An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

# 2019R617 – Blue whiting protein hydrolysates for management of sarcopenia

## Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

## SUMMARY

The overall objective of this project was to investigate the potential of a specific Blue Whiting Protein Hydrosylate (BWPH) as a functional food ingredient which could be used in the prevention and management of sarcopenia, a debilitating medical condition primarily associated with ageing. A range of BWPHs were generated and characterised in-situ using a novel cell-based assay system and tested for their ability to promote anabolic signalling of a key pathway associated with muscle protein synthesis (MPS). This allowed the identification of a specific BWPH for inclusion in a two site (UU and UL) double blind placebo controlled human intervention study. This BWPH was generated at large scale under GMP conditions and was formulated into an acceptable format based on taste, smell and consumer acceptability.

The human intervention study in free living older adults, which was carried out across two sites (UL and UU). The primary aim of the study was to determine the effect of the BWPH on body composition and musculoskeletal health, however, importantly this intervention study also determined the acceptance of the subjects to consuming the BWPH as part of their everyday life.

Specific peptide sequences were identified using mass spectrometry combined with a bioassay guided fractionation strategy within the BWPH sample employed in the human study. Synthetic derivatives of these peptides were generated, these peptides were then presented to muscle myotubes and the mechanism by which they could mediate anabolic signalling related to MPS was elucidated.

The results provide alternative and sustainable protein-derived ingredient development strategies to meet market demands for high quality bio-functional foods/food ingredients targeted at sarcopenia prevention. The outcomes from the project provide the marine industry with an opportunity to add value to an underutilised sustainably sourced fish species, i.e., to blue whiting.

## KEYWORDS

Fish, Hydrolysate, Sarcopenia

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| **ACRONYM** | SarcoBlu |
| **PROJECT COORDINATOR, INSTITUTION** | Prof Richard Fitzgerald, University of Limerick |
| **EMAIL** | dick.fitzgerald@ul.ie |
| **COLLABORATORS, INSTITUTION** | Dr Philip Allsopp, University of Ulster<br>Prof Emeir McSorley, University of Ulster<br>Dr P. Magee, University of Ulster<br>Dr D. Armstrong, University of Ulster<br>Prof Nora O'Brien, University College Cork<br>Prof Catherine Norton, University of Limerick<br>Prof Brian Carson, University of Limerick |
| **PUBLICATION DATE** | February 2024. |

## Section 1 - Research Approach & Results

**Start Date**

01 April 2020

**End Date**

31 March 2023

**Research Programme**

Food Institutional Research Measure

**TRL Scale**

TRL 3: Experimental Proof of Concept

**NRPE Priority area**

Food for Health

**Total DAFM Award**

€526,030.32

**Total Project Expenditure**

€450,136.18

**Rationale for undertaking the Research**

Fish is a source of high quality protein, i.e., it has a good balance of essential, non-essential and branched chain amino acids. High quality protein ingredients derived from underutilised marine sources such as blue whiting (BW) have potential applications as functional food ingredients to promote optimal health and wellbeing. The literature suggests that branched chain amino acids (particularly leucine) play a key role in stimulating muscle protein synthesis. The project aimed to investigate the potential of BW protein hydrolysates (PHs) to beneficially modulate markers of sarcopenia, a debilitating condition associated with muscle loss mainly in the elderly. In Ireland, BW is an example of an underutilised fish species having a high protein content (approx. 19 % (w/w)). However, due to its small size and bony structure, it is not readily amenable for consumer utilisation. Furthermore, due to recently enacted EU Fisheries landing obligations, underutilised fish species (such as BW) when caught at sea must now be landed. Therefore, the development of new added-value streams from BW in the form of BWPHs represents an opportunity to utilise sustainable protein resources for the development of new functional food ingredients. Therefore, this project builds on the capability, knowledge and expertise developed within previous DAFM-funded projects to valorise the high quality proteinaceous components from BW as functional ingredients. Furthermore, no previous studies have investigated the role of BWPH in the management of sarcopenia. Therefore, this proposal aimed to generate scientific substantiation on the potential of BWPHs as functional food ingredients for sarcopenia prevention/management.

**Methodology**

This project was composed of 4 main tasks as follows:

Task 1: Sample Generation, Characterisation and Prioritisation - this involved the in situ evaluation (using muscle myotubes) and selection of the most bioactive BWPHs in terms of their ability to stimulate muscle protein synthesis using an xCELLigence™ platform. The most bioactive BWPH in terms of its ability to stimulate muscle

myotube growth and muscle protein synthesis was produced in large amounts under GMP manufacturing conditions for a human intervention study and was then extensively characterised (protein/nitrogen, lipid, ash analyses, molecular mass distribution and peptide profile).

