13F454 - Beverage formulation/reformulation/reformulation targeted at older population using in vitro assay to design whey protein structure for optimum glutathione (GSH) generation and increased antioxidant potential.

Final Report

SUMMARY

This project delivered nutritional beverages at pilot plant scale. The beverages were based on the dairy protein, whey, formulated with well-established antioxidants (plant polyphenols, vitamins, and marine carotenoids). Pasteurization, ultra-high temperature, or spray drying was used to prepare beverages which were representative of current product formats. Antioxidant activities of beverages and ingredients were determined using ABTS, FRAP and ORAC. A 24-week shelf-life study was performed on final products. In vitro gastrointestinal digestion of beverages and individual ingredients was performed, using the standardised INFOGEST method. Bioavailability studies were performed using Caco2-HT29 monolayers. The project revealed that combining whey with additional antioxidant ingredients increased the bioactivity of formulated products; however, this greater bioactivity was altered after gastrointestinal transit, depending on processing type and antioxidant methodology. The whey protein, a-lactalbumin, had the highest antioxidant capability post gastrointestinal digestion. From a health perspective, b-lactoglobulin and a-lactalbumin were able to protect intestinal cells from free radicals. Several whey peptides were identified capable of crossing the in vitro gut barrier, four of which provided redox protection to liver and muscle cells.

Industrial Impact: Whey protein-based antioxidant beverages could benefit the older consumer to meet their increased protein requirements and boost their dietary antioxidant intake. Our novel combination of plant and marine antioxidants increased antioxidant activity of whey protein-based formulations. Furthermore, to suit export markets this work generated powders by spray drying.

KEYWORDS

Whey, antioxidant, beverage.

ACRONYM WheyGSH

PROJECT COORDINATOR, Dr Linda Giblin, Teagasc. INSTITUTION

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COLLABORATORS, Dr Vitaly Buckin, University College Dublin.
INSTITUTION

PUBLICATION DATE July 2023.

Section 1 - Research Approach & Results

Start Date

01 December 2013

End Date

31 May 2019

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 3: Experimental Proof of Concept

NRPE Priority area

Food for Health

Total DAFM Award

€591,508.50

Total Project Expenditure

€577,614.24

Rationale for undertaking the Research

Bovine whey proteins are considered high quality proteins. They have a well-balanced amino acid profile, are a rich source of branch chain amino acids, have encrypted bioactive peptides and well documented health benefits on muscle. This project aimed to investigate their antioxidant potential. Questions posed included:

- A. Have individual whey proteins antioxidant capabilities?
- B. Can this antioxidant capability survive upper gastrointestinal digestion?
- C. Once consumed, can whey promote antioxidant (i.e., GSH) pathways in gut cells?
- D. Can the bioavailable fraction promote antioxidant pathways in muscle and liver cells?
- E. Can we use the knowledge gained to create a nutritional beverage, at pilot plant scale, rich in protein and antioxidants for the older consumer.

Studying antioxidant capabilities of whey proteins can expand the uses of whey in the 'food for health' sector, particularly foods designed for the older consumer. WheyGSH also meets the consumer need for nutritional products with blended ingredients of dairy, plant and marine.

Methodology

This project was multidisciplinary in nature spanning pilot plant processing to protein chemistry to cellular biology. At project start, individual proteins within the whey fraction were assessed for antioxidant activity by the well-established techniques of ABTS, FRAP and ORAC. The antioxidant bioactivities of individual whey proteins were then tracked as they transited the upper gut using the INFOGEST static model of gastrointestinal digestion. The health benefits of whey peptides to gut calls was evaluated using cellular assays which monitored antioxidant enzymes (glutathione peroxidase, catalase, superoxide dismutase, thioredoxin, peroxiredoxin), free radical production, DPP-IV activity and satiety hormone production. The release of free amino acids during digestion of

whey proteins was determined by Joel JLC-500/V amino acid analyser. LCMS/MS methodology identified peptides released from whey proteins during gastrointestinal digestion. Epithelial cells Caco2 and HT29 were co-cultured and allowed to differentiate into polarised monolayers as an in vitro representation of the gut barrier. These monolayers were treated with whey digesta. Peptides were identified in the basolateral fraction by LC MS/MS. Free amino acids in this fraction were also quantified. The basolateral fraction represents the bioavailable fraction of whey predicting what reaches the bloodstream post consumption of whey protein. A selection of bioavailable peptides were assessed for their ability to promote antioxidant pathways in liver and muscle cells and to promote immune modulation in THP-1 macrophages. Formulated beverages were processed at pilot plant using pasteurization, ultra-high temperature and spray drying. The effect of the addition of Vitamin B12, astaxanthin and marjoram to whey-based formulations was evaluated by particle size distribution, viscosity, dispersion analysis, colour, powder moisture, microstructure, microbiological analysis and shelf-life studies.

