REPORT ON TRAVEL GRANT TO ACQUIRE PROTEIN CHARACTERISATION SKILLS FOR TEACHING AND HELPING THE FOOD INDUSTRY

Awardee: Dr Samson A. Oyeyinka
Affiliation: University of Lincoln, Holbeach Campus
Date: 1-16 June 2024.

A. Executive Summary

This report provides a concise overview of a two-week professional development experience in Manitoba, Canada, involving laboratory research and farm visits. The visit to the Richardson Centre for Food Technology and Research at the University of Manitoba was generously funded by The Farmers Club Charitable Trust in the UK. Hosted by Professor Rotimi Aluko, a leading expert in food protein and peptide chemistry, the primary objectives were to enhance my protein characterization skills to benefit teaching, research, and UK food businesses, and to learn sustainable pulse farming practices applicable to UK agriculture.

During the visit, I engaged in hands-on laboratory work under Prof. Aluko's guidance, toured the Richardson Centre, and visited three farms, including the Ian N. Morrison Research Farm in Carman, Winnipeg. These experiences provided valuable insights into advanced agricultural practices, such as no-tillage methods, stubble retention, crop rotation, and both intercropping and monocropping approaches for pea cultivation.

This short but impactful trip significantly improved my protein extraction and characterization expertise and enabled me to share best practices with students and professionals in protein chemistry and ingredient development. The knowledge gained will enhance my contributions to

academic and industry collaborations, fostering innovation and sustainable practices in food production.

B. Introduction

Proteins are important components of foods including legumes which are essential for immunity, building up cells, catalysation of reactions, provide structure, transport molecules, and may regulate gene expression and cellular activities in the body. Traditionally, the two main sources of proteins are plant and animal, with insect-based proteins being another promising nutritious option. Insect and plant-based proteins, for example, faba beans (FB) are more environmentally friendly with potential benefits for food security and sustainability. The knowledge of protein structure and functional properties is vital for their application in the food industry. Different protein characterisation methods have been widely used to understand the behaviour of proteins under different processing conditions. This is crucial for the industry as it ensures the quality, safety, and efficacy of protein-based products, including pharmaceuticals, and food items. Detailed analysis of protein structure, function, and stability helps in understanding their behaviour and interactions, which is vital for nutritional formulations.

FB are a good source of proteins which are cheaper options to animal protein and can contribute to reduction in global food insecurity. The growing level of food insecurity and projected increase in human population, which may reach about 9.8 billion by 2050 has spurred global interest in plant-based foods (PBF). The PBF market is expected to reach USD 162 billion worldwide by 2030, an increase from USD 29.4 billion in 2020, and FB, which are grown in the UK may contribute to this market niche in no distant future. It is an agronomically resilient crop that can be grown without the need for additional chemical-based fertilisers, while increasing soil fertility and ensuring environmental sustainability through a reduction in carbon-foot print associated with animal protein consumption.

Growing more FB in the UK would contribute to sustainable development goals (SDGs) by enhancing health and well-being (SDG s-3), reducing hunger (SDGs-2), creating employment, and improving the livelihoods of farmers who are involved in the cultivation of FB, hence, contributing to poverty reduction (SDGs-1).

C. Objectives of the Trip

As a researcher within the National Centre for Food Manufacturing (NCFM) at the University of Lincoln (UoL), my research focus in the last 7 years has been mainly carbohydrate chemistry. However, since joining NCFM, I have been engaged in a wide range of research cutting across chemistry and safety of alternative proteins including insect and plant-based proteins as well as food fraud. As an emerging researcher in this field, it is imperative to acquire knowledge in protein chemistry to broaden my expertise in the field. Thus, the primary objective of this trip was to acquire protein characterisation skills to enhance my teaching and research skills and to support food business in the UK. Another important objective of the trip was to visit some farms in Canada growing pulses with the aim to learn best practices on how to increase yield of pulses that will be beneficial to UK farmers.

D. Details of the Visit

I arrived in Canada on the First of June and lodged at Queen Bees Hotel in Winnipeg, the closest to the Richardson Centre for Food Technology and Research (RCFTR) at the University of Manitoba, Canada where my host, Professor Rotimi Aluko is based. The University of Manitoba, located in Winnipeg, Canada, is renowned for its research excellence and diverse academic programs. The university is home to the RCFTR, a leading research facility dedicated to advancing food quality and human nutrition through traditional and innovative food processing techniques. The centre supports the food and agriculture value chain by engaging in collaborative

research and development activities with the food industry and providing services. This is in addition to advancing knowledge in functional foods and nutraceuticals.