Task 2: Human Intervention Study - A two site double blind randomised controlled human intervention study investigated the effect of the selected BWPH, in combination with exercise, on measures of body composition and musculoskeletal health in free living older adults.

Task 3: Mechanism of Action Studies - this involved bioassay guided fractionation, peptide identification and bioactive confirmatory studies using myotube cells grown in culture with peptide sequences which were identified using mass spectrometry in the most active fraction.

Task 4: Project Dissemination - this involved both technical and non-technical oral and written communications of the project's outputs.

**Project Results**

A range of BW-SPH samples were generated and characterised in terms of their chemical composition, technofunctional and biofunctional properties. All samples had protein equivalent contents > 70 g/100g with minimal lipid contents. All samples were > 80 soluble, displayed good heat stability on heating at 140˚C, and had favourable apparent viscosity and colour properties. All samples displayed good antioxidative (AO) activity, i.e., using the DPPH scavenging assay. Cell viability analyses (WST-1 assay) of skeletal muscle myotubes (C2C12 cells) when grown in DMEM + 10% FBS showed no cytotoxic effects in the presence of 0.1, 0.5 and 1.0 mg/ml test sample. Cell analysis of the simulated gastrointestinal digestions (SGID) of the BW-SPHs showed that a membrane processed sample displayed highest muscle stimulating effects. This sample was generated at large scale under GMP conditions and had a protein equivalent of 87.2% with minimal lipid and carbohydrate contents. Amino acid (AA) analysis showed that it contained all the essential AAs with a branched chain AA content of 11.2%. Microbiological analysis certified it to be safe for human consumption. This sample was masked with a citrus flavour and sensory analysis showed that reconstitution in a fruit juice led to a sensorically acceptable beverage. Test (12.5 g protein equivalent) and isocaloric control samples where then sacheted. Ethical approval for an 8 week double blind placebo controlled human intervention study was obtained and the study was registered (NCT05356559). The main human intervention study confirmed the safety of consuming 25g of this hydrolysate for 8 weeks in 150 free-living adults (75 UU site, 75 UL site) and it had no negative effects on health biomarkers. Whilst there were no significant differences in body composition noted between the BWPH group and the control group, reductions were noted in mean body weight and body fat percentage in the BWPH group (-4% and -1.77%, respectively), albeit these changes did not significantly differ from the control. Additionally, there was a small reduction in blood pressure, most notably a 4.9% reduction in the BWPH group, and whilst not statistically significant in this study it could warrant further investigation into the antihypertensive properties of BWPH.

Fractionation of the SGID treated BW-SPH sample used in the human study using semi-preparative reverse -phase high performance liquid chromatography (25 runs) yielded 5 fractions. The most active fraction was identified and was assessed in situ using the C2C12 muscle myotube assay. Mass spectrometry analysis of this fraction identified the peptides therein and 22 peptides were prioritised for further study in the in situ assay. Assessment of the anabolic signalling potential, i.e., activation of the mTOR signalling pathway, and muscle protein synthesis (using the puromycin incorporation assay) has allowed identification of the bioactive potential of specific peptide sequences within the complex BW-SPH sample.

The main conclusion arising from this project is that ingestion of a nutrient composition containing a BW protein hydrolysate was shown, for the first time, to be safe for human consumption while supporting muscle mass/function.

## Section 2 - Research Outputs

**Summary of Project Findings**

Many benefits have accrued from this project as follows:

Industry: a lab-scale enzymatic hydrolysis process has been successfully transferred from small to pilot scale

Consumers: a nutrient supplement has been developed & validated to be safe for human consumption and it supports muscle mass/function during exercise, a strategy has been developed to mask the bitter and fishy flavour of the hydrolysate providing an acceptable format for long term ingestion of the nutrient supplement.

Regulatory authorities: the project has shown that a BW-SPH ingredient can be developed which is safe for human consumption and which can be used in conjunction with resistance exercise to support/maintain muscle mass/function. The BW-SPH has also been extensively characterised which is a critical factor for pursuing any claimed benefits to health in the future.

Policymakers: the project has demonstrated that a BW-SPH ingredient can be developed which acts as an alternative sustainable source of high quality protein for human nutrition.

Scientific: the ability of a novel cell-based strategy has been demonstrated in the screening of food protein hydrolysates to indicate their anabolic stimulation potential for muscle cells, a lab-scale hydrolysis process has been successfully transferred to pilot manufacture scale, a flavour masking strategy has been elucidated which allows the long-term acceptance of the BW-SPH for consumers in the 50-70 age category, the project provided high level training for undergraduate, post-graduate and postdoctoral fellows, the project generated data for the submission of peer reviewed scientific manuscripts. The data derived from the human intervention trial will also add to the current evidence base on the role of protein and exercise on muscle strength and function in relation to the prevention of sarcopenia.

