesting. Plants produce flavonoids to prevent microbial infection, defend against plant-eating animals and insects, and absorb surplus energy from sunlight after photosynthesis (Ryan et al., 2002; Rissini et al., 2011). For humans, flavonoids are typical functional food components (Kanazawa, 2007; Kanazawa, 2011). Preventing the decrease in flavonoids during storage of vegetables and fruits under home refrigeration will likely contribute to health promotion in consumers.

This author stimulated the function of plant cells by providing surplus light to vegetables, using light that was near the maximum absorption wavelength for flavonoids. The vegetables and fruits were irradiated with UV-B light in a refrigerator at an energy level of 98 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) for 5 min (1 joule), which is the average daily solar irradiation level in Japan. The expression of flavonoid synthesis genes, \( \text{PAL, C4H, 4CL, CHS} \), and \( \text{FNS} \) (Dixon and Paiva, 1995; Blount et al., 2000), was stimulated 6-24 h after the single 5-min irradiation, and flavonoids were synthesized and increased by 1.2-fold to 8.6-fold in the plants after 6 days of storage in the refrigerator (Kanazawa et al., 2012).

Unripe green strawberries (Fragaria X ananassa Duch) maturated to a red color after 6-day storage and irradiation with UV-B for 5 min/day in a refrigerator at 10°C, and significantly accumulated the strawberry flavonoid, anthocyanin pelargonidin glycoside, at 2-fold higher than for strawberries kept in a
refrigerator in the dark. However, anthocyanin possesses its maximum absorption level at wavelengths near to green light, and thus irradiation was tested again with a green light at 560 nm. There was a 4-fold increase in pelargonidin, and vitamin C, which possesses the maximum absorption at this wavelength, increased to 125% compared to that after 6-day storage in the dark.

Irradiated storage of vegetables and fruits has been applied to commercial refrigerators equipped with UV-B LED lights, and has been marketed since 2005 following a patent application (no. 2004-121228). This refrigerator has sold well, and the concept has likely contributed to the design of industrial growing systems for crops.

Significance of functional food and functional chemicals in food science Food and nutrition scientists have identified tertiary functions in daily food that have human health benefits. Consumers have requested functional foods, and functional ingredients have been prepared and marketed. To ensure repeat customers, the functional food must be so effective that it produces a bodily sensation in the user. These effective components are highly bioavailable chemicals that are incorporated into the body largely in the original form without undergoing modification and decomposition; however, these effective chemicals can produce side effects in humans. To discuss these issues, the author provides four examples of food functional research and supplement development.

The first example, artepillin C, is a component in folk medicine and is generally known to be effective. Our research found that artepillin C is a highly bioavailable chemical, but produced side effects in a human study.

The second example, quercetin, is partly incorporated into the body after undergoing conjugation reaction and is not so highly bioavailable. Quercetin can be prepared from onion bulb skin, thus reducing agricultural waste, and has been developed into functional cosmetics and toiletries, but not functional foods. However, consumer have demanded for functional foods but does not for cosmetics, and at present, quercetin is being prepared from sources other than food, and is marketed as a supplement.

The intestinal absorption of bioavailable chemicals in food can be disturbed by other food components and the incorporation of
nutrients is influenced by dietary fibers (Imai et al., 2014). Disturbance of intestinal absorption likely reduces the bioavailability of quercetin. On the other hand, in supplement form, a large supply of quercetin in one dose could potentially produce a similar effect to artemin C.

In the third example, the authors designed a novel food wrapping using functional fucoxanthin and functional F-fucoidan from kombu. However, this processed food was not cost-effective. Recently, fucoxanthin has been prepared from sources other than food, including microbial cultures, which can be easily prepared to produce pure fucoxanthin on a large scale.

In the fourth example, the author applied the results of functional food research to design a refrigerator equipped with an irradiating LED, which prevented a decrease in flavonoid contents during storage. This refrigerator is useful for consumers in that it provides health benefits and promotes food factors. On the other hand, the attaching of LED in refrigerator requires a small cost, and consequently the patent royalties for the developer are very low.

These four examples clearly demonstrate that research on the health benefits and promoting functions of food factors are not associated with the development of functional foods. Food is a mixture of various ingredients possessing primary nutritional functions, secondary taste functions, and tertiary functions. A product composed solely of tertiary functions is not considered to be a food because of the absence of primary and secondary functions. Among the four examples, artemin C and quercetin from onion skin are functional chemicals, while the novel reconstructed kombu and the vegetables in the irradiating refrigerator are functional foods. Research into artemin C and onion skin quercetin is unlikely to contribute to the health promotion of consumers, although it may facilitate the development of supplements. The importance and necessity of research into the development of functional foods will be discussed in the conclusion.

Conclusion

Humans are heterotrophs that must consume food for energy production and physiological processes. Food should be a mixture of energy sources and chemicals to reform body cells, such as carbohydrates, lipids, proteins, vitamins, and minerals. In order to maintain healthy lives, people select their everyday foods themselves. Research into the health benefits and promoting functions of food helps people to select the most suitable food among various kinds of everyday foods. In addition, the knowledge gained from research into functional foods can facilitate agricultural production as well as support sustainable agriculture in the supply of food for humans.

On the other hand, the application of functional food research has also led to the development of supplements and medicines, which do not provide energy sources for the maintenance of life. Medicines can save the lives of patients; for example, it is estimated that streptomycin has saved a few hundred million lives. However, agricultural production can save many more people; for example, crop (rice and wheat) breeding is estimated to have saved a few thousand million lives in the feeding of starving people following WW2. Thus, research into functional food is important for allowing people to select desirable everyday foods, rather than for the development of medicine-like functional foods.

A good example is the health benefits of tea catechins, which have been widely investigated (Natsume et al., 2003; Ueda et al., 2008; Ueda et al., 2010; Okabe, 2013) and have helped facilitate the breeding of tea species to produce the bioavailable catechins, methyl catechins (Maeda-Yamamoto et al., 2009). Green tea is a drink that does not supply large amounts of bioavailable chemicals all at once, and thus consumers can avoid potential side effects from the excess intake of bioavailable chemicals. Similarly, consumers can themselves control the intake amount of everyday foods. This behavior limits the intake of bioavailable components, preventing side effects in consumers following the intake of everyday food.

Thus, research into the functions of food ingredients is necessary to provide information on the health benefits of everyday food to society. Furthermore, this research can promote sustainable agriculture. However, the use of research information to develop supplements is not considered to be such an important issue, since functional chemicals can be obtained from other sources. Supplements can be beneficial in the context of consumers’ personal needs, but not societal needs.

References