On the first day (3rd June 2024), I visited the RCFTR and was received by the Director, Professor Rotimi Aluko, who conducted a tour of the facility (Fig. 1) and laboratory (Fig. 2). The centre is well-known for its research in dry milling and fractionation, oil extraction, protein quality, food functionality, phytochemicals, extrusion cooking and sustainable packaging. This is in addition to clinical trials, glycaemic response testing, food intake testing, metabolic and body composition analysis.

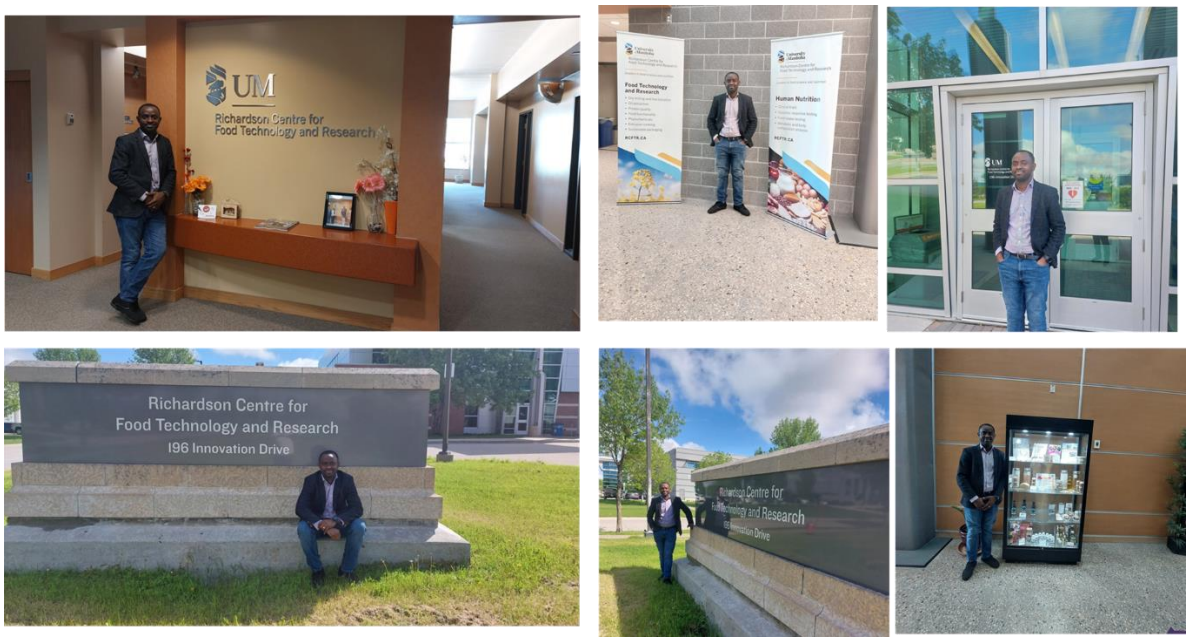

Fig. 1: Arrival at the Richardson Centre for Food Technology and Research, University of Manitoba

Fig. 2: Pictures of some facilities used for protein characterisation.
A: UV spectra spectropolarimeter; B: Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) fractionation system; C: Image scanner for Gel electrophoresis; D: Spectrofluorometer; E: HPLC system

On the second and third day of my visit to the lab, I had hands-on training on how protein hydrolysates are produced from protein isolate or concentrates. Protein hydrolysates are mixtures of amino acids, peptides, and proteins that are derived from the hydrolysis (breakdown) of proteins. This process can be achieved through various methods, such as enzymatic hydrolysis, acid hydrolysis, or alkaline hydrolysis. The resulting hydrolysates are used in a variety of applications due to their unique functionality. For example, protein hydrolysate and peptide fractions have been shown to be display bioactive properties with potentials to reduce high blood pressure and oxidative stress (Arise et al., 2016; Arise et al., 2017). Hydrolysates and peptide fractions of protein have also been reported show potentials as food-based therapeutic agents against metabolic syndrome (Suwanangul et al., 2022).