Project Results

This project delivered nutritional blended beverages at pilot plant scale. The beverages were based on the dairy protein, whey, formulated with well-established antioxidants (vitamin B12, marjoram (plant) and astaxanthin (marine)). The project revealed that the whey protein, a-lactalbumin, had the highest antioxidant capability post gastrointestinal digestion. Hence our beverage base was composed of 4.4% (w/v) whey protein isolate with an additional 0.1% alactalbumin. Ultra-high temperature processing produced beverages of greater clarity, stability and antioxidant activity compared to pasteurization. Incorporation of additional antioxidant ingredients had no effect on viscosity or instability index but did increase sedimentation and redness. From a health perspective, b-lactoglobulin and a-lactalbumin post gastrointestinal digestion were able to protect intestinal cells from free radical formation and promoted antioxidant enzyme production. Our study identified whey peptides capable of crossing the in vitro gut barrier. Four of these whey peptides were capable of inhibiting free radical formation in muscle and liver cells. Gastrointestinal digestion of blactoglobulin resulted in the highest concentration of free amino acids arriving on the basolateral face of the intestinal barrier with notable levels of branch chain amino acids and sulphur containing amino acids. With in vitro models, this project tracked whey bioactivity from processing, to digestion, to bioavailability to health benefit. WheyGSH research outputs meets the consumer need for nutritional products with blended ingredients from dairy, plant and marine.

Section 2 - Research Outputs

Summary of Project Findings

Benefits to

Industry: WheyGSH delivered nutritional beverages, at pilot plant scale, for uptake by the food industry. These formulated beverages were processed using pasteurization, ultra-high temperature and spray drying. The beverages were based on the dairy protein, whey, formulated with well-established antioxidants (vitamin B12, majoram (plant) and astaxanthin (marine)). Ultra-high temperature processing produced beverages of greater clarity, stability and antioxidant activity compared to pasteurization. Consumer acceptance for whey protein-based beverages often improves with clear formulations. Incorporation of additional antioxidant ingredients had no effect on viscosity or instability index but did increase sedimentation and redness. Studying antioxidant capabilities of whey proteins can expand the uses of whey in the 'food for health' sector, particularly foods designed for the older consumer. Furthermore, to suit export markets this work spray dried powders were generated.

Consumer: WheyGSH produced nutritional beverages suitable for the older consumer to delay oxidative stress of aging (via antioxidant ingredients) and reduce frailty (via whey). The older consumer is a growing population group with estimates suggesting 1.6 million people over the age of 65 will be living in Ireland by 2051. Whey protein-based antioxidant beverages could benefit the elderly consumer to meet their increased protein requirements and boost their antioxidant status. By providing these nutritional beverages we hope to decrease frailty (and sarcopenia), allowing this cohort to live healthier for longer.

Scientific community: WheyGSH provided a significant evidence base for the fate of whey in the upper gut and how processing can influence bioactivity. The scientific highlights included the development of a co-culture gut barrier model in house for bioavailability studies, the identification of bioavailable whey peptides and the development of cellular assays to assess bioactivity.

