

## REVIEW ARTICLE

# Fish Hydrolysates: A Regulatory Perspective of Bioactive Peptides

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**Abstract:** For the first time introduced on the Japanese market, bioactive fish hydrolysates are now available all over the world as food supplements, functional food ingredients or nutraceuticals. They are generally produced from low value fish waste, an almost inexhaustible source of raw material, and are sold as high value products, making them economically interesting from a manufacturer's view point. Most of these products have health or structure/function claims on their packages with different actions like antihypertensive, blood-glucose lowering, anxiolytic, and skin anti-aging activities. Although the different regional legislations all aim to assure consumer safety and prevent misleading of the consumer, the number of legally approved fish hydrolysate containing products drastically differs among different regions. This is because products that have been positively evaluated based on safety and efficacy in one region were found to have not enough evidence for efficacy in another region. These findings call for further international harmonization of the regulation and classification of these products. Moreover, interaction studies of these bioactive products with the normal diet or medicines are generally not performed, keeping the consumer uninformed of the possible risks of combining these products with medicinal products or other food ingredients.



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## INTRODUCTION

Today, much interest is given to the use of fish waste as a source of bioactive compounds. In 2014, the world capture fisheries and aquaculture production was 167.2 million tons and keeps increasing every year [1]. However, a significant part of the biomass, ranging from 30-60%, depending on the type of seafood and processing method, is not used for consumption. These parts, including carcasses, heads, intestinal organs, fins and skin, are used as animal feed or crop fertilizer, or are discarded without processing [2, 3]. As the worldwide fishery resources are declining due to overfishing, there is a general trend in fish waste recuperation and processing as nutritional source but also as a source of bioactive molecules.

Different companies are focusing on the production of fish hydrolysates by processing protein rich fish materials with acids, bases, heat, or more often with exogenously added or endogenously produced enzymes. Depending on the used fish material, enzyme, and the reaction conditions, a variety of different peptides are produced which can be purified, for example, by membrane filtration or chromatography

[2]. A general flow for the production of fish hydrolysates by enzymatic hydrolysis is as follows: after mincing the protein rich fish waste with water and homogenizing it, enzymes are added and the digestion is started. When the desired grade of cleavage is obtained, the enzymes are inactivated by altering temperature or pH. The oil-water mixture is then centrifuged and/or filtered to remove the oil phase and/or insoluble particles and consequently, the water fraction is removed by drying (e.g., lyophilization) resulting in the dry fish hydrolysate powder [4].

Today, several of these peptide mixtures isolated from fish hydrolysates are marketed worldwide or studied for their favorable actions in several health domains like cardiovascular, dermatological, neurological, and metabolic areas. The search for fish-derived food products with activity in these domains receives much attention because of the high incidence of lifestyle and aging-related diseases in the Western society. Therefore, this review gives an overview of the fish hydrolysates currently available for the consumer, including their claimed beneficial effects on health. Furthermore, the existing regulations and classifications regarding these food-derived products with nutritional, physiological, and pharmacological activities are evaluated and suggestions for improvement in their legal-regulatory classification are made.

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## THE USE OF HEALTH CLAIMS ON FOOD PRODUCTS THROUGH THE WORLD

Function foods, including food supplements, with health claims concerning cardiovascular, neurological or metabolic activities are available for the consumer. Examples include fish oil rich in omega-3 fatty acids, fermented red rice, garlic containing products, polyphenols from green tea and coenzyme Q10 [5]. Since the implementation of Regulation (EC) No. 1924/2006, the use of health claims on food products in Europe is better regulated and more harmonized [6]. The Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) of the European Food Safety Authority (EFSA) has provided guidelines to manufacturers and suppliers of food supplements and functional foods for placing health claims on their products. These guidelines include that the provided claims need to be well substantiated and a cause-effect relationship demonstrated (i.e., 'generally accepted scientific evidence') [6, 7]. Although the evaluation of these health claims is beneficial for the consumers' safety, the stringency has resulted in a very low success rate of accepted claims. Especially the demand for generally accepted scientific evidence is not easy to fulfil. Taking the different scientific opinions on the substantiation of health claims of food products reported in the EFSA journal into consideration, the weakness of some studies (such as not double-blinded, animal and *in vitro* studies, ...) can be identified as the main reason of failure. A well-founded clinical trial design is thus of the utmost importance.