In Prof. Aluko's laboratory, we used different enzymes such as papain, chymotrypsin, and trypsin for the hydrolysis process. Initially, the protein is extracted using an established method, followed by determining the protein content. For the hydrolysis, small workstations (Fig. 3) are set up with a beaker containing a sample-to-water ratio of 5% (w/v) and an enzyme added at 4%

relative to the protein weight in the sample. The protein isolate is added to the beaker containing distilled water and stirred on a magnetic stirrer heated to 37°C, consistently monitored with a probe. The pH of the mixture is adjusted to the enzyme's optimum pH before adding the enzyme. The sample mixture is allowed to hydrolyse for 4 hours before terminating the process by changing the pH to 4.5 and heating the sample in a water bath at 90°C for 15 minutes. The samples are then immediately cooked down using ice and centrifuged to obtain the hydrolysate in the supernatant. The supernatant can be freeze-dried to obtain the powder before further analysis.

Fig. 3: Production of peptides from protein concentrates or isolates

The fourth day in the laboratory exposed me to the separation of peptides into different fractions using membrane separation process. Protein hydrolysates produced from the above process (Fig. 3) contains a wide range of peptides with

different chain length, hydrophobicity, net charge, and bioactivity. Hence, separation methods such as membrane ultrafiltration and gel permeation chromatography to isolate these peptides into different fractions have been developed (Aluko, 2018). Membrane filtration separates peptides from protein hydrolysates by utilising semi-permeable membranes with specific pore sizes ranging from 500-100000 Da (Fig. 4A). During the process, a solution of hydrolysed proteins is forced through these membranes under pressure. On a laboratory scale, the most common equipment set up uses the Amicon separation chamber with a capacity for approximately 300 mL of sample (Fig 4 B-D). The ultrafiltration system contains an opening to collect the permeate while being flushed by a Nitrogen gas (Fig. 4D). Smaller peptides pass through the pores, while larger protein fragments and undigested proteins are retained. Membrane filtration is preferred over other separation techniques for its ability to handle large volumes, maintain peptide integrity, and operate without extreme temperatures or chemicals that might degrade the peptides. There are two methods to running the set above. The first being permeate collection and second, retentate collection. In the first method, the membrane of smallest molecular cutoffs (MWCO) is firstly used, then the retentate is passed through the membrane with higher MWCO. For the second method (retentate), the process is reversed such that the protein hydrolysate is first passed through the highest MWCO membrane. The permeate is then passed through the next lower sized MWCO membrane until the smallest size membrane has been used. For both methods, an additional step involves adding water, a process called diafiltration, after each run and before the next separation occurs.