Policymakers: WheyGSH was presented at Dept of An Taoiseach-Programme of Actions for Smart Ageing.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	2	0	2
Post Doctorates	1	1	2
Research 0 Technicians/Assistants		1	1

Summary of Academic Outputs

Research	Total	Details
Outputs	Number	
PhD Theses	2	Alberto Corrochano. PhD thesis. 2018 Bioavailability and Antioxidant activity of Whey Proteins.
		Mark Dizon. PhD thesis. 2023. Application of high-resolution
		ultrasonic spectroscopy for real-time non-destructive monitoring of
		hydrolysis of whey proteins.
		nyurorysis or writey proteins.
Publications in	14	Arranz E, Corrochano AR, Shanahan C, Villalva M, Jaime L,
Peer Reviewed		Santoyo S, Callanan MJ, Murphy E, Giblin L (2019).
Scientific		Antioxidant activity and characterization of whey protein-
Journals		based beverages: effect of shelf life and gastrointestinal
		transit on bioactivity. Innovative Food Science and Emerging
		Technologies. doi.org/10.1016/j.ifset.2019.102209.
		2. Corrochano AR, Ferraretto A, Arranz E, Stuknytė M, Bottani
		M, O'Connor PM, Kelly PM, De Noni I, Buckin V, Giblin L
		(2019). Bovine whey peptides transit the intestinal barrier to
		reduce oxidative stress in muscle cells. Food Chemistry. 288;
		1, 306-314. doi: 10.1016/j.foodchem.2019.03.009.
		3. Corrochano AR, Sariçay Y, Arranz E, Kelly PM, Buckin V, Giblin
		L (2018). Comparison of antioxidant activities of bovine whey
		proteins before and after simulated gastrointestinal digestion.
		Journal of Dairy Science. 102(1), 54-67. doi: 10.3168/jds.2018-
		14581.
		4. Corrochano AR, Arranz E, De Noni I, Stuknytė M, Ferraretto A,
		Kelly PM, Buckin V, Giblin L (2018). Intestinal health benefits
		of bovine whey proteins after simulated gastrointestinal
		digestion. Journal of Functional Foods 49, 526-35.
		doi.org/10.1016/j.jff.2018.08.043.
		5. Chen Z, Kondrashina A, Greco I, Gamon L, Lund M, Giblin L
		and Davies M (2019). Effects of protein derived amino acid
		modification products present in Infant formula on metabolic
		function, oxidative stress and intestinal permeability using cell
		models. Journal of Agricultural and Food Chemistry.
		DOI:10.1021/acs.jafc.9b01324.
		6. Corrochano AR, Buckin V, Kelly PM, Giblin L 2018. Invited
		review: Whey proteins as antioxidants and promoters of
		cellular antioxidant pathways. J Dairy Sci. 2018
		Jun;101(6):4747-4761. doi: 10.3168/jds.2017-13618.
		 Giblin L, Yalcin A, Bicim G, Kramer A, Chen Z, Callanan M, Arranz E, Davies M. (2019). Whey proteins: targets of
		oxidation, or mediators of redox protection. Free Radical
		Research. doi 10.1080/10715762.2019.
		8. Margarida C. Altas, Evgeny Kudryashov, Vitaly Buckin. 2016.
		Ultrasonic Monitoring of Enzyme Catalysis; Enzyme Activity in
		Formulations for Lactose-Intolerant Infants. Analytical
-		rominiations for Lactose-intolerant linalits. Alialytical

Chemistry 88 (9), 4714–4723.doi.org/10.1021/acs.analchem.5b04673.

- Vitaly Buckin and Margarida Caras Altas. 2017. Ultrasonic Monitoring of Biocatalysis in Solutions and Complex Dispersions. Catalysts 7(11), 336. doi.org/10.3390/catal7110336.
- 10. Vitaly Buckin.2018. High-resolution ultrasonic spectroscopy. Journal of Sensors and Sensor Systems. Review 7:1, 207–217, 2018. doi.org/10.5194/jsss-7-207-2018.
- 11. Lynch R., Burke A., Byrne J., Buckin V.2020.. Osmolality and Molar Mass of Oligosaccharides in Breast Milks and Infant Formula During Hydrolysis of Lactose. Application of High-Resolution Ultrasonic Spectroscopy' J. of Food Chemistry. Food Chem. doi: 10.1016/j.foodchem.2020.126645.
- 12. R.Lynch, V.Buckin. 2020. Ultrasonic analysis of effects of varying temperature, pH, and proteolytic enzymes on hydrolysis of lactose by neutral lactase formulations in infant milk and in the infant digestive system. Food Research International, 157(2022)111004,1-20. doi: 10.1016/j.foodres.2022.111004.
- 13. Mark Dizon, Vitaly Buckin. 2023. Ultrasonic monitoring of enzymatic hydrolysis of proteins. 1. Effects of ionisation. Food Hydrocolloids, 114. doi.org/10.1016/j.foodhyd.2023.108866.
- 14. GI Papoutsidakis, V Buckin. 2023. Real-time monitoring of enzymatic hydrolysis of 1, 3 (4)- β -glucan with high-resolution ultrasonic spectroscopy. Food Hydrocolloids, 138. doi.org/10.1016/j.foodhyd.2022.108426.

