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Review Article

A review on the analysis of ingredients with health care effects in health food in Taiwan

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ABSTRACT

This review article discusses the analysis of ingredients with health care effects in health food in Taiwan. The top 10 items on the list of registered health food products up to 2014 in Taiwan are described, including monocolin K, ω-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid), β-glucans, inulin, catechins, oligosaccharides, resistant maltodextrin, amino acids, medium chain fatty acids, and polysaccharides. Some analytical methods for the analysis of ingredients with health care effects are announced to the public on the website of health food section of the Taiwan Food and Drug Administration for the application and the postmarket surveillance of health food. Each application of health food should include the appropriate analytical method for the analysis of the ingredient or specific compound that has the health care effect, for the sake of quality assurance. Self-management of each applicant is required for regulation, the reputation of its own, and social responsibility to the consumers.

1. Introduction

"Health Food" is a legislative term used in Taiwan. The total number of products designated as health foods from 1999 to 2014 is 310, including 50 health foods with specification standards (Fig. 1) [1]. There are two tracks to apply to register a health food. Track 1 is the application for individual case review deliberated by the Health Food Advisory Committee, including safety assessments (Table 1), health care effects (Table 2), and quality stability. However, Track 2, for the specification standard, skips the reviews of the Health Food Advisory Committee due to the safety and health care effects of the ingredients. Up to now, red mold rice and fish oil are the two health food specification standards [2]. The management system of health food in Taiwan, similar to that of Japan and China, belongs to premarket registration [3–6]. Applications should be deliberated by the Health Food Advisory Committee which examine safety, effective efficiency, and evaluate the stability of the ingredient with the health care effect.

The quality of ingredients with health care effect in health foods should be assessed in order to ensure the health benefits to the consumers. The ingredients with health care effects should be qualified and quantitated. Therefore, appropriate analytical methods for the ingredients with health care effects are necessary to assess the compliance with specifications. There are more than 100 items of ingredients with health care effects in the 310 registered health food products up to 2014 in

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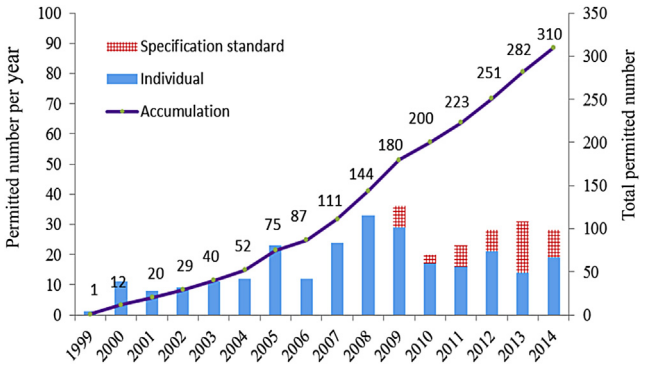

Fig. 1 – Number of permitted health foods from 1999 to 2014.

Taiwan. In the regulation of health food, it is necessary to provide the analytical methods used to examine ingredients with health care effects for each application, so that the method may be recognized as having scientific reliability and accuracy [7]. Because of the complexity and diversity of matrixes, the available and reliable analytical methods for various types of health food effects are not adequate for specific ingredient identification or composition confirmation. The appropriate analytical methods of ingredients with health care effects for each product application should be validated and verified. The quality of ingredients with health care effects in health food is an issue of great concern.

2. Ingredients with health care effects

There are 13 health care effects announced by the competent authority (Table 2). The experimental results represent the health care effects of the complete health food product rather than just a single compound or ingredient. Because of the diverse properties, there are various ingredients with health care effects in permitted health foods. However, for some cases, the effective components cannot be clearly identified. Thereby, the index compounds are presented for the quality control of the product. This excludes the probiotics group, the top 10 ingredients with health care effects: monacolin K, ω-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid), β-glucans, inulin, catechins, oligosaccharides, resistant maltodextrin, amino acids, medium chain fatty acids, and polysaccharides (Table 3). For the sake of postmarketing surveillance of health foods, and the necessity of commonality for the application, there are some analytical methods of efficient compounds available to the public in the section on health food analysis on the Taiwan Food and Drug Administration (TFDA) website (Table 4) [8]. The availability of those

Table 2 – Thirteen health care effects of the permitted health foods.