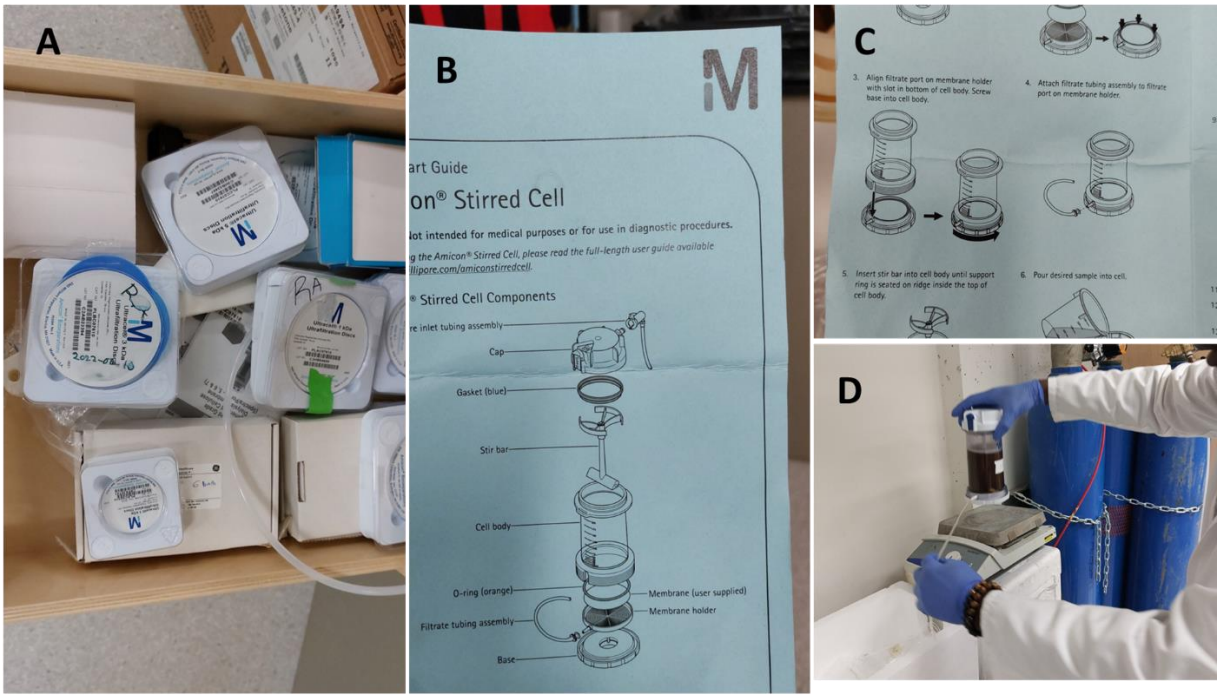

Fig. 4: Pictures of some facilities used for protein characterisation.
A: Membrane filters with different pores; B: Set up manual; C: Fixing the Ultrafiltration system; D: Running the system under nitrogen

Day 5 (7th of June 2024): The activities on this day include, observing defatting of flour using hexane, a pre-extraction step prior to alkaline extraction of protein. This was in addition to repeating the hydrolysate with another enzyme (trypsin) like what was done on day 2 and 3. Furthermore, I was also involved in the measurement of protein solubility using different reagents. Protein solubility is defined as the concentration of protein in a solution at equilibrium under specified conditions of solvent, temperature, pH, ionic strength, and the presence of other solutes. It is an important functional property of protein that determines the use of protein in foods. In most foods such as beverages, foams and emulsions, a protein must be soluble to have functionality. Foaming, gelation, and water binding capacities of protein are also influenced by solubility. The solubility of a protein is mostly determined by its primary structure, i.e., the sequence of amino acids in the protein chain. For example, if a protein has a polar surface due to the presence of polar amino acids, it will have good solubility.

Day 6 (10th of June): On the sixth day, I was privilege to learn the use of the CD Spectrometer and Spectrofluorometer used for secondary and tertiary structure measurement of protein. A standard bovine albumin dissolved in distilled water was used as the reference sample.

Day 7 (11th of June): On the seventh day saw I was involved in protein isolation from pea flour, and I also visited one of the teaching and research farms in the University. Protein isolation from pea flour involves extracting proteins using methods like alkaline or acidic extraction, followed by separation through techniques such as precipitation or filtration. These methods aim to separate proteins from other components like carbohydrates and lipids, yielding a protein-rich fraction. After extraction, proteins are dried and used for varied applications. The laboratory extraction involved defatting of the flour to remove fats which may interfere with the extraction process. The defatted flour was mixed with double distilled water (DDW) at a 5:100 (w/v) ratio and adjusted to pH 10 using 1 M NaOH to solubilise the proteins. Proteins are mostly soluble at alkaline pH and may also solubilise at acidic pH. The mixture was continuously stirred for 1 h followed by centrifugation at 5600× g for 30 min. The supernatant was collected, filtered with cheesecloth (grade 90, 40 × 36 thread count), adjusted to pH 4.5 with 1 M HCl, stirred for 30 min, and then centrifuged. The resulting precipitate was washed with water to remove contaminating non-protein materials and centrifuged again to obtain the final precipitate, which was mixed with DDW and adjusted to pH 7.0 before freeze-drying as the isoelectric isolate (ISO).

After extraction, Prof Aluko and I travelled to the Ian N. Morrison Research Farm in Carman (Fig. 5), a 7200-square-foot space that houses the research farm's field research equipment which is critical to the agronomic programs of researchers, graduate students, trainees, and research collaborators across multiple disciplines. This is the experimental research farm for the faculty of Agriculture at the University of Manitoba.

