



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

Food Institutional Research Measure

Final Report

**'CardioRubus Project: Beneficial effects of blackberry polyphenols
on cardiovascular and metabolic health'**

DAFM Project Reference No: 13 F 539

Start date: 01/12/2013

End Date: 31/12/2017

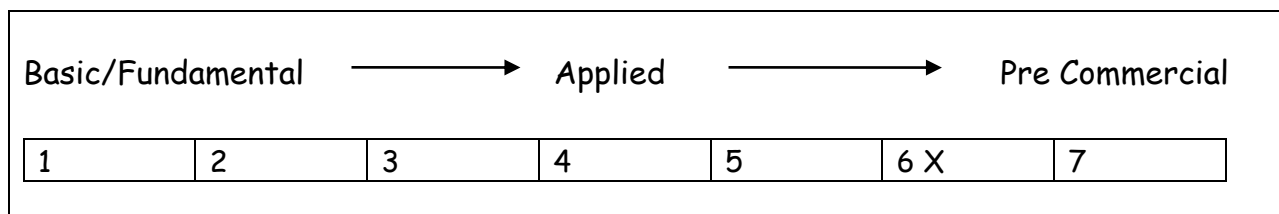
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Collaborating Research Institutions and Researchers:

- 1) University College Cork, Partner: Dr. Seamus O'Mahony
- 2) Teagasc Food Research Centre, Ashtown, Partner: Dr Dilip Rai

Please place one "x" below in the appropriate area on the research continuum where you feel this project fits



Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report:

Priority Area (s)	H Food for Health I Sustainable Food Production and Processing
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Key words: (max 4): *Berry fruits, Polyphenols, Cardiovascular health, Functional Foods.*

1. Rationale for Undertaking the Research

Cardiovascular disease (CVD) is responsible for one-in-three deaths in Ireland¹ and 64% of Irish adults aged >50yrs have high blood pressure². Pragmatic evidence-based food solutions are an attractive option for the maintenance of normal blood pressure and for mitigating cardiovascular risk. While increased intakes of fruits and vegetables are consistently associated with reduced mortality and chronic disease; 91% of Irish adults do not consume the WHO recommended intake for fruit and vegetables^{3,4}. Epidemiological data consistently indicate cardio-protective effects associated with increased berry fruit consumption⁵. Berry fruits are a rich source of bioactive polyphenols which also have well established health benefits and provide a niche opportunity for the development of fruit-based functional foods.

Blackberry is a berry fruit widely available in Ireland (native species *Rubus fruticosus*) and the purchase of blackberry fruits has recently increased in Ireland (*Bord Bia, personal communication, 2017*). Blackberries are rich in micronutrients and contain a myriad of phytochemicals, particularly polyphenol compounds⁶. Blackberries have potent antioxidant properties and are ranked third, after strawberry and black raspberry in terms of antioxidant capacity (Wada & Ou, 2002), however, their distinct effects on cardiovascular and metabolic health remain to be established. Therefore, as polyphenolic compounds are increasingly associated with beneficial health effects, there is an ever-increasing need to develop the scientific and technological fundamentals required to facilitate their incorporation into a wider range of functional foods.

Furthermore, a critical factor to inform functional food design and the establishment of recommended dietary intakes for polyphenols is to have an appreciation of dietary exposures to polyphenols at population level. For example, findings from the large-scale Pan European PREDIMED dietary intervention study indicate that a mean polyphenol intake of $\geq 1,200$ mg/d was associated with a 46% reduced risk of a major cardiovascular event in comparison to adults with polyphenol intakes ≤ 560 mg/d (Tresserra-Rimbau et al., 2014). Data on intakes of polyphenols at Irish population level are unavailable; therefore, dietary targets linked to health outcomes cannot be employed or extrapolated to the Irish population. Knowledge of polyphenol intakes and key food sources across population groups is a critical starting point to advise both nutrition policy and functional food design.

Therefore, as markets for food-based solutions to support healthy ageing continue to expand; there is tangible opportunity to provide innovative functional beverages underpinned by robust scientific evidence to this rapidly broadening sector. Furthermore, such scientific data would underpin and support positive messages on polyphenol intakes and berry fruit consumption, their fundamental role in the diet as well as their contribution to overall health and well-being.

References

¹Irish Heart Foundation. <http://www.irishheart.ie/iopen24/about-us-t-1.html> Accessed 03/09/19.