1. Modulating blood lipids
2. Improving gastrointestinal functions
3. Regulating immune system
4. Adjuvant modulating allergic constitution
5. Protecting bone health
6. Maintaining dental health
7. Modulating blood sugar
8. Protecting liver functions (chemically induced liver damage)
9. Reducing body fat formation
10. Anti-weariness
11. Postponing aging
12. Adjuvant modulating blood pressure
13. Promoting iron absorption

Table 3 – Top 10 ingredients with health claim/index compounds for quality control in permitted health foods.

Ingredient with health claim/index compound for quality control	Number of permitted health foods
Monacolin K	45
EPA and DHA	22
β-Glucan	18
Inulin	16
Catechins	13
Oligosaccharides	12
Resistant maltodextrin	9
Amino acids, medium fatty acids, polysaccharides	8

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

Table 1 – Categories of safety assessments.

Category	Content	Toxicity test
I	The raw materials of the products are conventionally consumed and the product is in the form of commonly processed food; or there is complete academic literature and reports on the toxicology and safety, as well as records of consumption, of the product	May be exempted under any of the circumstances listed on the left
II	The raw materials of the product are conventionally consumed and the product is not in the form of commonly processed food	Genotoxicity study, 28-d feeding toxicity study
III	The raw materials of the product are not conventionally consumed	Genotoxicity study, 90-d feeding toxicity study, teratogenicity
IV	The raw materials of the product are not conventionally consumed and contain carcinogen analogues	Genotoxicity study, 90-d feeding toxicity study, teratogenicity, carcinogenicity study, reproduction study

Table 4 – Analytical methods of the ingredients (index compounds/specific components) with health claims in health foods.

Effective index / specific compound	Scope	Method	Attribute	Equipment/principle
DHA, EPA	Fish oil	Method of test for eicosapentaenoic acid and docosahexaenoic acid in fish oils. MOHW (Ministry of Health and Welfare) Food No. 1021950329 Amended, September 6, 2013	Promulgated method	Analysis by using gas chromatography after saponification and methylation
Oleic acid, linoleic acid, linolenic acid	Food	Method of test for fatty acids in food. MOHW Food No. 1021950978 Amended, November 28, 2013	Promulgated method	Analysis by using GC after extraction of oil, saponification and methylation
Vitamin D	Capsule, tablet, powder	Method of test for vitamin D in food. October 6, 2013	Recommended method	Analysis using HPLC after extraction
HMG-CoA inhibitor (Monacolin K)	Red mold powder, cereal powder	Red mold rice health food with specification standard. DOH Food No. 0960406448 promulgated, December 24, 2007	Promulgated method	Analysis using HPLC after extraction
Citrinin	Red mold powder, cereal powder	Method of test for mycotoxin in foods – test of citrinin. MOHW Food No. 1021950329 Amended, September 6, 2013	Promulgated method	Analysis using HPLC after extraction
Catechins	Tea beverage	Method of test for catechins in drink. November 16, 2010	Recommended method	Analysis using HPLC after dilution
β-carotene	Algae powder	β-Carotene in supplements and raw materials reversed-phase HPLC method. AOAC Official Method 2005.07	Recommended method	Analysis using HPLC after extraction
Xylitol	Chewing gum	Method for test xylitol in chewing gum. June 30, 2010	Recommended method	Analysis using HPLC after extraction
Calcium	Tablet, milk powder, liquid milk	General method of test for heavy metals. MOHW Food No. 1031901169 Amended, August 25, 2014	Promulgated method	According to the conditions of element species, sample matrix, quantitative limits and laboratory equipment, a complete analysis method is combined by choosing the appropriate digestion method and content determination method.
Inulin, fructan	Beverage, powder food	Measurement of fructan in foods-enzymatic / spectrophotometric method AOAC official method 999.03	Recommended method	Analysis using spectrophotometry after enzymatic treatment
b-glucans	Cereal food	β-D-glucan in oats, streamlined enzymatic-spectrophotometric method. AOAC Official Method 995.16	Recommended method	Analysis using spectrophotometry after enzymatic treatment
Lactic acid bacteria	Dairy product, milk powder, capsule, tablet, powder, crystalline powder	Methods of test for food microorganisms-test of lactic acid bacteria. MOHW Food No. 1021950329 Amended, September 6, 2013	Promulgated method	After serial dilution, cultivated with selective medium
Bifidobacterium longum		Methods of test for food microorganisms - Test of lactic acid bacteria - *Bifidobacterium longum*. June 6, 2013	Recommended method	Strains identification with real-time polymerase chain reaction after isolation and purification and
Bifidobacterium lactis		Methods of test for food microorganisms - Test of lactic acid bacteria - *Bifidobacterium lactis*. June 6, 2013	Recommended method	DNA extraction
Bifidobacterium breve		Methods of test for food microorganisms - Test of lactic acid bacteria - *Bifidobacteirum breve*. June 6, 2013	Recommended method	
Bifidobacterium bifidum		Methods of test for food microorganisms - test of lactic acid bacteria - *Bifidobacterium bifidum*. June 6, 2013	Recommended method	
Enterococcus faecalis		Methods of test for food microorganisms - test of lactic acid bacteria - *Enterococcus faecalis*. November 22, 2012	Recommended method	
Streptococcus thermophilus		Methods of test for food microorganisms - test of lactic acid bacteria - *Streptococcus thermophilus*. November 22, 2012	Recommended method	