Peer Reviewed Conference Papers

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- R. Corrochano, E. Arranz, A. Ferrareto, M. Bottani, M. Stuknyte, V. Buckin, P. Kelly, I. De Noni and L. Giblin.
 Antioxidant activity of whey protein: survival during gut transit and bioavailable to target cells? NutRedOx COST Action CA16112. 27-29 September 2017, Strasbourg (France). Oral presentation.
- Arranz, A. R. Corrochano, M. Villalva, L. Jaime, S. Santoyo, E. Murphy and L. Giblin. Antioxidant activity of whey-based beverages: effect of shelf life and gastrointestinal transit on bioactivity. The NutRedOx COST Action CA16112 WGs Meeting. 15-16 February 2018, Palma (Spain). Oral presentation.
- R. Corrochano, E. Arranz, A. Ferrareto, M. Bottani, M. Stuknyte, V. Buckin, P. Kelly, I. De Noni and L. Giblin. Whey proteins: bioaccessibility and biofunctionality. Walsh Fellowships Seminar. 9 November 2017, Dublin (Ireland). Oral presentation.
- Alberto R. Corrochano, Vitaly Buckin, Phil M. Kelly and Linda Giblin. Antioxidant activity and bioavailability of whey proteins. DubChem Third Year PhD Talks. 15 May 2017, Dublin (Ireland). Oral presentation.

- Mark Dizon, Margarida C Altas, Vitaly Buckin. Monitoring of hydrolysis of whey proteins with high-resolution ultrasonic spectroscopy. Degree of polymerization and molar mass. International Workshop: Recent applications of highresolution ultrasonic spectroscopy for monitoring of hydrolytic activities in milks. Nov Dublin 2017. Oral presentation.
- Buckin V, High-Resolution Ultrasonic Spectroscopy for Monitoring of Biocatalysis, International Conference ULTRASONICS 2018, June 2018, Lisbon, Portugal. Oral presentation.
- 7. Vitaly Buckin. 2017. Ultrasonic Monitoring of Hydrolytic Reactions in Milks. International Workshop: Recent applications of high-resolution ultrasonic spectroscopy for monitoring of hydrolytic activities in milks. Nov Dublin 2017. Oral presentation.
- Buckin V., High-resolution ultrasonic spectroscopy for monitoring of enzyme reactions, International, Conference and School on High-Resolution Ultrasonic Spectroscopy (HRUS 2018), October 2018, Brno, The Czech Republic. Plenary Lecture. Oral Presentation.
- Lynch R, Buckin V, The application of HR-US spectroscopy for monitoring of hydrolysis of lactose in milks and development of enzyme formulations for lactose intolerant infants.
 Conference: From routine to novelty – analysis methods in dairy industry, June 2018, Budapest, Hungary. Oral Presentation.
- 10. Dizon M., Application of high-resolution ultrasonic spectroscopy for real-time monitoring of enzymatic hydrolysis of whey proteins. Conference and School on High-Resolution Ultrasonic Spectroscopy (HRUS 2018), October 2018, Brno, The Czech Republic. Oral presentation.
- 11. Lynch R., Ultrasonic analysis for the monitoring and control of lactose hydrolysis in infant milks, Conference and School on High-Resolution Ultrasonic Spectroscopy (HRUS 2018), October 2018, Brno, The Czech Republic. Oral presentation.
- 12. Papoutsidakis, G., Dizon, M., Scanlon, A., Buckin, V., Ultrasonic Monitoring of Hydrolysis of β-Lactoglobulin by Digestive Enzymes. International Workshop: Recent applications of high-resolution ultrasonic spectroscopy for monitoring of hydrolytic activities in milks, November 2017, Dublin, Ireland. Poster presentation.
- 13. Dizon M., Buckin V, Novel ultrasonic technology for real-time monitoring of enzymatic hydrolysis of whey proteins. Conference: From routine to novelty analysis methods in dairy industry, June 2018, Budapest, Hungary. Poster presentation.
- 14. Szabó, T., Trif, L., Molnár, N., Csupor, B., O'Driscoll, B., Buckin, V., Keresztes, Z., Protein stabilised nanoparticle assisted detection of proteolytic activity studied with photometric and