²Murphy, CM, Kearney, PM, Shelley EB et al. (2016). *Journal of Public Health*, 38(3), 450-8.

³Cummins C, Bannon S, Walton J, et al. (2011). *Proceedings of the Nutrition Society*. 70(OCE3), E50.

⁴Irish Universities Nutrition Alliance. National Adult Nutrition Survey, Summary Report 2011 Accessed 11/09/2019 at <http://www.iuna.net/wp-content/uploads/2010/12/National-Adult-Nutrition-Survey-Summary-Report-March-2011.pdf>

⁵Basu A, Rhone M, Lyons TJ. (2010). *Nutrition Reviews*, 68, 168-77.

⁶Kaume, L., Howard, L. R. & Devareddy, L. (2012). *Journal of Agricultural and Food Chemistry*, 60, 5716-27.

⁷Wada L & Ou B (2002). *Journal of Agricultural and Food Chemistry*, 50, 3494-500.

⁸Tresserra-Rimbau, A. et al. (2014). *Nutrition, Metabolism and Cardiovascular Diseases*, 24(6), 639-47.

2. Research Approach

The CardioRubus project was coordinated by UCC with Teagasc Food Research Centre, Ashtown as its project partner. Adopting a farm-to-fork multi-disciplinary approach, five distinct yet complementary research tasks were undertaken in this project which included the development of a blackberry polyphenol enriched functional beverage and extract which was comprehensively characterised in terms of nutrient profile and polyphenol composition. This novel beverage was subsequently tested for physiological efficacy in relation to cardiovascular health in a dietary intervention study in a sample of Irish adults aged 45 years plus. Additionally, this project has conducted a systematic review to investigate the cardio-protective effects of berry fruit consumption and has also estimated for the first time intakes of polyphenols in Irish population groups and has conducted dietary modelling of the impact of polyphenol-enriched foods on Irish population intakes of polyphenols.

The CardioRubus project is strongly committed to advancing research in the area of polyphenols and health and the establishment of productive integrative links between the disciplines of Food Science and Nutrition, thus, enabling opportunities for future scientifically validated polyphenol-based functional foods.

Research approaches focused on the following core objectives:

Informed design and development of a functional beverage and extract:

- Literature-based approaches: Conduction of a systematic review which focuses exclusively on the effects of berry-fruit consumption on cardiovascular and metabolic health outcomes (evaluating data from food-based interventions). This review has incorporated a stringent quality assessment protocol which evaluated and scored the level of scientific quality of each published dietary intervention study included within this review. The objective of this review was to inform on the types of berry fruits and the quantities required in the diet to support cardio-metabolic health. This review was pivotal to inform on the optimal quantities of berry fruits required for the development of the CardioRubus beverage.
- Development of novel design approaches for 1) the delivery of fruit-derived polyphenols within foods, and 2) enhancing polyphenol stability within functional beverages.

The process employed for development of the polyphenol-enriched ingredients was designed and executed as per the original proposal and is described in brief here. Pre-treatment of the blackberry purée with pectinase, followed by centrifugation to generate a clarified purée, increased down-stream recovery of polyphenols using membrane filtration. On ultrafiltration using a 1000 Da cut-off membrane, permeate flux was increased 3.7-fold by increasing the feed temperature from 9 to 55°C; however, minimal enrichment of polyphenols was achieved under the temperature (9-55°C) and pH (2-6) conditions tested. Nanofiltration conducted at a transmembrane pressure of 10 bar at 45-50°C, using a 450 Da cut-off membrane, yielded a retentate with a polyphenol content which was 29.2% higher and an antioxidant capacity 25.1% higher than that of the feed (i.e., clarified blackberry purée). Polyphenol rejection by the membrane was 91%; therefore, the majority of phenolic compounds were retained in the retentate.

- Extensive polyphenol characterization of these novel food prototypes

Advanced analytical techniques involving high-resolution mass spectrometry methods were employed in the structural characterisation of blackberry polyphenols. These assessments were carried out on both beverage prototypes and the blackberry polyphenol extract and were subsequently assessed to provide data on polyphenol stability across product shelf-life. Quantification of 13 most abundant polyphenols in blackberry were achieved using high throughput ultra-high pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods in each sample in less than 10 minutes. The UPLC-MS/MS quantification data correlated the spectrophotometric data of total phenolics, flavonoids and anthocyanins.