Regulations about health claims on food are also available in Japan and the United States of America (USA). The Japanese Ministry of Health, Labor and Welfare (MHLW) introduced the Foods for Specified Health Uses (FOSHU) system, which is an approval system for the regulation of all health claims on packages of food products launched in Japan [8]. This status is granted to food that contains ingredients with a proven beneficial effect on health and a demonstrated safety. FOSHU-approved products can put physiological claims on their packages which must be priority approved by MHLW [8]. Furthermore, these products can also put the FOSHU label on their package, which can influence the consumers' purchasing behavior and is therefore an important marketing factor. The use of these labels is unique compared to other regions and countries in the world [8, 9].

The US Government proclaimed the Nutritional Labeling and Education Act (NLEA) to regulate health claims and food labeling. Such health claims describe a relation between food, a food component, or a dietary supplement ingredient and the reduced risk of a disease or health-related condition [10]. The authorization of a claim is generally triggered by the submission of a health claim petition and the evaluation is performed based on an extensive scientific literature review [8, 10, 11]. Next to the health claims, which are to be pre-approved by the food and drug administration (FDA), structure/function claims can also be placed on the packages of food or dietary supplements in the USA. The structure/function claims are regulated under the Dietary Supplement Health and Education Act of 1994. They define the effect of a dietary supplement on the structure or function of the body e.g., 'helps promote bone health'. These claims do not need to be pre-approved by the FDA and must be ac-

companied by the following disclaimer: "This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease"[10, 11]. The main difference between health and structure/function claims can be illustrated using the food ingredient fiber: "Three grams of soluble fiber from oatmeal daily in a diet low in saturated fat and cholesterol may reduce the risk of heart disease. This cereal has 2 grams per serving." is accepted by the FDA as health claim: there is a clear effect relationship between the use of this food ingredient and the prevention of the heart disease. Compared to the claim "fiber maintains bowel regularity", which is a structure/function claim describing the relationship between the intake of a food ingredient and the structure or function of the human body [10, 12]. Most of the dietary supplements containing fish hydrolysates in the USA, e.g., Stabium 200, Vasotensin, PepTACE (all trademarks), have claims that fall in the structure/function class.

## MARKETED FISH HYDROLYSATES

The market of food supplements and functional foods is growing significantly in recent years. In a study of Leatherhead Food Research, the global functional food market is forecasted to grow to € 43 billion (US\$ 47 billion) in 2017, which represents a 25% increase compared to 2013 [13]. The same growth is seen for dietary supplements, which had an estimated market of US\$ 32 billion only in the USA in 2012 [14]. This growth is related to the consumer's awareness of the role of food in the prevention and development of diseases like obesity, diabetes, cardiovascular diseases, osteoporosis, intestinal cancer, *etc.* Today, Japan remains the most important player with 40% of the total global food market. In 2013, 1095 FOSHU brands were marketed with a market size of ¥ 6275 billion (US\$ 63 billion). Products with claims related to 'intestinal and lactic acid bacteria' were the most important (60.5%) followed by products with claims concerning 'neutral fat and body fat' (22.4%) [8].

Although today, Japan remains the leading market, the high occurrence of obesity in the USA will be one of the main incentives for a shift from the East to the West. Furthermore, the immense healthcare costs, related to these diseases have also motivated the governments in their promotion of the use of functional foods [15].