We had a Postgraduate student on the farm who guided us during the visit. He showed us a farm where he currently grows peas (Fig. 5) and explained that some of the best practises used on the farm for growing peas is crop rotation and leaving crop residues such as pea stubble on the farm after harvest. This includes stems, leaves and any other parts of the pea plant that were not collected during harvest. Stubbles from plants such as peas and rice are very beneficial in several ways. For instance, an earlier report found that incorporation of rice straw into soil increased grain yield to the up to between 15 and 18% compared to burning (Chutiwat, 1997). Furthermore, leaving 10-30 cm standing stubbles of rice in the field has been reported to enhance soil-quality restoration and improvement (Das et al., 2020). Stubbles are rich in organic matter and can decompose over time, improving soil structure and fertility. Furthermore, stubbles can also help with erosion control, weed suppression by acting as a mulch, and moisture retention by reducing evaporation. The student also noted that farmers can either leave the stubble on the surface as mulch or till it into the soil, depending on their specific farming strategy and environmental conditions. The stubble can benefit farmers that have not grown peas before. According to him, wheat, pea, and canola are being rotated yearly so that each crop can benefit from one another. A common approach is to plant wheat on the soil where canola was grown previously, peas go on wheat and canola on peas soil. His research focused was to assess how preceding stubbles from each of these crops (pea, wheat and canola) affected the yield of the crops grown on them. From the initial observations, peas grown on wheat stubble had more effect on yield than other crops. The improved crop yield was likely due to higher soil moisture levels and increased activity of mycorrhizal fungi in the soil. These fungi form symbiotic relationships with plant roots, enhancing water and nutrient absorption, particularly phosphorus, which is crucial for plant health and growth. While mycorrhizal fungi do not directly produce phosphorus, they play an essential role in breaking down organic matter, making phosphorus more available to plants. Additionally, the healthier soil environment

supported by mycorrhizal fungi may have contributed to better nodulation in legumes, indirectly boosting plant development and yield.

Fig. 5: Pictures of Ian N. Morrison Research Farm in Carman, Winnipeg

Day 8 (12th of June)

The farm visits, led by Dr. Daryl Domitruk, Executive Director of Manitoba Pulse and Soybean Growers, provided invaluable insights into diverse management practices tailored to local climate and soil conditions. Dr. Domitruk shared extensive knowledge and provided bulletins, which were delivered to The Farmers Club Charitable Trust and confirmed received by Lisa on July 2, 2024.

Dr. Domitruk emphasised the importance of conservation practices, such as leaving crop residue to prevent soil erosion, conserve moisture, and protect against wind erosion. He highlighted that peas thrive on well-drained land with moderate moisture levels. While some farmers use fertilizers, Dr. Domitruk noted that this is not ideal for long-term soil health. Instead, practices that enhance soil organic matter and carbon content, such as no-

tillage farming, were advocated. This regenerative approach preserves natural root channels, improving water infiltration and retention for crop use.

Crop rotation, an essential practice observed on multiple farms, is instrumental in managing soil-borne diseases and pests. Farmers avoid consecutive planting of the same crop, a strategy confirmed by discussions with a postgraduate student at the Ian N. Morrison Research Farm. Key crops grown in Manitoba include wheat, barley, oats, canola, flaxseed, soybeans, peas, edible beans, sunflowers, potatoes, and vegetables. Although fungicides are widely used to protect crops like peas, which are highly disease-prone, insecticides are less commonly applied.

Dr. Domitruk also discussed challenges with regenerative agriculture (RA). While some farmers adopt RA practices, others dismiss it as impractical or align with traditional organic farming, avoiding affiliation with RA movements. For instance, a farmer demonstrated spraying of fungicides (Fig. 6) to manage canola plants remaining from the previous season's crop, incorporating canola stubble into the soil for nutrients while controlling fungal threats.

Overall, Manitoba’s farming practices underscore a balance between conventional and innovative approaches, with a focus on sustainable crop management and high-yield, disease-resistant, protein-rich pea varieties. These insights offer practical implications for UK agriculture, particularly in enhancing sustainability and productivity.

Fig. 6: Picture of a visit to a Commercial Farmer led by Dr Domitruk
A: Samson Oyeyinka and the farmer; B: Samson Oyeyink, Prof Aluko and a view of the farm and machinery; C: Dr Domitruk, the farmer, a view of the farm and machinery; D: Dr Domitruk showing the nodes of an uprooted pea plant; E: Samson Oyeyinka, Dr Domitruk and Prof Aluko before taking off for the farm visit.