Validation and Substantiation: Delivery of robust data from a randomized control trial (RCT) with a crossover design which investigated the effects of the CardioRubus functional beverage in comparison with a specifically designed control beverage (minimal polyphenols present) on cardiovascular and metabolic health outcomes in a sample of 83 middle-aged Irish adults with modestly elevated blood pressure. This Dietary Intervention study was conducted by the PI and Coordinator on the project at the Human Nutrition Studies Unit based at the School of Food and Nutritional Sciences, UCC.

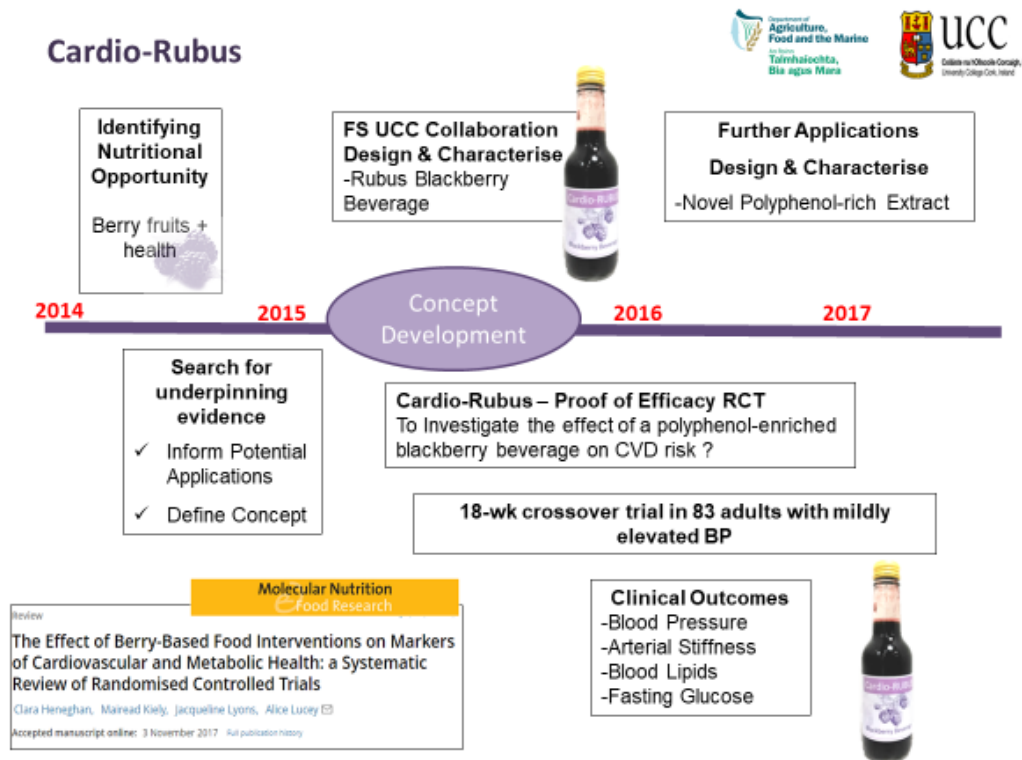
Two blackberry beverages: a high-dose (treatment) and a low-dose (control) blackberry polyphenol beverage were formulated and manufactured at laboratory, pilot and commercial scale to support the CardioRubus dietary intervention. The CardioRubus polyphenol enriched blackberry beverage contained an equivalent of 84 blackberries per 250ml serving and had a Total Polyphenol Concentration (TPC) of ~700mg Gallic Acid Equivalents (GAE)/250ml while the control beverage which was specifically designed for this RCT by the Food Science team, was similar in appearance and taste to the CardioRubus beverage but contained an equivalent of ~10 blackberries per 250ml serving and a TPC of <100mg GAE/250ml. Given the crossover study design, each participant consumed one bottle of their assigned beverage per day for two study phases of six-weeks each.

Health outcomes of this study included blood pressure and arterial stiffness (primary outcome), blood lipid profile and glucose homeostasis. The underpinning of health claims on novel foods with robust scientific evidence is fundamental for the protection of the consumer. RCTs or dietary interventions in humans provide the gold standard evidence base to support relationships between food and health.

Dietary Modelling and Application: The estimation of habitual polyphenol dietary exposure is a cornerstone for the development of dietary recommendations. This project has estimated dietary intakes of polyphenols in Irish adults, teens and children using nationally representative data. The key food group contributors to polyphenol intakes were also identified. A data-modelling assessment was undertaken to investigate if hypothetically a polyphenol-rich beverage similar to that used in the CardioRubus RCT could meaningfully increase polyphenol intakes in the Irish population.