- HR-US spectroscopic methods. International Workshop: From routine to novelty analysis methods in dairy industry, June 2018, Budapest, Hungary. Poster presentation.
- 15. Szarka, G., Bisztrán, M., Kasza, G., Stumphauser, T., Dizon, M., O'Driscoll, M., Keresztes, Z., Iván, B., Buckin, V., The synthesis of polymer-enzyme nanoparticles and their reaction with beta-casein, International Workshop: From routine to novelty analysis methods in dairy industry, June 2018, Budapest, Hungary. Poster presentation.
- 16. Osváth, Z., Láng, A., Szabó, B., Dizon, M., Buckin, V., Iván, B., Poly(N-isopropyl acrylamide) interactions with proteins, investigated by UV-Visible, NMR and Ultrasonic spectroscopy, International Workshop: From routine to novelty analysis methods in dairy industry, June 2018, Budapest, Hungary. Poster presentation.
- 17. Papoutsidakis G, Dizon M, Melikishvili S, Hianik T, Buckin V, Application of High-Resolution Ultrasonic Spectroscopy for Monitoring the Hydrolysis of bovine β-Casein by Trypsin, Conference and School on High-Resolution Ultrasonic Spectroscopy (HRUS 2018), October 2018, Brno, The Czech Republic. Poster presentation.
- 18. Lynch, R., Burke, A., Buckin, V., Application of High-Resolution Ultrasonic Spectroscopy for monitoring of osmolality and average molar mass in infant milks during lactose hydrolysis, International Symposium on Food Rheology and Structure, June 2019, Zurich, Switzerland. Poster presentation.
- 19. Lynch, R., Papoutsidakis, G., Dizon, M., Buckin, V., Application of High-Resolution Ultrasonic Spectroscopy for real-time monitoring of enzymatic hydrolysis of globular and non-globular proteins, International Symposium on Food Rheology and Structure, June 2019, Zurich, Switzerland. Poster presentation.

Other 3

3

Three Postgraduate student short term scientific missions:

- 1. WheyGSH Principle Investigator L. Giblin hosted PhD student Zhifei Chen from 7 May- 4 July 2018 from University of Copenhagen, Denmark.
- 2. WheyGSH Principle Investigator L. Giblin hosted PhD student Ariadna Gasa Falcon from April-9 August 2018 from Department of Food Technology, University of Lleid, Spain.
- WheyGSH PhD student Alberto Corrochano went on a shortterm scientific mission to Prof Ivano de Noni's lab at University of Italy from 1 Sept-31 Dec 2016 to learn in vitro gut barrier models.

Other

WheyGSH Principal Investigator organised three international conferences:

 2019. L. Giblin co-organised conference entitled Natural Products and the Hallmarks of chronic diseases. COST Action CA16112 NutRedOx. 25-27 March 2019. Luxembourg

2.	2017 V. Buckin organized International Workshop: "Recent
	applications of high-resolution ultrasonic spectroscopy for
	monitoring of hydrolytic activities in milks", Dublin 2017.
3.	2018 V. Buckin chaired the organization of the International
	Conference and School "High-Resolution Ultrasonic
	Spectroscopy for analysis of biomolecular processes" (HRUS
	2018), Brno 2018.

Intellectual Property

Two Teagasc Technology Expertise (Food Bioactives, Food Digestion) have been offered to Industry as a result of WheyGSH.