When the sample matrix is not the same as described in the scope of the recommended method, the assessment should be evaluated according to the *Guidelines for the Validation of Chemical Methods for Food* or *Guidelines for Laboratory Quality Management – Test Results of Quality Control (Microbial Field)*.
If there is any interference on test result, a further study is required.
AOAC = Association of Official Analytical Chemists; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; GC = gas chromatography; HPLC = high performance liquid chromatography.

analytical methods is based on the ingredients can be identified; applied more than two permitted applications or applicants, or for the other reasons. The analytical methods on the TFDA website are either promulgated methods for the foundations of food sanitation standards and health food with specification standards (fish oil and red mold rice); or recommended methods modified and validated as necessary, after which the limit of quantitation (LOQ) should be equal to the original references. In addition, the international published methods, such as the Association of Official Analytical Chemists (AOAC) official methods of analysis, are recognized after validation for each application. Some analytical methods for ingredients with health care effect in health foods are interpreted in detail as follows.

2.1. *Monacolin K in red mold rice*

As reported, monacolin K (MK) can lower blood cholesterol concentrations effectively [9,10]. There are 45 health food products that claim MK as an ingredient with a health care effect, of which 33 belong to specification standards [1]. The daily intake of MK for red mold rice health food with a specification standard should be in the range of 4.8~10 mg [11], which is summed up with its lactone form (MKL) and hydroxy acid form (MKA). The physiologically active form of MK is its hydroxyl acid form converted from the lactone ring. MKL transfers easily into MKA with bioactivity *in vivo* [12–14]. Both forms coexist in red mold rice [15,16]. The ratios of MKL over MKA are in the range of 20:1 to 3:1 for the 12 red mold rice products (data are not published). A high performance liquid chromatography-diode array detection (HPLC-DAD) method for determining monacolin K in red mold rice capsules is promulgated in 2011 [17]. The MKL acetonitrile stock standard solution is stored at −20°C, and the working standard solution and sample are treated with methanol. MKL and MKA remain at 95% and 98%, respectively, after 24 hours. Nevertheless, MK methanol working standard solution should be prepared fresh daily. Moreover, analysis of MK should be completed as soon as possible because of MKL standard acetonitrile stock solution that could be converted to MKA in the appropriate alkali condition. The MK contents of the 12 health food products with red mold rice powder are in the range 1.49–25.0 mg/g [18]. After calculation of the net weight of each capsule and daily intake dose, nine specification standard products are in the range of 5.4~10 mg, which are compliant with the regulations.

