Risk–benefit assessment of seaweed Allergenicity risk assessment of novel protein

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Abstract

As the world population rapidly grows, there is a clear need for alternative food sources, particularly for the provision of protein. Seaweed is one such alternative source of protein that requires greater investigation. In this context, a working programme within the European Food Risk Assessment (EU-FORA) Fellowship Programme framework was developed at National Food Institute – Technical University of Denmark. This Programme is an initiative of the EFSA with the aim to build a European risk assessment community. The purpose of this technical report is to describe the activities in which the fellow was involved. As part of the Research Group for Risk–Benefit, the fellow performed a risk–benefit assessment of seaweed *Palmaria palmata* gaining an in-depth expertise in all the steps. The health impact of *Palmaria palmata* consumption was estimated, considering its high nutritional value but also highlighting concerns towards some components. Simultaneous to the work on the risk–benefit, the fellow also worked within the Research Group for Food Allergy, specifically on the allergenicity risk assessment of a plant-based novel protein (seaweed protein) using different laboratory assays. Seaweed protein digestibility was assessed, and its digestion products were characterised and assessed for immunogenicity. Finally, the fellow collaborated with the Research Group for Microbial Biotechnology and Biorefining in the development of a novel food (alfalfa protein) application dossier to be submitted to EFSA, gaining expertise in the risk assessment of a novel food. In conclusion, the present working programme, together with additional activities and training provided by different institutions, enabled the fellow to gain a broader perspective in food safety, particularly concerning seaweed, novel foods and the safety assessment of novel proteins.

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1.   Introduction

European Food Risk Assessment (EU-FORA) Fellowship Programme is an initiative of European Food Safety Authority (EFSA) with the aim to build European risk assessment capacity and knowledge community. This technical report describes the activities of the programme conducted at National Food Institute – Technical University of Denmark, as follows: (i) Risk–benefit assessment (RBA) of seaweed, (ii) allergenicity risk assessment of a plant-based novel protein (seaweed) and (iii) risk assessment of novel food (alfalfa protein).

1.1.   Seaweed

The world's population is continuously growing and there are studies concluding that total food production should be increased by at least 60% to feed a world of more than 9 billion people by 2050.[1] One of the challenges will be rising food production to meet the protein demand, showing the clear need to find alternative protein sources and to develop means of production that have less negative environmental impacts (European Commission, 2021). Seaweed is considered an alternative source of protein (Leandro et al., 2020).

Seaweed has a high nutritional value due to its content of several dietary components (Leandro et al., 2020) and has been found to be a source of potential bioactive compounds (Holdt and Kraan, 2011). Seaweed is characterised by a high level of protein (up to 47% of dry weight in red species) (Mouritsen et al., 2013), low fat content (0.3–3.8% dry weight) and relatively high concentration of omega-3, omega-6 fatty acids and dietary fibre (Leandro et al., 2020). Seaweed is a source of minerals and vitamins (Leandro et al., 2020), particularly iodine, which is present in significant high concentrations in some species (Roleda et al., 2018) and could be of health concern. Furthermore, seaweeds could also be a source of potential harmful contaminants such as cadmium, mercury, inorganic arsenic and lead, due to their cell wall structure conferring them high absorption properties (Banach et al., 2020). Seaweed allergenicity has not been widely studied, but there are some reports in literature describing allergic reactions to it (Thomas et al., 2019). This toxicological profile has raised concern from several food authorities regarding the exposure to excessive levels of these contaminants upon seaweed consumption (ANSES, 2018; Duinker et al., 2020). EFSA identified seaweed as a potential emerging risk (EFSA, 2017). Therefore, it is a food which requires further investigation.

1.2.   Risk–benefit assessment

A risk–benefit assessment (RBA) is an evaluation of combined adverse and beneficial health effects associated with food in order to inform food safety and public health strategies (Verhagen et al., 2012). It is necessary to perform a RBA for seaweed in order to evaluate its health impact, considering its high nutritional value but also the concerns regarding some of its components. A risk–benefit balance for different intake levels of red seaweed *Palmaria palmata* (L.) Weber & Mohr had not been assessed previously, and therefore, it was included in this project under the EU-FORA Fellowship Programme.