Day 9 (13th of June)

The third visit was to a large-scale commercial farmer managing over 6,500 hectares of land across 35 fields, cultivating peas, wheat, corn, soybeans, and oats (Fig. 7). Crop rotation is a primary strategy employed, complemented by intercropping in some areas and a focus on using well-drained soil. Excessive soil moisture, as evidenced by leaf yellowing, is carefully managed. Weed control is achieved using herbicides. The farmer integrates multiple best practices, including the use of organic manure from his dairy farm to enhance soil fertility. He employs a mix of 50% zero-tillage farming and traditional tillage, spreading manure on the fields and incorporating it into the soil. Unlike some farms, he practices monocropping but utilises a diverse approach by planting three different crop varieties across mapped plots. Residual stubble is also used to improve soil health. Seed procurement is facilitated through partnerships with industry suppliers, who provide seeds and support services, maintaining trust-based

relationships with annual farm visits. Technology plays a significant role in farm management; the farmer uses an app to monitor field locations, crop conditions, and farm operations in real time. The app also connects with chemical suppliers for inputs and enables remote monitoring.

This visit highlighted the integration of traditional and modern practices, showcasing how scale, innovation, and sustainable strategies can coexist in large-scale farming operations. The insights are highly relevant for enhancing agricultural practices in the UK, particularly in improving efficiency and resource management.

Fig. 7: Picture of a visit to a Commercial Farmer

Day 10 (14th June)

The final day was dedicated to a tour of the Richardson Centre for Food Technology and Research, a state-of-the-art facility renowned for advanced research in protein chemistry. The centre is equipped for

sophisticated techniques, including peptide fractionation, metagenomics, and structural analyses, complementing the work conducted in Professor Rotimi Aluko's laboratory.

A notable feature of the centre is its dry protein fractionation system, which separates proteins without solvents, simulating industrial processes. This makes the centre a pivotal hub for developing functional ingredients for industrial applications, working in close collaboration with farmers and industry stakeholders.

The exposure provided by this visit broadened my perspective on research opportunities to enhance the legume farming sector. It underscored the potential to support farmers with nutritional analyses and the development of value-added products, contributing to the creation of healthier food options for the UK population.

Fig. 8: Pictures of different facilities within the Richardson centre for research

E. Impact and Benefits

Upon returning, the training significantly enhanced my understanding of protein structure and functionality, particularly in relation to legumes. I am now proficient in using several advanced analytical instruments, including the spectrofluorometer for analysing surface hydrophobicity of proteins. This instrument, recently acquired by NCFM, has already been utilized in a student training session. The experience has also highlighted the need to acquire additional instruments through future grant applications.

The knowledge gained has directly impacted my teaching and research. For the MSc Agri-Food Programme, I have identified key updates to the New Product Development modules and proposed a dedicated session to share insights from the trip with members of The Farmers Club Charitable Trust. My postgraduate students have greatly benefited, with improvements in protein extraction methods leading to higher yields and increased protein content.

For example, an MSc student noted a significant improvement in productivity using techniques I shared after the trip: "*I had struggled with the extraction of protein isolates before Dr Samson went on the trip. The knowledge he shared was a game changer in my research, saving me a lot of productive hours*."

Additionally, the expertise gained has been pivotal in the recruitment and mentoring of a new PhD student focusing on protein extraction from UK-grown pulses. This training will enrich their research and further advance our collective work in protein chemistry and its application in agri-food systems.

F. Challenges and Solutions

The primary challenge before the trip was the lengthy process of securing a Canadian visa, which took approximately 12 months to approve. During the waiting period, I requested a change in location to South Africa to avoid losing this valuable opportunity. Thanks to the support of The Farmers Club Charitable Trust, this request was granted. Fortunately, before the South African visa was finalised, the Canadian visa was approved.

Another significant challenge was the limited duration of the visit. Protein chemistry is a complex and technical field that requires more time to fully grasp these characterisation skills. Although the allocated funding was appreciated, the time was insufficient to learn as much as I would have liked, highlighting the need for additional support from other funding bodies. Such support could provide more extensive learning opportunities for researchers like me and contribute to transforming both the UK agricultural and educational sectors. To address this, I concentrated on mastering the most crucial technical aspects during the visit and aim to return in the future to further refine these skills. I took detailed notes on each protocol within the lab, which will assist in setting up our laboratory at the University of Lincoln upon my return.

G. Future Plan

Professor Aluko played a pivotal role in facilitating this visit by connecting me with farmers and allowing me to gain protein chemistry experience in his lab. He has extended a hand of mentorship and proposed collaboration with my research group at the University of Lincoln. I am looking forward to returning soon to spend more time in the lab, gathering data that can be published and presented at conferences.