Currently, fish hydrolysates, in contrast to hydrolysates of milk and soy proteins, correspond to a small share of the food supplement and functional food consumption [16]. Although already popular in Japan, also in Europe and the USA, more and more fish hydrolysate containing products are becoming available for the consumers under different trade names. A typical example is the antihypertensive product Valturon. Valturon, a sardine muscle digest preparation with angiotensin converting enzyme (ACE) inhibiting properties, was first marketed in Japan by the company Senmi Ekisu Co. The product obtained the FOSHU status for blood pressure regulation. Valturon is incorporated as supplement in health drinks, vegetable juices and beverages. In 2010, the EFSA-NDA panel decided that the product is safe to use as a novel food ingredient, i.e., defined as food that has not been consumed to a significant degree by humans in the EU prior to 1997 and regulated by Regulation (EU) 2015/2283, at a

level of 0.6 g/serving [17, 18]. Today, dietary supplements containing Valturon are easily available through the internet all over the world. In 2011, Valturon meeting food-grade specifications and manufactured according to cGMP was self-affirmed as generally recognized as safe (GRAS) (USA). It is used as an ingredient in a variety of food products at levels up to 30% or 0.6 g per serving [19]. Next to sardine peptide hydrolysates, also hydrolysates of other fish, like the bonito fish, are known for their antihypertensive effects and are available as dietary supplements on the Japanese (Peptide ACE 3000), American (Vasotensin and PeptACE) and Canadian (Levenorm) market [20].

The health claims on marketed fish hydrolysates are not limited to antihypertensive activities. As can be seen in Table 1, there are also products available, which may alter high blood glucose, stress, skin youth conditions, etc.

The products listed in Table 1 can be classified according to the claimed physiological activities. Stabilium 200, Serenlider, and Procalm claim to possess anxiolytic properties and are considered as beneficial for mental health and stress conditions. The active ingredient in Serenlider is Protizen, which is a pollock and coalfish digest produced by enzymatic hydrolysis. No information regarding its composition nor any clinical trials have been published, though the manufacturer describes the product as 'mood food' as it is proclaimed to be used in stress relief and provides a natural feeling of well-being while not lowering the attention [27]. Molval, a dietary supplement with claimed beneficial effects on dyslipidemia and cardiovascular risk evolution, contains next to omega-3 fatty acids, the protein hydrolysate Gabolysat PC60. This fish peptide mixture also claims anxiolytic properties. In rats, the product has demonstrated stress responsiveness by influencing the pituitary adrenal axis, the sympathoadrenal activity, and the  $\gamma$ -aminobutyric acid (GABA) content in hippocampus and hypothalamus in a similar way as the anxiolytic drug diazepam [36]. Another marketed fish hydrolysate containing product with anxiolytic properties is Stabilium 200, which contains *Garum armoricum* and is available in the USA. According to the supplier, the product may be useful for reducing fatigue and supporting normal psychological functions, such as memory, concentration, and cognitive abilities [37]. Furthermore, Messaoudi *et al.* found anxiolytic and antidepressant-like effects of *Garum armoricum* in rats. [38]. Like most food supplements, the functional/structural claims and statements of Stabilium have not been evaluated by the FDA.

Several products containing fish hydrolysates have also reported metabolic activities. Slimpro contains the enzymatic hydrolysate of the northern blue whiting fish (*Micromesistius poutassou*) and has been studied for its beneficial activity on body weight. In a randomized placebo-controlled and two-dose treatment study on 120 slightly overweighted subjects (body mass index (BMI) between 25 and 30 kg/m<sup>2</sup>), the product was found to improve the body weight composition and to increase the blood concentration of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) [39]. These two anorexigenic signal molecules are produced by the duodenal I-cells and the intestinal L-cells, respectively, as a response to nutrient ingestion and are thus partly regulating energy homeostasis. Also the cod fish hydrolysate Nutripeptin is

studied for its effect on weight management. However, in a randomized, double-blinded crossover study (n = 7; BMI > 30 kg/m<sup>2</sup>), no beneficial effect of Nutripeptin (3 g of hydrolysate) was found for the measured metabolic parameter absolute fat oxidation rate [40].

Two other important classes are fish collagen hydrolysates, used for their beneficial effect on skin health and photo-aging (e.g. Collactive and hydro MN peptide), and the ACE inhibitory, antihypertensive fish hydrolysates (e.g., Vasotensin, PeptACE, Valturon, Protensin, Tensideal and Katsuboshi), which are discussed more in detail and in relation to their effect and regulatory framework in section 5 of this publication.