1.3.   Allergenicity risk assessment of novel proteins

Food allergy is considered as an adverse immune response to food (Bøgh and Madsen, 2016). Immunoglobulin E (IgE)-mediated immune reaction (type I), the most common one in food allergy, consists of two phases: (i) sensitisation, whereby the immune system develops hyper-reactivity to the allergen upon exposure without symptoms, (ii) elicitation, clinical manifestations of immune response occur after re-exposure to the allergen, which can be gastrointestinal, respiratory or on skin (Fernandez et al., 2013).

Taking into account that seaweed is considered an alternative source of protein, and there is little information on the potential allergenicity of seaweed protein, it was considered relevant to include a project investigating the allergenicity of seaweed protein within the EU-FORA Programme.

1.4.   Risk assessment of novel foods

Novel foods are defined as foods that had not been consumed to a significant degree by humans in the European Union before 15 May 1997. Their safety for consumers has to be assessed in terms of nutritional composition, microbiology, toxicology and allergenicity (EFSA NDA Panel, 2021). In the

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[1] https://www.fao.org/news/story/en/item/35571/icode/

specific case of alfalfa, it is widely consumed as food, but not as isolated alfalfa protein. Therefore, an application will be submitted to EFSA in order to evaluate its safety for consumers as novel food. The fellow collaborated on the development of this dossier as part of the EU-FORA Programme.

2. Description of work programme

2.1. Aims

The aims of the work programme for the fellow were the following ones:

1) Gaining expertise in performing RBA of food. This includes working on a specific RBA case on seaweed as part of the Risk–Benefit Group.
2) Gaining expertise in allergenicity risk assessment of a plant-based novel protein (seaweed) within the Research Group for Food Allergy Group. Introduction to novel proteins allergenicity risk assessment and experimental work were included.
3) Learning about risk assessment of novel foods (alfalfa protein) in collaboration with the Research Group for Microbial Biotechnology and Biorefining, including thorough examination of all aspects of a novel food risk assessment.

The activities described in Section 2.2 are aligned with the aims of the programme.

2.2. Activities/Methods

The fellow's main project was to perform the RBA of seaweed, gaining experience in searching scientific databases, performing systematic data extraction from scientific publications and its evaluation, generation of data using dose–response modelling, discussion of results and their presentation and drafting the final output with results and conclusions in an harmonised format. The fellow collaborated with the Research Group for Food Allergy for a period, gaining practical experience in allergenicity assessment techniques. Finally, the fellow collaborated in the development of a novel food application, gaining experience in data extraction and collation and resolving practical issues in the preparation of an application.

2.2.1. Risk–Benefit Assessment of seaweed

Initially, the fellow conducted a literature search including risk–benefit methodology together with previously performed risk–benefit cases in order to understand different steps of the assessment. Following this, the fellow was involved in the development of a specific risk–benefit assessment case on *Palmaria palmata* in collaboration with other members of the Research Group for Risk–Benefit. The overall RBA was based on five stages (Figure 1).
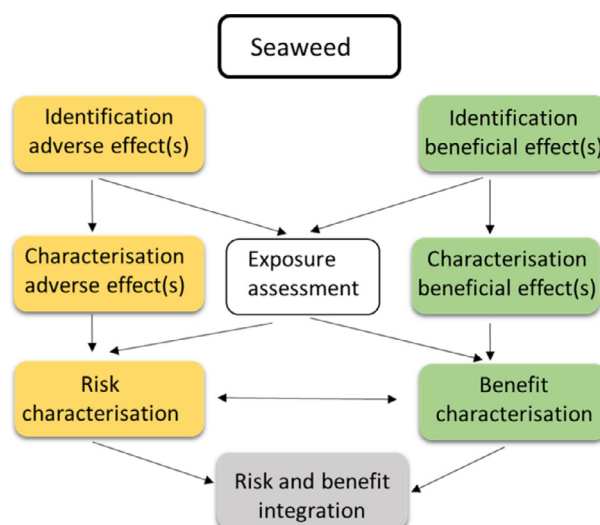
Figure 1: Risk–benefit assessment paradigm. Adapted from EFSA Scientific Committee (2010)