In addition, the products applied to red mold rice health food with a specification standard should not only examine MK, but also citrinin, a type of nephrotoxic mycotoxin, of which content should less than 2 ppm and analyzed by using HPLC with fluorescence detection [10,19].

2.2. *Fatty acids*

Functional fatty acids, including linoleic acid, linolenic acid, γ-linolenic acid, and eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA), are applied as the effective ingredients/index compounds for quality control in the permitted Health Food, which claim health benefits of lowering lipid or cholesterol in blood. The oil sample or the oil extraction from foods are done using the processes of hydrolysis, methylation to fatty acid methyl esters, and analysis using capillary gas chromatography with flame ionization detection (GC-FID) [20–22]. The analytical methods for testing for fatty acids in food [23] and EPA and DHA in fish oils are available on the TFDA website [24]. Both of these methods are catalyzed by boron trifluoride methanol solution, whereas they have different internal standards and separation conditions.

2.3. *EPA ethyl ester and DHA ethyl ester in fish oil*

In compliance with the regulation of standardized fish oil health food, the contents of EPA and DHA are regulated in the range of 30–50% and the daily intake allowance of them are summed up in the range of 1–2 mg/g in triacylglycerol (TG) form [25]. The size exclusion method is applied to distinguish the glyceride forms of oil [26]. EPA and DHA exist in human body in triglyceride form naturally, rather than the ethyl ester form from refined processing [27,28]. Therefore, the mild catalyzation with tetramethylammonium hydroxide (TMAH) is applied to identify and quantify EPA ethyl ester and DHA ethyl ester in fish oil [29,30].

2.4. *Catechins*

Catechins are a group of flavonoid compounds with some derivatives, containing biological activity related to blood lipid adjustment or reducing body fat formation [31–33]. Eleven health foods declare catechins as the effective ingredients; most of the products are tea beverages. An event occurred in 2010, where the catechin content of some tea beverages with the "Health Food" logo were noncompliant with labeling. Therefore, a HPLC method with good resolution of eight catechins (gallocatechin, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, gallocatechin gallate, catechin gallate, and epicatechin gallate) and caffeine is developed and shown on the TFDA website as the recommended analytical method for application and surveillance [34].

2.5. *Polysaccharides*

The polysaccharides is a complicated category because of its diverse structure [35]. In permitted health food products that claim polysaccharides as the ingredients with health care effects, they usually contain ganoderma, mushroom, ginseng, or a mixture of these [7].

The analytical procedures of determination of polysaccharides usually digest these macromolecular compounds by enzymes or chemicals into smaller compounds, which are then detected using chromogenic reactions or chromatographic instruments [36–38]. However, the aforementioned methods do not seem precise enough to coordinate with the efficiency for these compounds. Recently, the analysis of the precise effective parts of polysaccharides was studied. The determination of (1,3/1,6)-β-D-glucan extracted from fungus and yeasts containing immune modulating properties [39–41], interpreted the content and the degree of branching of the branched (1,3/1,6)-β-D-glucan simultaneously without tedious isolation and purification procedures [42,43]. The requirement of effective polysaccharide compounds for

health food applications will be more precise and compliant to keep pace with developments.

In addition, there are further developments on the specific categories of effective polysaccharides described as follows.

2.6. β-Glucans

(1,3/1,4)-β-D-glucan extracted from the bran of some grains, such as oats, is a rich source of the water-soluble fiber (1,3/1,4)-β-D-glucan, which has health claims related to the effects on lowering blood cholesterol and regulating the immune system [44–46]. AOAC methods are widely adopted for the analysis of β-glucan in oats and its related products; the principle of analysis of these methods is hydrolysis by lichenase and beta-glucosidase to glucose, then measurement using a glucose oxidase–peroxidase buffer mixture [47]. The absorption response increases as the glucose amount increases. This method was found to be applicable for the measurement of β-glucan in cereal products containing high levels of glucose after pre-extraction with aqueous ethanol. However, there is no description of the definition of high glucose levels or the concentration of ethanol used. One study demonstrated that the detected amounts of β-glucan in oat products had significant differences when more than 5% glucose was added. In this situation, pretreatment with 50% ethanol resulted in steadier data. Added sucrose or milk powder in oat products did not influence the determination of β-glucan [48].