Summary of other Project Outputs

Project Outputs	Details			
New Products	Two model beverages and two nutritional powders have been 2 produced at pilot plant scale			
New Industry Collaborations Developed	 Five projects in collaboration with industry were initiated as a direct result of this project: Adisesso (Teagasc) MDBY0532, 1 Oct 2017-31 Jan 2018. Crosscare Ltd (UCD) 2017-2021. Danone (UCD) 2018. Carrig Brewing Co. (UCD) 2018-2019. FoodBIBS. MF2018-0151 (Teagasc) CareerFit Co-funded by Enterprise Ireland and Horizon Europe with Kerry Ingredients as Industry Partner. €244,800. 15 Jan 2018-21 June 2020. 	5		

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental	Producing nutritional beverages with blended ingredients (whey, marine and
Sustainability	plants) will reduce the overreliance of dairy in the 'Food for Health' sector. As
	the dietary shift occurs away from meat and milk, the digestion,
	bioaccessibilty and bioavailability research capacity built in WheyGSH can
	offer a service to food manufactures to test nutritive and bioactivity potential
	of novel alternative foods.
Socio-	The older consumer is a growing population group with estimates suggesting 1.6
Economic	million people over the age of 65 will be living in Ireland by 2051. Whey protein-based
	antioxidant beverages could benefit the elderly consumer to meet their increased

protein requirements and boost their antioxidant status. By providing these nutritional beverages we hope to decrease frailty (and sarcopenia), allowing this cohort to live healthier for longer. To inform policy makers of the importance of protein and antioxidants in the diet of older adults, WheyGSH was presented to policy makers via Dept of An Taoiseach Programme for Action for Smart Ageing (2016-2018). Industry WheyGSH offers industry two nutritional beverages and two powders produced at pilot plant scale. The research capacity built in WheyGSH has been offered to Industry as two Teagasc Technology Expertises. The interest and relevance to industry is evidenced by five follow-on industry projects. As a direct result of WheyGSH three early career scientist are employed in a fulltime permanent position in industry and 2 in full time permanent positions in academia in Europe. Other WheyGSH impact on the scientific community is considerable with two PhD dissertations, 14 peer reviewed publications, 19 conference presentations, three conferences organized, a leadership role in EU COST Action CA16112 NutRedOx, seven follow-on publicly funded research projects and two student awards. **Dissemination Activities Activity Details** Other Three Popular Press Articles: 1. L. Giblin, P. Kelly, A.R. Corrochano. 2015. Antioxidant Boost. Moorepark News Issue 41. 2. Alberto Corrochano, Elena Arranz, Linda Giblin. 2017. The benefits of whey. T-Research Vol 12 N: 3 page 28-29. 3. V. Buckin. produced an Information flyer 'High-Resolution Ultrasonic Spectroscopy for Real-Time Non-Destructive Monitoring of Bioprocesses in Dairy Industry', was distributed among EU dairy industrial and academic research institutes and manufacturers in English, Hungarian, Slovak and Czech languages.

1. R. Corrochano. "The magic behind whey proteins". Researchfest,

2. Alberto Corrochano, Food Tent, Moorpark Open Day, 04 July 2017.

1. 2018-ongoing: L. Giblin is a member of MTU Industry Advisory Board

2. 05 Oct 2017: L. Giblin presented at Loretto Secondary School (all-girls

Industry Panel for BSc (Hons) Nutrition and Health Science

school), Fermoy Cork, Career Guidance information session.

3. 11 Oct 2017: UCC undergraduate career day (L. Giblin).

Inspirefest. 6-7 July 2017, Dublin (Ireland).

Two Public Engagements:

Nine Education Outreach Events:

programme.

Other

4. 08 Dec 2017: L. Giblin invited lecture to MTU BSc Nutritional Sciences.
 5. 15 March 2018: L. Giblin attendance at UCC Food Science Graduate Day.
 6. 30 Jan 2018: L. Giblin and A Corrochano delivered an Interactive Lab Day to 5th and 6th class Castlelyons Primary School, Cork.
 7. 23 Oct 2018: L. Giblin attended Loreto Secondary School for Girls Career Day, Fermoy, Cork.
 8. Nov 2018: L. Giblin lab Hosted one transition year secondary school student.
 9. Jan-June 2018: L. Giblin Hosted one undergraduate student (Mark Kearney CIT) for 20 weeks research project.
 Other
 L. Giblin presented WheyGSH to policy makers via Dept of An Taoiseach - Programme for Action for Smart Ageing (2016-2018).

Knowledge Transfer Activities

Identify knowledge
outputs generated
during this project.

There were five main outputs from this project (four products and one methodology).

- 1. Two whey-based antioxidant beverages.
- 2. Two whey-based antioxidant powders for export market.
- 3. One methodology to track food bioavailability using co-cultures of epithelial cells.

Identify any knowledge transfer activities executed within the project.