### SAFETY ASPECTS OF FISH HYDROLYSATE CONTAINING DIETARY SUPPLEMENTS AND FUNCTIONAL FOODS

As already mentioned, in Europe an application needs to be submitted and evaluated by the EFSA-NDA panel according to Regulation (EC) No. 1924/2006, before any health claim can be used in combination with a dietary supplement or functional food. Although this panel will advise the European Commission about scientific substantiation of the health claim, it does not constitute an evaluation of the authorization for marketing the product, a decision on proper classification of the product as foodstuff nor a positive assessment of its safety in its scientific opinion, which is not foreseen in the framework of Regulation (EC) No. 1924/2006. The decision whether a food ingredient is considered as safe is made after the European Commission requests the EFSA-NDA panel a scientific opinion on the safety of the food ingredient (e.g., in the context of Regulation (EC) No. 258/97). In its evaluation of the safety of the sardine peptide product Valturon, the panel concluded that there is sufficient evidence to consider the product to be safe as food ingredient based on the absence of side effects in the different studies and the fact that the peptide product will be hydrolyzed in the small intestine into single amino acid constituents prior to systemic absorption [17]. However, the arguments used for safety evaluation are in contrast with the FoSHU status granted to this product in Japan. This status was granted based on its safety and on its effect: if no toxicity is expected because of the absence of intact peptides for systemic adsorption, no activity is expected as well. Differences in evaluation between Europe and Japan are also seen for other fish hydrolysates, such as antihypertensive bonito peptides. Bonito is a fish that has been traditionally consumed in Japan and other Asian countries [41]. Its thermolysin digest has antihypertensive activities in animals and on tissues, with the pentapeptide LKPNM being the main active component [41-43]. This hydrolysate gained the FoSHU status in 1997 [42]. However, during its evaluation of the scientific substantiation of the bonito peptide related health claim 'maintenance of normal blood pressure', the EFSA-NDA panel decided that no cause-effect relationship was demonstrated between the consumption of bonito peptide (LKPNM) and maintenance of normal blood pressure [43].

In the safety evaluation of food ingredients with claimed bioactivities, the interaction with other products, most importantly medicines, should be considered as well. The

Table 1. Market products containing fish hydrolysates.

Product name/ trade name	Company (region)	Source	Production method	Use according to supplier	Form (according to supplier)	Reference
Seacure	Proper nutrition Inc. (USA)	Pacific whiting (cod) muscles	Fermentation by microorganisms (yeast)	Intestinal health	Dietary supple- ment	[21]
Collective	CTPP-Copalis (pro- duction, France) PLT Health Solutions, Inc (USA).	Wild caught fish skins	Not available	Hydration, anti-ageing, anti-oxidant	Supplement, bev- erage and func- tional food prod- ucts Nutricosmeceutical	[22]
Hydro MN peptide (part of Celergen)	Celergen (Switzer- land)	Not available	Not available	Prevention of photo-aging, reduces the dietary glycemic index	Nutritional sup- plement	[23]
Slimpro	Compagnie des pêches Saint-Malo Santé (France) Nutraceuticals Interna- tional Group (USA)	Northern blue whiting fish ( <i>Mi- cromesistius pou- tassou</i> )	Enzymatic hydrolysis	Hunger control by increased production of CCK and GLP- 1	Authorized for applications in food and dietary supplements	[24]
Nivelglu (con- tains Nu- tripeptin)	Tongil (Spain) Nutrimarine Life Science AS, (Norway) (supplier nutripeptin)	Cod	Enzymatic hydrolysis	Help to promote healthy blood sugar levels, can lower postprandial blood sugar to normal levels, helps to regu- late sugar peaks after a meal, helps to improve blood glu- cose control, helps to reduce plasma glucose levels, helps to limit the postprandial glu- cose rise	Dietary supple- ment	[25]
Fortidium li- quamen	Biothalassol (France)	White fish ( <i>Molva molva</i> )	Autolysate	Reducing oxidative stress, lowering glycemic index, anti-stress	Dietary supple- ment	[26]
Procalm	Copalis (France)	White fish	Not available	Improve dog well-being	Veterinary (Ca- nine) supplement	[27]
Stabilium 200 (contains Ga- rum armoni- cum)	Yalacta (France)	Blue ling viscera ( <i>Molva dypterygia</i> )	Enzymatic autolysis	Supports the body's response to stress, provides nutritional support for memory and cognitive function	Dietary supple- ment	[28]
Serenlider (contains Proti- zen)	Naturlider (Spain) Copalis (France)	Pollock Coalfish	Enzymatic hydrolysis	'Mood food' Stress relief action, provides a natural feeling of well-being while not lowering the atten- tion	Dietary supple- ment	[29]
Molval (con- tains Ga- bolysat)	Dielen (France)	Blue ling ( <i>Molva dypterygia</i> )	Not available	Cardiovascular protection and proper functioning of the heart, blood pressure and cholesterol balance, stress support	Nutritional sup- plement	[30]
Valtyron (main active peptide VY)	Senmi Equis Co. (Japan)	Sardine ( <i>Sardinops sagax</i> )	Alkaline prote- ase hydrolysis	Helps to maintain a healthy cardiovascular system	Dietary supple- ment and func- tional food ingre- dient	[19]