3. Research Achievements/Results

Cardio-metabolic disease is a critical health challenge at global scale. The development of food-based strategies to benefit cardiovascular and metabolic health is a pragmatic and cost effective measure at population level. The CardioRubus project investigated the effects of blackberry-derived polyphenols on blood pressure and cardiovascular risk using blackberry beverages formulated at UCC. Guided by the findings of a systematic review conducted within this project, a novel polyphenol-rich blackberry beverage was developed and characterised for use in the dietary intervention.



Informed design and development of a functional beverage and extract:

- *Literature-based approaches:* A comprehensive systematic review of published dietary intervention studies which investigated the effects of berry fruit consumption on cardio-metabolic health outcomes was conducted. Of 1,348 publications reviewed a total of 23 dietary intervention trials were initially included. After a stringent quality assessment, 17 of the 23 studies were included in the final review. We observed that more than two-thirds of high-quality berry-based dietary intervention trials demonstrated beneficial effects of berry consumption on markers of cardiovascular and metabolic health risk. Specifically, improvements in vascular function (endothelial function and blood pressure), blood lipid concentrations and fasting glucose concentrations were observed in studies categorized as of "higher scientific quality".

This systematic review contributes moderate to strong evidence for the inclusion of berry fruits as part of a cardio-protective diet. A benefit of this review was the identification of the types of berry fruits and the quantities required in the diet to support cardiovascular and metabolic health, which was also important to inform the optimal quantities of berry fruits required for the development of the CardioRubus beverage.

- *Development of novel design approaches for 1) the delivery of fruit-derived polyphenols within foods, and 2) enhancing polyphenol stability within functional beverages.*

This project has developed and comprehensively characterised a novel functional polyphenol-rich blackberry beverage and an extract with potential for use as a food ingredient. A membrane filtration-based process has been developed for the first time for the selective enrichment of polyphenols from blackberry puree using ceramic membrane technology.

Additionally, a considerable amount of new data to inform the formulation and stability of blackberry-based nutritional beverages has been generated. The impact of storage conditions (temperature and time) on the stability of polyphenols and antioxidant properties in these beverages has been elucidated and published. A number of prototype blackberry-based nutritional beverages have been formulated and manufactured at laboratory, pilot and commercial scale in support of the human dietary RCT.

Specifically, blackberry beverages were formulated to different total polyphenol concentrations, and their nutrient and physical stability during 6 months of storage at 20°C or 40°C were determined. Results showed that ≥86% of the total polyphenol content and ≥82% of the antioxidant activity of the beverages was retained during storage at either temperature, while storage at 40°C resulted in greater loss of anthocyanins than at 20°C. There were large decreases in total flavonoids, vitamin C concentrations and red colour during 6-months of storage, while pH and microbiological quality remained stable. Storage influenced polyphenol profiles, particularly the flavanol quercetin xyloside, the level of which increased substantially during storage, and the phenolic acids which generally decreased in concentration during storage. In addition, a process was developed and scaled-up for the enrichment of polyphenols from blackberry purée using clarification, ultrafiltration (UF) and nanofiltration (NF).

- *Extensive polyphenol characterization of these novel food prototypes*

Quantification of 13 most abundant polyphenols in blackberry fruit was conducted on a routine basis. Anthocyanins, associated with the dark pigmentation, were the major polyphenols in blackberry. However, this class of polyphenols and flavanols in the blackberry beverages were susceptible to degradation over time and storage temperature. Evidence of degradation, most likely deglycosylation of flavanols and anthocyanins, has been demonstrated for the beverages. This project has focused on identifying and characterising the predominant polyphenols present in blackberries which will facilitate the elucidation of the links between blackberry polyphenols and health and thus will facilitate the underpinning of health claims for berry-based food products.

Validation and Substantiation:

The CardioRubus Dietary Intervention was an 18-week RCT with a crossover design which investigated the effects of the CardioRubus functional beverage in comparison with a specifically designed control beverage (minimal polyphenols present) on cardiovascular and metabolic health outcomes in a sample of 83 middle-aged Irish adults with modestly elevated blood pressure. Eighty-two participants completed this dietary intervention. The main findings of this study indicate that BP, arterial stiffness, concentrations of circulating glucose and blood lipids did not change post consumption of this polyphenol rich blackberry beverage with a total polyphenol concentration (TPC) of ~700mg GAE/d over a 6-week period. However, in a sub-group analysis there was a significant reduction in arterial stiffness in the hypertensive participants receiving the treatment that was not observed in participants with a normal blood pressure. Importantly, no adverse effects associated with these beverages were observed. These findings further support the growing

research consensus which suggests that the large inter-individual variation in the response to polyphenol intakes may obscure beneficial associations between polyphenol-based food intakes and health outcomes in dietary interventions. This is a significant challenge facing polyphenol-based dietary interventions and the development of polyphenol enriched functional foods at global level which warrants imminent consideration.