WheyGSH outcomes were showcased to Industry at two Teagasc Gateways events:

- 1. 10 Dec 2015 'Foods for Health' Stand.
- 2. 16 Nov 2017 Food Digestion Stand.

WheyGSH research capacity was offered to industry by Teagasc TTO as two Teagasc Expertises (Food Bioactives, Food Digestion) in 2015-2017.

In addition, several individual meetings were held with industry to transfer WheyGSH activities:

- 11-13 April 2016: P. Kelly had discussions with ORNUA on the potential of whey ingredients for health market.
- 14 March 2017: L. Giblin presented to Danone Scientific Meeting at Moorepark.
- 3 May, 9 June, 26 June, 20 July, 5 Oct 2017: L. Giblin offered In Vitro Bioavailability Assays, as a service, to Adisseo.
- 14 June 2017: L. Giblin presented to Halo Life Sciences at Teagasc Moorepark.
- 27 June, 27 July, 9 Aug, 6 Sept 2017: LGiblin met with Crème Global, Irish start up.
- 23 Aug 2017: L. Giblin presented to Biostyme H&H group.
- 1 March 2018, 7 June 2018: L. Giblin met with Infant formula Start up.
- 13 March 2018: L. Giblin met with Innovate UK's Global Business Accelerator Programme.

WheyGSH was presented to policy makers via Dept of An Taoiseach - Programme for Action for Smart Ageing (2016-2018).

Identify any knowledge transfer activities executed within the project. Impact of knowledge transfer to Industry was evidenced by five follow-on collaborative projects with industry. Impact of knowledge transfer to academia was evidenced by seven follow-on projects funded by public research bodies. WheyGSH strengthened Teagasc & UCD research reputation which is evidenced by hosting three international conferences and a leadership role for LGiblin in EU COST Action CA16112 NutRedOx March 2017-Oct 2021.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer	€244,800.00	FoodBIBS. MF2018-0151, CareerFit Co-funded by Enterprise Ireland and
National		Horizon Europe. Kerry Ingredients as Industry Partner. 15 Jan 2018 - 21
Funding		June 2020.
Exchequer		TOMI 15F604. Irish Department of Agriculture, Food & Marine,
National	€560,964.00	Nov 2016 – Nov 2021,
Funding		
Other	€100,000.00	Four client contract projects:
		1. Oct 2017 - 31 Jan 2018. Adisseo project (Teagasc) MDBY0532).
		2. 2017 – 2021 Crosscare Ltd (UCD)
		3. 2018 Danone (UCD)
		4. 2018-2019 Carrig Brewing (UCD)
Non-Exchequer	€164,000.00	Teagasc funded PRO4FOOD MDBY0015. 1 Jan 2017-30 April 2021.
National Funding		
Non-Exchequer		Teagasc funded project BIOPROTEIN MBDY0360 1 Oct 2018-30
National Funding	€146,000.00	Sept 2022.
EU R&I	€300,000.00	2016, Innovative technology for the detection of enzyme activity in milk
programmes		(FORMILK) EU HORIZON 2020.
Exchequer	€96,000.00	2017, IRC Grant Overcoming lactose intolerance. Enzyme
National		formulations for hydrolysis of lactose and synthesis of galacto-
Funding		oligosaccharides (GOS).
Non-Exchequer	€90,000.00	2016, Internal funding scheme University College Dublin (UCD), and HEA
National Funding		Consumables award, Novel Ultrasonic Technologies for Monitoring of
		Enzymatic Hydrolysis of Polysaccharides and Proteins.

Future Strategies

The methodologies developed in WheyGSH are at the cutting edge of science tracking food bioactives as they transit the upper gut and cross the gut barrier. As the consumers looks to alternative sustainable foods to replace meat and milk in their diet, it is critical that the methodologies developed in WheyGSH are employed to track digestion and bioavailability of these alternative foods. As a direct result of WheyGSH the scientific reputation of the Principal Investigators has been enhanced both within industry and academia. The Principal Investigators intend to leverage this reputation to continue to attract Horizon Europe and Industry funding in this research space. From a research perspective, it is our intention to develop in vitro digestion and bioavailability models that best represent the older adult gut.

Project Publications

List publications numerically.