(Table 1) Contd....

Product name/ trade name	Company (region)	Source	Production method	Use according to supplier	Form (according to supplier)	Reference
Vasotensin (main active ingredient LKPNM)	Metagenics (USA)	Bonito fish ( <i>Sarda orientalis</i> )	Thermolysin digest	Support healthy blood pres- sure levels	Dietary supple- ment	[31]
PeptACE (main active ingredient LKPNM)	Natural factors (USA)	Bonito fish ( <i>Sarda orientalis</i> )	Thermolysin digest	Natural support to lower high blood pressure, increases blood and oxygen flow to the heart, may improve heart function	Dietary supple- ment	[32]
Protensin	Copalis (France)	Not available	Not available	Inhibition properties of ACE	Dietary supple- ment	[27]
Tensideal	ABYSS' ingredients (France)	Mackerel	Enzymatic hydrolysis	Inhibition properties of ACE	Dietary supple- ment	[33]
Katsuobushi	Nippon supplements (Japan)	Bonito fish ( <i>Sarda orientalis</i> )	Not available	Support healthy blood pres- sure levels already within the normal range	Dietary supple- ment and func- tional food	[34]
Amizate	Zymtech (Norway)	Salmon ( <i>Salmo salar</i> )	Autolysate	Amino acid source in diverse areas as malnutrition, wound treatment, skin care, sports nutrition, wasting diseases, severe trauma following surgery	Dietary supple- ment	[35]

antihypertensive fish hydrolysate peptides work by inhibiting the ACE enzyme, which decreases blood pressure. These fish hydrolysates share their mode of action with some well-known antihypertensive drugs like captopril, enalapril, and lisinopril, all structurally derived from peptides (**Figure 1**). Because of their common target, the interaction of antihypertensive fish hydrolysates with these drugs must be evaluated as well. Long term combination of enalapril and the bonito peptide hydrolysate (LKPNM) resulted in a significantly reduced efficacy compared to enalapril monotherapy in male spontaneously hypertensive rats. The effect of the two substances was thus not synergic but rather opposite (competitive): the hydrolysate partially adversed the effect of enalapril, possibly by interacting competitively with the catalytic site of ACE [44] (**Figure 1**). Similar results were obtained when co-administering dipeptide VY, the main active ingredient of Valturon, and the ACE-inhibitor captopril in spontaneously hypertensive rats. Although single oral administration of captopril (2.5 mg/kg) or VY (25 mg/kg) alone resulted in a decrease of systolic blood pressure of 23 and 20 mm Hg, respectively, **but no significant effect on blood pressure was seen when both** products were combined [45]. The competition for peptide transport 1 (PEPT1), resulting in a decreased systemic adsorption from the gastro-intestinal tract, is found to lie on the basis of the loss in hypotensive effect (**Figure 2**).