Dietary Modelling and Application:

The CardioRubus project has generated new data on polyphenol intakes and food sources in the Irish population and has demonstrated that the mean daily intake (MDI) (SD) of total polyphenols was 1008 (\pm 612) mg/day in Irish adults; 568 (\pm 419) mg/day in teens, and 438 (\pm 238) mg/day in children, this output enables us to compare our intakes with other populations and with dietary targets for health outcomes.

This project has demonstrated that beverages (tea, coffee & fruit juices) were the predominant contributor to polyphenol intakes in the Irish population; providing a feasible method by which to increase polyphenol intakes. The TPC of the CardioRubus beverage was compared with the predominant non-alcoholic beverages consumed in Ireland; per 100ml of beverage consumed, the CardioRubus blackberry beverage (~267 mg GAE) has a substantially higher concentration of polyphenols in comparison to fruit juices (orange (~65 mg GAE)/ apple (~34 mg GAE)) or tea (~104 mg GAE).

A modelling assessment demonstrated that hypothetically a polyphenol-rich beverage similar to the CardioRubus beverage could meaningfully increase polyphenol intakes in the Irish population. For example - for tea, if 10, 20 or 30% of tea-drinking occasions were replaced by the CardioRubus blackberry beverage, the mean (SD) intake of total polyphenols in Irish adults would increase by 23%, 50% and 75%, respectively. To put this into context - for example, if one in ten mugs of tea was replaced by one bottle of the CardioRubus blackberry beverage, polyphenol intakes would increase by 23%.

To conclude, this project provides key data endorsing the blackberry fruit as a widely available native source of polyphenols for the Irish population with potential health benefits. Data generated reinforces the importance of berry fruit cultivation and horticultural practices in Ireland and will assist in the development of future consumer-orientated functional foods which could be sourced and produced within Ireland.

4. Impact of the Research

The output of CardioRubus is of relevance to the scientific community, the consumer, the food industry, and the horticulture sector, supporting the cultivation of berry fruits in Ireland. The endeavours of this project align well with the strategies for the NRPE 'Food for Health' and 'Sustainable Food Production' and FoodWise 2025. We have created, characterised and subsequently tested for physiological efficacy a consumer-orientated functional beverage.

CardioRubus has enhanced the research base in terms of the development of more highly-skilled researchers at UCC and Teagasc and has increased knowledge and understanding of the efficacy of bioactives in promoting health, this knowledge has directly informed the development of functional foods for improved consumer well-being.

Adopting a farm-to-fork approach, this project has delivered critical mass of expertise across the disciplines of food science, plant chemistry, and nutrition and health, including:

- The provision of leading food processing capability for the advancement of polyphenol delivery systems in foods, thus, facilitating future innovations for polyphenol-based functional foods which meet consumer expectations and demands. Specifically, this project has investigated for the first time the use of next-generation techniques for the enrichment of polyphenols in foods and has provided robust data to inform on polyphenol stability in food formulation and across product shelf-life.

- The CardioRubus RCT is the first dietary intervention study to investigate the effect of blackberry-derived polyphenols on blood pressure and cardiovascular risk. To the best of our knowledge, this is the largest berry-based dietary intervention with a crossover design reported to date.

- CardioRubus has generated for the first time estimates for dietary intakes of polyphenols in representative samples of Irish Adults, Teens and Children and has identified that beverages are the predominant source of polyphenols in the Irish diet.

- CardioRubus has also published the first systematic review to exclusively focus on berry fruit based dietary interventions and cardiovascular outcomes and has contributed moderate to strong evidence for the inclusion of berry fruits as part of a cardio-protective diet.

Beyond the Scientific Community, these research findings are of value to the food sector for the formulation of polyphenol-based functional foods, the Food Safety Authority of Ireland, the Irish Heart Foundation, SafeFood as well as other agencies (nationally and internationally) briefed with the development of healthy eating guidelines.