**Summary of Staff Outputs**

| Research Output | Male | Female | Total Number |
|---|---|---|---|
| MSc Students | 0 | 1 | 1 |
| Post Doctorates | 2 | 1 | 3 |
| Research Technicians/Assistants | 0 | 1 | 1 |

**Summary of Academic Outputs**

| Research Outputs | Total Number | Details |
|---|---|---|
| Publications in Peer Reviewed Scientific Journals | 2 | 1. Shekoohi, N., Amigo-Benavent, M., da Fonseca, G. W. P., Harnedy-Rothwell, P., FitzGerald, R. J., Carson, B. P. (2023) "A Cell-Based Assessment of the Muscle Anabolic Potential of Blue Whiting (Micromesistius poutassou) Protein Hydrolysates." International Journal of Molecular Sciences 24: 2001.<br>2. Shekoohi, N., Naik, A. S., Amigo-Benavent, M., Harnedy-Rothwell, P., Carson, B. P., FitzGerald, R. J., (2023) " Physicochemical, technofunctional, in vitro antioxidant and |

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| | in situ muscle protein synthesis properties of a sprat (Sprattus sprattus) protein hydrolysate.'' Frontiers of Nutrition. 10:1197274, doi: 10.3389/fnut.2023.1197274. |

**Intellectual Property**

It is currently planned to continue publishing the data arising from the project as part of the public good remit associated with the granting of the funding.

**Summary of other Project Outputs**

| Project Outputs | Details | Total No. |
|---|---|---|
| New Processes | A novel approach using an exCELLigence(TM) platform has been applied to the screening of food protein hydrolysates for their ability to promote anabolic stimulation in muscle myotubes grown in culture. | 1 |
| New Products | A BW-SPH ingredient with muscle mass/function maintenance capability has been developed and validated in a human intervention study.<br><br>Furthermore, considerable improvements have been made in developing a food vehicle to enhance the organoleptic properties and consumer acceptability of BWPH which will greatly enhance the success of future studies and product development. | 1 |

**Potential Impact related to Policy, Practice and Other Impacts**

| Impact | Details |
|---|---|
| Environmental Sustainability | It has been demonstrated that a low value underutilised fish species may be utilised to generate a nutrient supplement capable to supporting muscle mass/function in humans, i.e., converting a fishing discard into a high value food protein ingredient. |
| Socio-Economic | Addition of value to a fishing industry discard may help stabilise rural economies by the provision of new manufacturing jobs |
| Industry | A new process validated for the development of a BWE-SPH which is safe for human consumption and which is shown to help maintain muscle mass/function. |

**Dissemination Activities**

| Activity | Details |
|---|---|
| Seminars at which results were presented | Key scientific results generated to date in the project were disseminated to all project partners and interested industry stakeholders in BII and KI at project meetings which were held virtually on the 28th April 2021, 11th June 2021, 2nd September 2021 and 20th January 2022. |

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| | All research personnel on the project have presented their research on an ongoing basis to laboratory group and departmental audiences. Ms. Niloofar Shekoohi presented an oral presentation entitled 'Physiochemical and in vitro characterisation of muscle protein synthesis potential of blue whiting (Micromesistius poutassou) protein hydrolysates' at the University of Limericks Biological Sciences Department Research and Innovation Day 15th December 2021 (virtual).<br><br>1. A cell-based assessment of the muscle anabolic potential of blue whiting (Micromesistius poutassou) protein hydrolysates, 2022. The International Society for Nutraceuticals and Functional Foods (ISNFF), 2-5 October, Istanbul, Turkey. Presentation delivered by N. Shekoohi.<br><br>2. Blue whiting (Micromesistius poutassou) protein hydrolysate stimulates muscle growth and muscle protein synthesis in C2C12 cells. University of Limerick, Biological Sciences Department, Research and Innovation Day, 7th December 2022. Presentation delivered by N. Shekoohi.<br><br>3. Blue whiting (Micromesistius poutassou) protein hydrolysate stimulates muscle growth and muscle protein synthesis in C2C12 cells, 2022. Conference of the Western European Fish Technologies Association (WEFTA), 17-21 October, Rotterdam, Netherland. Presentation delivered by N. Shekoohi.<br><br>4. Richard J. FitzGerald, invited presentation at the International Symposium on Bioactive Peptides (ISBP2023), Niagara Falls, Canada, Sept 28-29th, 2023 entitled: Marine protein hydrolysates/peptides: beneficial modulation of health enhancement biomarkers.<br><br>5. Richard J. FitzGerald, invited presentation at the International Society for Nutrition and Functional Foods (ISNFF2024) Dec 2023.<br><br>6. Brian P. Carson, invited presentation on "Alternative Protein Sources for Health and Human Performance" at the International Society Exercise and Nutrition Conference (ISENC) 18-20th Dec 2023.<br><br>7. Brian P. Carson, invited presentation on "Novel Protein Hydrolysates supporting health and human performance" at Manchester Metropolitan University (MMU), April 2023.<br><br>8. Philip J. Allsopp. Seminar series for Editor of the British Journal of Nutrition: "The potential for underutilised marine fish as a source of bioactive protein ingredients for human health". Ulster University, 28th April 2023.<br><br>9. Philip J. Allsopp, invited presentation on benefits of fish on human health. FISH (Fish and Seafood for Health) Roundtable event. All-island strategic event to discuss future challenges for the seafood industry with stakeholders from DAERA, marine institute, Safefood, FSAI, FSA, BIM and a range of industry stakeholders. Friday 8th September 2023. |
| Other | 1. The Minister of State at the Department of Agriculture, Food and the Marine with responsibility for Research and Development, Martin Heydon, T.D meets Department of Biological Sciences researchers. Department of Biological Science's (UL) newsletter no 6-June 2022. Biol_Sci_UL: Newsletter No 6, June 2022 (biologicalsciencesul.blogspot.com)<br><br>2. Three minute short talk competition at the international society for nutraceutical and functional foods (ISNFF), 2022. Department of Biological Science's (UL) newsletter no 7- |