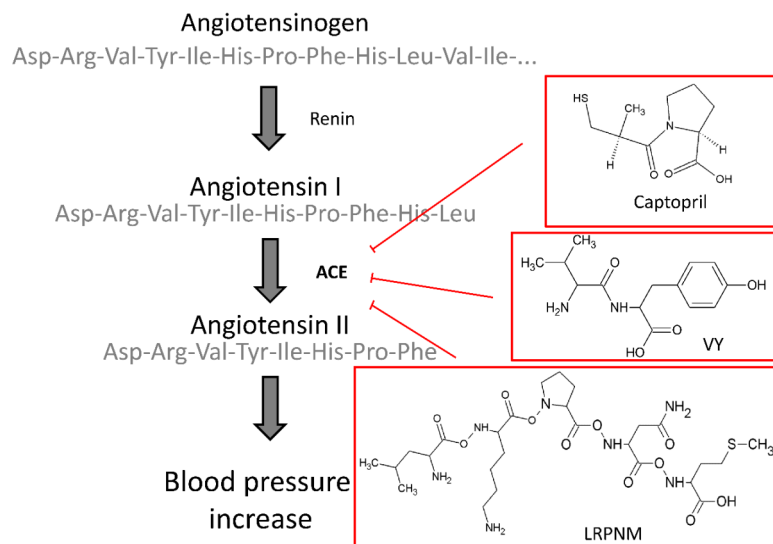
Several fish hydrolysates are available with claimed anxiolytic activities and effectiveness on stress, e.g., Gabolysat PC60 having a similar effect as the drug diazepam in rats

[36, 38]. Unfortunately, the effect of Gabolysat PC60 was only compared with diazepam, whereas the effect of co-administration was not studied. However, it is likely that an interaction between anxiolytic fish hydrolysates and benzodiazepines, or other psychoactive medicines, may occur as interactions are demonstrated between most of the psychoactive medicines as well as with some food ingredients like alcohol.

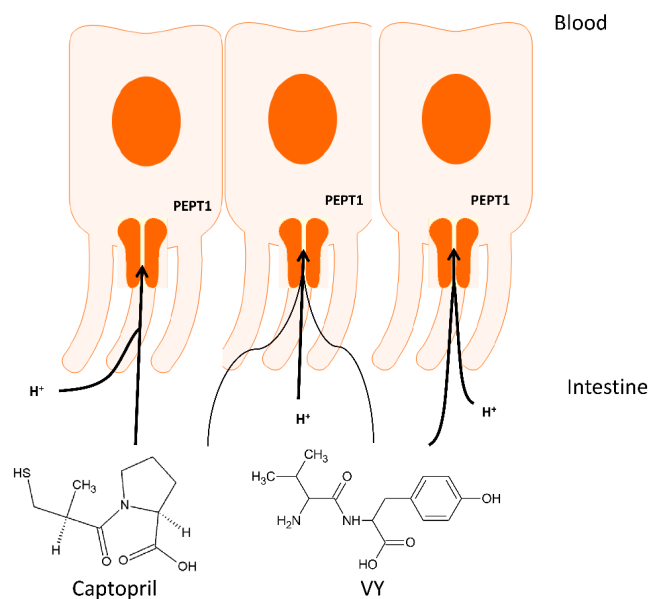
Another important functional group of fish-derived protein hydrolysates influence blood sugar and may have a role in the regulation of blood glucose homeostasis, such as Nutripeptin and Fortidium liquamen. Although no interaction studies of fish-derived peptides and DPP-IV inhibitors like stigliptin have been reported, such studies are available between whey-hydrolysate peptides and stigliptin, demonstrating that a **combination of the two products possess** an additive DPP-IV inhibition, which may result in unwanted side reactions [46].