First, the risk–benefit objective was formulated: to quantify the overall health impact in terms of Disability-Adjusted Life-Years (DALYs) of the consumption of seaweed *Palmaria palmata* in the adult Danish population. In order to identify its nutrients and contaminants and their concentration levels, a literature search was performed. The identification of potential health effects was based on assessments from international expert reports, including publications from the Food and Agriculture Organisation (FAO), World Health Organisation (WHO), regulatory agencies (EFSA; French Agency for Food, Environmental and Occupational Health and Safety (ANSES); Food Safety Authority of Ireland (FSAI)), Rapid Alert System for Food and Feed (RASFF) and scientific papers. For hazards and benefits characterisation, a literature search was also carried out to identify systematic reviews for epidemiological studies, including meta-analyses, in order to develop dose–response models. These literature searches were performed in PubMed, ScienceDirect and Web of Science including articles up to September 2021. *Palmaria palmata* nutrients and chemical contaminants and their health effects are summarised in Appendix A.

Three consumption scenarios were investigated, each one compared to zero intake of seaweed (reference scenario). Concentration data of different components were collected from the literature and internal databases, including seaweed samples from different European countries, years and seasons and the Danish food monitoring programme. An average value was calculated for each component taking into consideration the wide variation in their levels.

Mean daily exposure to nutrients and chemical contaminants in each scenario was calculated considering mean daily intake of *Palmaria palmata* and mean concentration of each component in *Palmaria palmata*. For contaminants, it was expressed in terms of μg per unit body weight (70 kg as default), whereas for nutrients, it was expressed in absolute terms. Background dietary exposure was also taken into consideration when dose–response models applied were not considered linear. Data were taken from the Danish National Survey of Diet and Physical Activity 2011–2013 and National Food Institute. Population statistics used in the models were obtained from Statistics Denmark.

The calculated daily exposure to nutrients and contaminants was combined with dose–response models to estimate the size of a given health effect associated with *Palmaria palmata* consumption in the three scenarios. The health effects were expressed and integrated in the composite health metric DALYs. DALY allows for the comparison of different health effects as it integrates the morbidity and mortality of a health effect in a single number, and one DALY is equal to one healthy life year lost (Devleesschauwer et al., 2014). Depending on the availability of data, 'Top-down' or 'Bottom-up' approaches were applied to estimate incidence and DALY of the different health effects (Gibb et al., 2015) (Appendix A). The last step was the integration of risks and benefits expressed in DALYs for each scenario and the comparison between different scenarios, through the calculation of the difference between alternative and reference scenarios (in ΔDALYs). ΔDALY > 0 implied a health loss due to *Palmaria palmata* intake, whereas ΔDALY < 0 meant a health gain. All modelling and calculations were performed using Microsoft Excel.

A manuscript, currently under preparation, includes a comprehensive description of the risk–benefit assessment, details on the risk and benefit characterisation and dose–response models, calculations and results of the assessment. It is expected to be submitted for publication in a peer-reviewed journal.

To be able to carry out this project, the fellow had regular meetings and discussions with the supervisor as well as other members of the group experienced in risk–benefit. The fellow also scheduled meetings with different groups of the Division of Risk Assessment and Nutrition at National Food Institute in order to gather and discuss data such as seaweed composition, consumption, health effects, etc.