December 2022. Biol_Sci_UL: Newsletter No 7, December 2022 (biologicalsciencesul.blogspot.com). N. Shekoohi achieved 3rd place in this competition.

3. Brian P. Carson Blog Post: BEYOND THE SCIENCE: COLLATERAL BENEFITS OF RUNNING LIFESTYLE INTERVENTION STUDIES https://pess.blog/2022/09/28/beyond-the-science-collateral-benefits-of-running-lifestyle-intervention-studies-dr-brian-carson/

4. Guilherme Fonseca Blog post: CAN SUSTAINABLE FISH PROTEIN SUPPORT RESISTANCE EXERCISE IN THE MANAGEMENT AND PREVENTION OF SARCOPENIA? https://pess.blog/2022/01/06/can-sustainable-fish-protein-support-resistance-exercise-in-the-management-and-prevention-of-sarcopenia/

**Knowledge Transfer Activities**

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| **Identify knowledge outputs generated during this project.** | A process has been developed to generate a nutrient composition which supports muscle mass/function in 50-70 year old free living community dwelling volunteers. |
| **Identify any knowledge transfer activities executed within the project.** | A lab scale enzymatic hydrolysis process has been successfully transferred to pilot scale. |
| **List any impacts resulting from the knowledge transferred during the project.** | The generation of a sustainable nutritional ingredient has been valorised as a muscle mass/function maintenance agent. This can enhance the added-value of low value underutilised components in the marine processing industry. |

## Section 3 – Research, Future Strategies & Reference

**Leveraging Metrics**

| Type of Funding | Funding € | Summary Resource |
|---|---|---|
| Exchequer National Funding | €80,500.00 | Department of Education, NI for a post-graduate studentship |

**Future Strategies**

The immediate strategy is to complete the publication of the remaining results arising from the project, i.e., the outcomes of the human intervention study.

Further research proposals have been submitted in conjunction with the UL research team to the recent DAFM FIRM call which aim to leverage the capability, facilities and expertise generated during the course of the SarcoBlu project to valorise other sustainable alternative sources of high quality food proteins.

The Enterprise Ireland Commercialisation Fund will be assessed for the further development/commercialisation of the technology and added value ingredients developed within the present project.

Opportunities for the submission of proposals for EU funding will be investigated to perform basic studies on the mechanism of action of the BW-SPH peptides in stimulation of anabolic pathways associated with muscle protein synthesis.

**Project Publications**


1. Shekoohi, N., Amigo-Benavent, M., da Fonseca, G. W. P., Harnedy-Rothwell, P., FitzGerald, R. J., Carson, B. P. (2023) "A Cell-Based Assessment of the Muscle Anabolic Potential of Blue Whiting (Micromesistius poutassou) Protein Hydrolysates." International Journal of Molecular Sciences 24: 2001. https://doi.org/10.3390/ijms24032001

2. Shekoohi, N., Naik, A. S., Amigo-Benavent, M., Harnedy-Rothwell, P., Carson, B. P., FitzGerald, R. J., (2023) " Physicochemical, technofunctional, in vitro antioxidant and in situ muscle protein synthesis properties of a sprat (Sprattus sprattus) protein hydrolysate.'' Frontiers of Nutrition. 10:1197274, doi: 10.3389/fnut.2023.1197274.