- 1. Arranz E, Corrochano AR, Shanahan C, Villalva M, Jaime L, Santoyo S, Callanan MJ, Murphy E, Giblin L (2019). Antioxidant activity and characterization of whey protein-based beverages: effect of shelf life and gastrointestinal transit on bioactivity. Innovative Food Science and Emerging Technologies. https://doi.org/10.1016/j.ifset.2019.102209.
- 2. Corrochano AR, Ferraretto A, Arranz E, Stuknytė M, Bottani M, O'Connor PM, Kelly PM, De Noni I, Buckin V, Giblin L (2019). Bovine whey peptides transit the intestinal barrier to reduce oxidative stress in muscle cells. Food Chemistry. 288; 1, 306-314. doi: 10.1016/j.foodchem.2019.03.009.
- 3. Corrochano AR, Sariçay Y, Arranz E, Kelly PM, Buckin V, Giblin L (2018). Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion. Journal of Dairy Science. 102(1), 54-67. doi: 10.3168/jds.2018-14581.
- 4. Corrochano AR, Arranz E, De Noni I, Stuknytė M, Ferraretto A, Kelly PM, Buckin V, Giblin L (2018). Intestinal health benefits of bovine whey proteins after simulated gastrointestinal digestion. Journal of Functional Foods 49, 526-35. doi.org/10.1016/j.jff.2018.08.043.
- 5. Chen Z, Kondrashina A, Greco I, Gamon L, Lund M, Giblin L and Davies M (2019). Effects of protein derived amino acid modification products present in Infant formula on metabolic function, oxidative stress and intestinal permeability using cell models. Journal of Agricultural and Food Chemistry. DOI:10.1021/acs.jafc.9b01324.
- 6. Corrochano AR, Buckin V, Kelly PM, Giblin L 2018. Invited review: Whey proteins as antioxidants and promoters of cellular antioxidant pathways. J Dairy Sci. 2018 Jun;101(6):4747-4761. doi: 10.3168/jds.2017-13618.
- 7. Giblin L, Yalcin A, Bicim G, Kramer A, Chen Z, Callanan M, Arranz E, Davies M. (2019). Whey proteins: targets of oxidation, or mediators of redox protection. Free Radical Research. DOI 10.1080/10715762.2019.
- 8. Margarida C. Altas, Evgeny Kudryashov, Vitaly Buckin. 2016. Ultrasonic Monitoring of Enzyme Catalysis; Enzyme Activity in Formulations for Lactose-Intolerant Infants. Analytical Chemistry 88 (9), 4714–4723.doi.org/10.1021/acs.analchem.5b04673.
- 9. Vitaly Buckin and Margarida Caras Altas. 2017. Ultrasonic Monitoring of Biocatalysis in Solutions and Complex Dispersions. Catalysts 7(11), 336. doi.org/10.3390/catal7110336.
- 10. Vitaly Buckin.2018. High-resolution ultrasonic spectroscopy. Journal of Sensors and Sensor Systems. Review 7:1, 207–217, 2018. doi.org/10.5194/jsss-7-207-2018.
- 11. Lynch R., Burke A., Byrne J., Buckin V.2020. Osmolality and Molar Mass of Oligosaccharides in Breast Milks and Infant Formula During Hydrolysis of Lactose. Application of High-Resolution Ultrasonic Spectroscopy' J. of Food Chemistry. Food Chem. doi: 10.1016/j.foodchem.2020.126645.
- 12. R.Lynch, V.Buckin. 2020. Ultrasonic analysis of effects of varying temperature, pH, and proteolytic enzymes on hydrolysis of lactose by neutral lactase formulations in infant milk and in the infant digestive system. Food Research International, 157(2022)111004,1-20. doi: 10.1016/j.foodres.2022.111004.
- 13. Mark Dizon, Vitaly Buckin. 2023. Ultrasonic monitoring of enzymatic hydrolysis of proteins. 1. Effects of ionisation. Food Hydrocolloids, 114. doi.org/10.1016/j.foodhyd.2023.108866.
- 14. GI Papoutsidakis, V Buckin. 2023. Real-time monitoring of enzymatic hydrolysis of 1, 3 (4)- β -glucan with high resolution ultrasonic spectroscopy. Food Hydrocolloids, 138. doi.org/10.1016/j.foodhyd.2022.108426.