2.2.2. Allergenicity risk assessment of seaweed protein

Initially, the fellow performed a literature review including novel protein allergenicity risk assessment in order to understand the approach to be followed. There are no validated methods to assess novel protein allergenicity at present. *In vitro* digestibility tests can provide information on the susceptibility of a protein to digestion, which can simulate its digestibility in the human gastrointestinal system and subsequent presentation to the host's immune system (Foster et al., 2013). Historically, pepsin resistance test has been considered an assay for allergenic potential assessment within a weight-of-evidence approach (Astwood et al., 1996; Codex Alimentarius Commission, 2009; EFSA GMO Panel, 2017). However, the predictability value of this test has been controversial because there is evidence showing that digestion may cancel, reduce, increase or not induce an effect on the

allergenicity of food allergens (Bøgh and Madsen, 2016). The main limitation of the pepsin resistance test is the difficulty to mimic human gastric digestion physiological conditions. Therefore, EFSA recommends using *in vitro* digestibility methods where human digestion process conditions can be simulated (gastric and intestinal digestion) (EFSA GMO Panel, 2017). Nevertheless, the classical pepsin resistance test provides information on stability of proteins including biochemical and physico-chemical properties contributing to the overall safety assessment of the novel protein (EFSA GMO Panel, 2021).

These limitations highlight the necessity of immunological tests (enzyme-linked immunosorbent assay (ELISA), immunoblots) in order to assess allergenic potential of food allergens and their digested products (Bøgh and Madsen, 2016). In order to be considered a complete allergen (in type I food allergy), a protein has to fulfil these properties: IgE-binding ability, allergic reaction elicitation and *de novo* sensitisation capacity, although not all allergens are complete allergens (Aalberse, 2000). This means the allergenic potential of digested products needs to be assessed on those levels in order to evaluate the effect of digestion on allergenicity (Verhoeckx et al., 2019).

In this context, the fellow carried out a 'hands-on' project on allergenicity assessment of plant-based protein from different red and brown seaweed species. Previously, the fellow performed the Chemical Risk Assessment relevant to the assays to be carried out. This project required writing of protocols for each assay, assay optimisation and presenting the results in the Research Group for Food Allergy meetings.

With the purpose to assess resistance to enzymes as a parameter in allergenicity assessment of novel proteins, the fellow performed *in vitro* digestibility tests with red and brown seaweed simulating gastrointestinal conditions. The protein profiles of intact protein and digestion products were characterised using electrophoretic techniques, which allowed them to separate peptides according to their size. Afterwards, immunoblots were carried out in order to assess the immunoreactivity of seaweed protein and its digestion products, and ELISA to evaluate the levels of animal antibodies reactive to these proteins (Figure 2). Results showed differences in protein digestibility of different seaweed species. Limited methods and results are presented in this report in order not to compromise the future scientific publication under preparation.
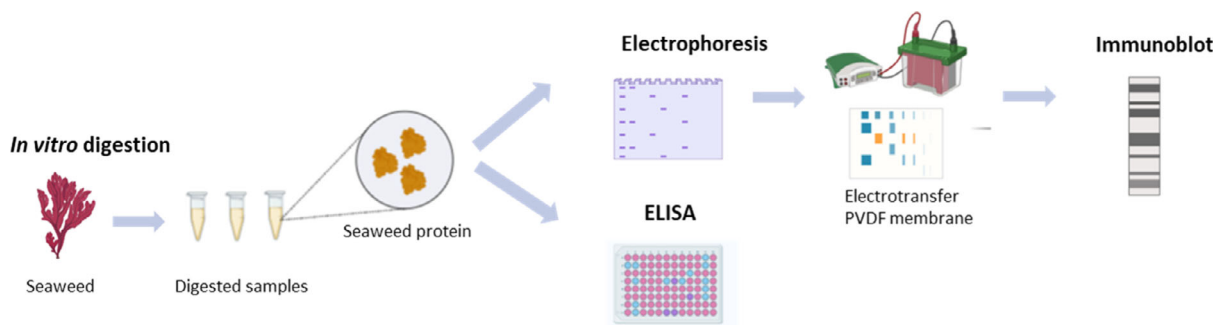
Figure 2: Stepwise approach followed in seaweed protein allergenicity assessment: seaweed protein *in vitro* digestion, protein characterisation throughout electrophoretic techniques and immunochemical techniques (ELISA and immunoblot). Pictures obtained from BioRender