I am also planning to organise a workshop with The Farmers Club Charitable Trust, inviting Dr. Daryl Domitruk, Executive Director of Manitoba Pulse and Soybean Growers, either for an in-person visit or a virtual meeting. This session will provide an opportunity for an engaging and impactful discussion on sustainable farming practices and the potential of pulses as a valuable protein and starch source in the UK.

Furthermore, I aim to continue exploring the potential of various pulses grown in the UK, such as sugar peas, runner beans, faba beans, and French Dwarf beans. This not only supports a sustainable farming system but also establishes a local, sustainable source of ingredients. Reducing the carbon footprint associated with importing legumes and enhancing farmers' incomes and food security in the UK are key goals of this initiative.

H. Conclusion

The visit to the University of Manitoba has significantly enriched my knowledge in protein chemistry, strengthened research capacities, and improved teaching methodologies. This experience aims to produce graduates with a positive impact on the UK agricultural sector. Through the Agricultural Educator Awards, I was able to engage with businesses, particularly in the development of value-added, protein-rich ingredients. Partnerships with food industries and research institutes have been established, fostering a knowledge exchange, and aligning practices across sectors.

Training sessions for research students and industry partners at the University of Lincoln have facilitated skill transfer and boosted research confidence. Enhancements to undergraduate modules and contributions to future program development at NCFM have been made, ensuring that the insights gained from this visit are integrated into our curriculum and benefit the next generation of agricultural professionals.

I. Acknowledgements

I sincerely thank The Farmers Club Charitable Trust for their generous funding and the University of Manitoba Management, especially Professor Rotimi Aluko, for their expert guidance in protein chemistry. I also extend my gratitude to all the research students within the laboratory for their support, training, and guidance during the two-week period. Finally, I appreciate the University of Lincoln for allowing me the time to undertake this invaluable learning experience.

J. Appendices

Detailed itinerary

Date	Activities	Location
1st to 2nd June 2024	Traveling from UK to Canada	In Transit
3rd to 7th June 2024	Visit to the University for induction and laboratory tour	University of Manitoba, Canada
	Hands-on training on protein extraction and characterisation	
11th to 14th June 2024	Visit to pea farmers to glean from their experience in growing FB bean and other pulses	University of Manitoba, Canada
15th to 16th June 2024	Traveling to UK from Canada	In Transit

List of contacts made.

Contacts	Location	Email
Professor Rotimi Aluko	University of Manitoba, Manitoba, Canada	Rotimi.Aluko@umanitoba.ca
Dr Daryl Domitruk	Manitoba Pulse and Soybean Growers, Manitoba, Canada	daryl@manitobapulse.ca
Dr Nandika Bandari	University of Manitoba, Manitoba, Canada	Nandika.Bandara@umanitoba.ca

K. References

Aluko, R. (2018). Food protein-derived peptides: Production, isolation, and purification. In *Proteins in food processing* (pp. 389-412): Elsevier.

Arise, A. K., Alashi, A. M., Nwachukwu, I. D., Ijabadeniyi, O. A., Aluko, R. E., & Amonsou, E. O. (2016). Antioxidant activities of bambara groundnut (Vigna subterranea) protein hydrolysates and their membrane ultrafiltration fractions. *Food & function, 7*(5), 2431-2437.

Arise, A. K., Alashi, A. M., Nwachukwu, I. D., Malomo, S. A., Aluko, R. E., & Amonsou, E. O. (2017). Inhibitory properties of bambara groundnut protein hydrolysate and peptide fractions against angiotensin-converting enzymes, renin and free radicals. *Journal of the Science of Food and Agriculture, 97*(9), 2834-2841.

Chutiwat, W. (1997). Effect of rice straw management on soil fertility and rice yield. *Naresuan J, 1*(3), 30-35.

Das, A., Basavaraj, S., Layek, J., Gandhiji Idapuganti, R., Lal, R., Rangappa, K., . . . Ngachan, S. (2020). Can conservation tillage and residue management enhance energy use efficiency and sustainability of rice-pea system in the Eastern Himalayas? *Archives of Agronomy and Soil Science, 66*(6), 830-846.

Suwanangul, S., Aluko, R. E., Sangsawad, P., Kreungngernd, D., & Ruttarattanamongkol, K. (2022). Antioxidant and enzyme inhibitory properties of sacha inchi (Plukenetia volubilis) protein hydrolysate and its peptide fractions. *Journal of Food Biochemistry, 46*(12), e14464.