## REGULATORY ASPECTS OF FISH HYDROLYSATES

User's safety is one of the major reasons, next to the fair competition and free movement of goods, for the European Union to create several regulations defining product classes of bioactive compounds. This classification is based on the product's effect, presentation, use and origin, which offers manufacturers of bioactive products new and interesting opportunities for regulatory classification. Although each



**Figure 1.** Captopril, Valturon (VY) and the bonito hydrolysate peptide (LRPNM) are all inhibitors of ACE, which convert angiotensin I in hypertensive angiotensin II. The simultaneous administration of an ACE-inhibitor (captopril or enalapril) with one of these fish hydrolysates results in activity loss of both components.



**Figure 2.** Captopril and Valturon (peptide VY) are absorbed from the gastro-intestinal tract and reach the systemic circulation by the PEPT1 transporter, co-transporting protons. However, when both products are administered simultaneously, they both influence each other's absorption.

product class has a clear definition with authored, but also obligated aspects, the creation of defined classes automatically results in the creation of borderlines or grey zones, containing products located between - and overlapping different product categories. Classification of products, including borderline products, is carried out by the manufacturer, based on the intended use of the product and verified by the local authorities of the country where the manufacturer intends to

market the product [47]. This is done case-by-case and therefore, especially for very innovative, complex products and because of the different perceptions of these regulations by the national authorities, identical cases may be differently classified. These differences increase the complexity of this matter and the regulatory uncertainty for manufacturers. Two examples are the C-109/12 case, concerning the classification of Gynocaps, i.e. *lactobacillus* containing vaginal capsules, as a medicinal product in one country and as medical device in other countries and the C-140/07 case, concerning the classification of Red Rice 330 mg capsules as medicinal product in one country and as food supplement in other countries [48, 49]. Proper classification by the national authorities should be done based on all aspects of the product and not only on the ingredients' identity without taking into consideration the classification of similar, already approved products.

Especially the border between medicinal products and food supplements is a matter of frequent debates. Both products are dosed in the same pharmaceutical form (capsules, tablets, and oral liquids) and contain bioactive substances. Although the presentational and functional aspects of medicinal products,

*"i.e. presented as having properties for treating or preventing disease in human beings or may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis"* [50]

are different than that of food supplements,

*"i.e. foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect"* [51]

it is demonstrated that borderline cases are unpreventable due to different interpretation of some aspects in the definitions (e.g., physiological effect), similar ingredients (e.g., botanicals), and the same presentation forms [47-49, 52]. In general, a medicine is linked to a disease (which is expected to be handled by the medicine), while a food product is associated with a healthy person (who wants to maintain or improve his healthy state), broadening this legal-regulatory product classification discussion also towards the “disease” semantics, e.g., from which blood pressure or cholesterol blood-level becomes a healthy person a diseased one. A decision needs to be made based on the definition of the product classes, the available studies and evidence, and similar or relevant precedents at the European Court of Justice (ECJ) or domestic courts. Indeed, in those cases with undisputable doubt, the ECJ can be requested to provide judgement. However, whenever the product falls within both the definition of a medicinal product and at least another product class, the strictest directive, i.e., concerning medicinal products, shall apply [50]. The proper classification of a product can also have important implications for the manufacturer. Especially the gap between the requirements for the regulation of a medicinal product and a food product is vast.

Some fish hydrolysates are also located at the borderline between foods and cosmetics. The European Union Cosmetics Directive defines a cosmetic as “any substance or preparation intended to be placed in contact with the various external parts of the human body or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition” [53]. The so called ‘nutricosmeceuticals’ contain food ingredients that have a beneficial effect on the external parts of our body by changing their appearance. Although they contain food ingredients and are presented in capsules, the fact that they have an esthetical action on the skin makes them lean close to the definition of cosmetics. Examples are fish hydrolysates Collactive™ and Hydro MN, which both contain hydrolyzed collagen and elastin protein from fish and are claimed to have an anti-wrinkle action [54]. Hydrolyzed collagen is digested into small peptides and amino acids, which are absorbed and distributed in the human body. When reaching the dermis, the amino acids function as building blocks in the collagen synthesis, whereas the oligopeptides can interact with receptors on fibroblasts and stimulate the production of new collagen, elastin, and hyaluronic acid [55].