2.2.3. Novel food application

The fellow collaborated with the Research Group for Microbial Biotechnology and Biorefining in the development of a plant-based novel food (alfalfa protein) application to be submitted to EFSA. Initial literature searches were performed including 'EFSA Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283' (EFSA NDA Panel, 2021) and scientific outputs (Ververis et al., 2020). After gathering all data requirements for the submission of novel food applications, several meetings took place where the fellow provided support and assistance guiding the responsible members of the project through the data requirements. Different practical aspects and the approach to be taken were also addressed. This task also included the exchange of knowledge with the supervisor, the professor and the PhD student in charge of the project.

2.2.4. Additional activities

During the EU-FORA fellowship, the fellow had the opportunity to take part in the following activities:

- Preparation of a manuscript including results from the RBA of seaweed and collaboration in the drafting of a manuscript on allergenicity risk assessment of seaweed.
- Scientific division seminars: lectures including a wide range of projects within the area of food research.
- Weekly group meetings: discussion of ongoing projects and presentation of results.
- EUROTOX Virtual Congress 2021.
- 9th BfR-Summer Academy 2021: online training course on risk assessment and risk communication in the area of food safety provided by The German Federal Institute for Risk Assessment.
- WHO webinar: Burden of foodborne diseases: how can we estimate it and why do we need it?
- 'Bridging European Science III' event in Copenhagen: international forum for scientific interactions between universities, companies and the public sector in the Nordic countries and Spain.
- National Food Institute - Technical University of Denmark Conference: digitalisation.

3. Conclusions

The work programme carried out at National Food Institute – Technical University of Denmark provided the fellow with expertise in RBA, novel foods and allergenicity risk assessment of novel proteins. Through participation in the research group for risk–benefit, the fellow gained broad 'hands-on' experience in performing a RBA and obtained deep knowledge through collaboration with colleagues experienced in different areas. Through collaboration with the research group for food allergy, the fellow was challenged to learn laboratory assays in the area of novel protein allergenicity assessment and to apply them in the assessment of specific seaweed protein samples. The outputs of the risk–benefit assessment and allergenicity assessment will be published in relevant peer-reviewed journals. At the same time, the fellow worked on the development of a novel food application. During this work, the fellow expanded the knowledge in plant-based novel foods and proteins, and got a thorough insight of the requirements for their risk assessment.

In addition to these projects and activities developed at the hosting site, the trainings provided by the different institutions throughout the programme were of great value in the learning process of the fellow. Therefore, EU-FORA programme was an excellent opportunity to promote knowledge exchange and future collaboration opportunities. The National Food Institute – Technical University of Denmark made it possible for the fellow to gain a broader perspective and contribute to food safety assessment, in a multicultural and pleasant atmosphere.

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Abbreviations

ANSES	French Agency for Food, Environmental and Occupational Health & Safety
DALY	Disability-Adjusted Life Year
DHA	Docosahexaenoic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
EU-FORA	European Food Risk Assessment
FAO	Food and Agriculture Organization
FSAI	Food Safety Authority of Ireland
IgE	Immunoglobulin E
IQ	Intelligence Quotient
PIF	Population Impact Fraction
RASFF	Rapid Alert System for Food and Feed
RBA	Risk-Benefit Assessment
RR	Relative risk
WHO	World Health Organisation
YLDs	Years lived with disability
YLLs	Years of life lost

Appendix A – Risk-Benefit assessment of seaweed

A.1. Components and health effects associated

Table A.1: *Palmaria palmata* nutrients and chemical contaminants of interest included in the RBA