2.7. Inulin

Inulin is a heterogeneous complex of fructose polymers, and its effect is related to the colonic microflora, gastrointestinal function, or immune system regulating [49,50]. An enzymatic and spectrophotometric collocation method is recommended to measure the fructan in foods [51], as according to theory inulin is excluded while other sugars can be hydrolyzed with enzymes (sucrase, α-amylase, maltase, and pullanase) then reduced to sugar alcohol, the remaining fructan hydrolyzed by fructanase (*exo*-inulinase plus *endo*-inulinase) and measured by the *p*-hydroxybenzoic acid hydrazide (PAHBAH) collocation method for reducing sugars.

2.8. Total dietary fiber

The definitions of dietary fiber have been discussed in depth [52–54], the common definition by the American Association for Clinical Chemistry (AACC) is "dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine". The analytical methods of dietary fibers depend on their scope of application, as analysis of the dietary fiber can refer to insoluble high molecular fiber [55] or soluble low molecular compounds [56–58].

For example, some food products containing resistant maltodextrin, which have health claims regarding maintenance of normal blood low density lipoprotein cholesterol concentrations or serum concentrations of triglycerides, and changes in bowel function [59], are usually analyzed with AOAC methods, in which the total fiber is calculated as the sum of insoluble dietary fiber measured by enzymatic-gravimetric method plus the high molecular weight soluble dietary fiber and low molecular weight resistant maltodextrin measured by an HPLC method [56–58].

2.9. Oligosaccharides and sugar alcohol

Oligosaccharides such as fructooligosaccharides, isomaltooligosaccharides, and galactooligosaccharides are claimed to improve gastrointestinal function [60–62]. The analytical methods for determination of oligosaccharides include enzymatic-colorimetric and HPLC methods, and comparison with specific standards [52,63–65].

Health foods containing xylitol as a sugar replacement have health claims for maintaining dental health [66], and may be analyzed using HPLC [67].

2.10. Amino acids

Amino acids are not only the building block of proteins, but also are involved in the nutrition metabolism and oxidative defense [68,69]. Some amino acids such as alanine, arginine, glycine, isoleucine, L-arginine, leucine, taurine, and valine are listed as the effective ingredients or the quality control index compounds in the permitted health food products, the health claims of which are related to antifatigue or immunity. The analyses of amino acids are usually detected by using either HPLC-UV or an amino acid analyzer after derivatization. The other choices are to analyze directly with LC-MS/MS [70–72].

2.11. Terpenoids

Some products containing ganoderma, ginseng, fuling, *Taiwanofungus camphoratus*, or other mushrooms claim the terpenoids (ganoderic acids, ginsenosides, lanostanes, and so on) as the ingredients with health care effects in health food applications [4]. These health foods show various health benefits, such as the immunostimulating activities via induction of cytokines and enhancement of immunological effectors, and protection of liver injury induced by tetrachloromethane. Most of these specific terpenoids are analyzed using HPLC-UV or LC-MS/MS [73,74]; however, some of them appeared as possible triterpenoids by using spectrophotometry [75].

3. Conclusion

The analytical methods for the determination of the efficacy of compounds in health foods are published on the TFDA on an ongoing basis. However, they are still not adequate for all the various types of applications. According to the self-management of food businesses, the control of raw materials is as important as the analysis of final products. As well as adopting the methods for health food analysis recommended by the TFDA or recognized by international authorities, each applicant can also develop an appropriate method following the validation guidelines [76,77]. More efforts are needed to develop validated analytical methods for the consistency of labeling of these products.

Each application of a health food should be accompanied by the appropriate analytical methods used to measure its ingredients or specific compounds that have health care effects, for the sake of quality assurance. The self-management for each product of applicant is required for the regulation, the reputation of its own, and the social responsibility to the consumers.

Conflicts of interest

The author has no conflicts of interest to declare.

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