Although regional differences exist for the acceptance of health claims on fish hydrolysates (e.g., bonito peptides, Valturon), the question remains if some of these hydrolysates are safe to be used by the consumer as dietary supplement or functional food in an uncontrolled way. The ACE inhibitor fish peptides for example, which interact with the ACE enzyme, share their target with some prescription medicines (e.g. captopril, enalapril, and lisinopril). ACE inhibitory fish peptides can thus be considered as borderline cases of products that are situated between medicinal products and food (dietary supplements and functional food). The classification of these hypotensive products resembles to the Hecht-Pharma case regarding the proper classification of yeast fermented red rice capsules containing 1.33 mg monacolin k,

which is synonymous with lovastatin, an inhibitor of the cholesterol synthesis and active substance in several prescription medicinal products [49]. According to the supplier, the product can be categorized as dietary supplement, because the dose of monacolin k (1-3 capsules a day = 1.33- 4 mg) is too low for exerting a pharmacological effect (compared to 10 – 80 mg as recommended daily dose of lovastatin). The ECJ agreed that the fermented red rice capsules are classified as dietary supplement and stated that “products containing a substance having a physiological effect cannot automatically be classified as medicinal products by function. Apart from the case of substances or combinations of substances intended for the purpose of making a medical diagnosis, a product cannot be regarded as being a medicinal product by function where, having regard to its composition – including its content in active substances – and if used as intended, it is incapable of appreciably restoring, correcting or modifying physiological functions in human beings.”

Some similarities can be found between the Hecht-Pharma case and the hypotensive sardine and bonito hydrolysates: both products originate from processed food with an active ingredient that is structurally related to marketed prescribed medicinal products (statins and ACE-inhibitors) and historically used for their beneficial effects on health in many regions worldwide. However, fish hydrolyzed products tested in clinical studies showed significant hypotensive effects for doses that are the same as those proposed by the suppliers of these products as daily intake [43, 56, 57]. A pharmacological action is thus demonstrated. Furthermore, the available case reports of patients encountering (serious) side-effects after using these ‘pharmacological inactive’ dietary supplements, e.g., fermented red rice, demonstrate that the classification of physiological active products should be done with care [58].

## CONCLUSION

For the first time introduced on the Japanese market more than 25 years ago, bioactive fish hydrolysates are now available all over the world as food supplements, functional food ingredients, and ‘nutricosmeceuticals’. Different actions like antihypertensive, blood-glucose lowering, anxiolytic, and skin anti-aging activities have been demonstrated in animals and humans, but most scientific evidence has not been rigorously controlled by competent authorities making them possibly misleading to the consumer. Using the current regulatory classification systems and regulations, inconsistency in the interpretation and application of these regulations as well as in the requirements for authorization exists. This results in international differences in the number and use of available fish hydrolysates containing products. Interaction studies with normal diet and/or medicines are available only for a limited number of products and they demonstrate that the use of these bioactive fish hydrolysates is not without risk. Currently, these studies are not required and as a result, even for most authorized products, consumer safety is not guaranteed.

## FUTURE CONSIDERATIONS AND/OR RECOMMENDATIONS

In contrast to the international harmonization of pharmaceuticals for human use (e.g., International Conference on

Harmonisation (ICH) guidelines and the international pharmaceutical regulators forum (IPRF)), the harmonization for food with physiological functions (functional food, food supplements) is lagging behind. The existing differences among the USA, EU, and Japan in the evaluation of products with health- and/or functional claims have led to unbalanced numbers of available products in different regions and countries. To ensure the availability of these products to the consumer, having possible beneficial health effects, and to promote international trade eliminating discrimination, while still maintaining an acceptable level of safety and information, a uniform science based assessment should be the basis for the evaluation of the safety and functionality of fish hydrolysates and derived products. Furthermore, as is obligated for medicinal products, the market responsible for these fish hydrolysates must inform the consumer about the possible risk when combining these biologically active substances with medicinal products and/or other food ingredients.

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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