Component	Health effect	Reference
Lead	↑ Intellectual disability (lowered IQ)	EFSA CONTAM Panel (2010)
Cadmium	↑ Chronic kidney disease	EFSA CONTAM Panel (2009a,b)
Methylmercury	↑ Intellectual disability (lowered IQ)	EFSA CONTAM Panel (2012)
Inorganic arsenic	↑ Lung, bladder and skin cancer	EFSA CONTAM Panel (2009a,b)
Iodine	↑ Intellectual disability (lowered IQ)	EFSA NDA Panel (2014)
	↑/↓ Goiter	Yu et al. (2008)
	↑/↓ Thyroid cancer	Franceschi et al. (1999)
Kainic acid	↑ Neurotoxicity	Jørgensen and Olesen (2018)
Docosahexaenoic acid, (DHA), Eicosapentaenoic acid (EPA)	↓ Fatal coronary heart disease	Mozzafarian and Rimm (2006)
Dietary fibre	↓ Fatal coronary heart disease	Reynolds et al. (2019)
	↓ Type 2 diabetes mellitus	
	↓ Colorectal cancer	

↑: increased effect (adverse effect of the component and associated health outcome); ↓: decreased effect (beneficial effect of the component and associated health outcome); IQ: intelligence quotient.

A.2. Disability-adjusted life years (DALY) calculation

A.2.1. 'Bottom-up' approach

It was applied when estimates of current incident cases or DALY of a given health effect irrespective of risk factors were not available. Incidence of disease was estimated due to the exposure using dose–response models (Nauta et al., 2018). The disease burden of each health effect was quantified in DALYs, composed of years lived with disability (YLDs) and years of life lost due to premature mortality (YLLs). YLDs are calculated as follows:

$$YLD_{d,s,a} = AC_{d,s,a} \times D_d \times DW_d,$$

where $AC_{d,s,a}$ is the annual number of incident cases for health outcome d for sex s and age a, D_d is the duration of health outcome d and DW_d is the disability weight for health outcome d. The disability weight can be interpreted as a measure of good health, ranging from zero (perfect health) to one (death) (Devleesschauwer et al., 2014). YLLs are calculated as follows:

$$YLL_{d,s,a} = AD_{d,s,a} \times SEYLL_{s,a},$$

where $AD_{d,s,a}$ is the annual number of deaths due to the health outcome d for sex s and age a, and $SEYLL_{s,a}$ is the standard expected years of life lost for a death for sex s and at age a (Thomsen et al., 2018) (WHO, 2020). DALYs were calculated as the sum of YLD and YLL:

$$DALY_d = \sum_s \sum_a (YLD_{d,s,a} + YLL_{d,s,a}).$$

A.2.2. 'Top-down' approach

It combines epidemiological and incidence data and estimates the number of attributable cases of a certain health outcome due to exposure to *Palmaria palmata* (Nauta et al., 2018). It was applied when disease or DALY envelopes were available.

Dose–response models already available in literature were applied with modifications. When relative risk (RR) estimates were available in literature, dose–response functions were derived following these equations (Barendregt and Veerman, 2010):

$$\beta = \ln(RR_{literature})/dose$$
$$RR = \exp(\beta \times exposure),$$

where $RR_{literature}$ is the relative risk for each health outcome obtained from literature, RR is the relative risk for each scenario and exposure is the intake of each component in each scenario.

In order to calculate the change in DALYs for each scenario, population impact fraction (PIF) was used to estimate the proportion of health outcome cases that could be prevented by the change of the exposure to different components of *Palmaria palmata* from the reference to alternative scenarios (Murray et al., 2003). RR shift methodology was applied, assuming the changes in the RR of scenarios describe the change in exposure (Barendregt and Veerman, 2010), according to the following equation:

$$PIF_d = (RR_{d,ref} - RR_{d,alt})/RR_{d,ref},$$

where $RR_{d,ref}$ is the relative risk of reference scenario for health outcome d and $RR_{d,alt}$ is relative risk of alternative scenario for health outcome d.

The health effect in DALY for each component and health outcome was calculated using the following formula:

$$DALY = PIF_d \times DALY_{GBD},$$

where PIF_d is the population impact fraction for health outcome d and $DALY_{GBD}$ is DALY value obtained from GBD Results tool.