Our multidisciplinary collaborative efforts have addressed several research objectives, resulting in the delivery of two MSc Theses: 1. *Formulation of nutritional beverages and development of polyphenol-enriched ingredients from blackberry puree*; and 2. *Contribution of berries to cardio-protective bioactive intakes, and one PhD Thesis: "Dietary bioactives and cardiovascular disease"*. This project has enabled opportunities to expand research activities which has facilitated participation in EU Cost Actions and competitive funding calls, and has generated high impact multi-disciplinary output including the publication of findings in high calibre peer reviewed Food Science and Nutrition journals as well as the presentation of project findings at key international conferences. Additionally, CardioRubus has provided key data on the blackberry fruits for use in the eBASIS database developed by EuroFIR AISBL, an international, member-based, non-profit association which focuses on ensuring sustained advocacy for food information in Europe (<http://ebasis.eurofir.org/Default.asp>).

Benefit to consumer, citizen and societal needs

- Findings from our systematic review indicate that berry fruits consumed as part of a healthy diet may confer additional cardio-protective benefits for health.
- Increased berry fruit and berry polyphenol consumption in the Irish population.
- The delivery of consumer-orientated innovative polyphenol enriched foods to support health.
- The provision of estimates of polyphenol intakes in Irish population groups enables comparison with polyphenol intakes in other countries (particularly the Mediterranean diet) and with links to multiple health outcomes.
- These data have significant societal value for the development of nutrition policy, as establishing dietary recommendations for polyphenols must consider habitual polyphenol intakes at population level.

Benefit to the Irish Agri-Food Sector

- The novel process developed for selective enrichment of polyphenols from a fruit puree will assist Irish fruit processors and beverage product formulators in developing next-generation polyphenol-enriched foods.
- Provision of greater scientific capability to enable a wider economic development of new foods with proven performance/health benefit tailored to specific applications.
- Promotion of Irish horticulture- increased requirement for berries to support a thriving bioactive ingredients market.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

This project has fostered ongoing and extended collaborations at both National and International level in terms of Food and Health research. This project has successfully enabled the development of a productive inter-disciplinary collaboration between Nutrition and Food Science research teams at UCC and with Scientists in the area of Analytical Chemistry at Teagasc, Ashtown; collaborations which extend beyond the life of this project.

At International level, the CardioRubus project has strongly benefited from its involvement with the EU FP7-funded BACCHUS project (2012-2016) which investigated the Beneficial Effects of Bioactive Compounds on Cardiovascular Health. The Principal Investigator and Coordinator were partners on this BACCHUS Project which was highly advantageous to the CardioRubus project in terms of access to the expertise of the BACCHUS Consortium for the provision of technical guidance and support, knowledge sharing, access to the EuroFir network and dissemination opportunities. These connections across 28 research institutions have underpinned the development of successful working relationships to include with the Quadrum Institute, Norwich UK, University of Gent, Belgium, CSIC-CEBAS, Murcia, Spain and Nofima, Norway and have provided the CardioRubus project with a valuable opportunity to develop tangible multi-disciplinary research collaborations which will extend beyond the CardioRubus project. This study facilitated the development of collaborative linkages between UCC and Teagasc research centres at Ashtown (polyphenol analysis) and Moorepark (technology scale up). Collaborative linkages were also established between UCC and Inopor (German membrane filtration technology developer) through 2e Technical Development Ltd.

Furthermore, a Task Leader (D Rai) was actively linked with two EU COST Actions on plant bioactives and health, namely FA1403 (POSITIVE) and CA16112 (NutRedOx), both of which were of direct relevance to CardioRubus. D Rai was co-author of an extensive meta-analysis entitled "Meta-analysis of the effects of foods and derived products containing ellagitannins and anthocyanins on

cardiometabolic biomarkers: Analysis of factors influencing variability of the individual responses" produced as part of CA POSITIVE (published in International Journal of Molecular Science 2018, 19, 694).

CardioRubus has also exclusively provided data on blackberry polyphenol composition and the biological effects of blackberries to the European eBASIS database, an internet-deployed database developed by EuroFIR (2007). eBASIS is a unique resource that is widely used by the food industry and policy makers as it contains critically evaluated published data on the content and biological effects of bioactive constituents in plant based foods and is available in 15 EU languages (<http://ebasis.eurofir.org/Default.asp>).

- (ii) Outcomes where new products, technologies and processes were developed and/or adopted

This project has delivered complementary food science, nutrition, and health objectives in terms of the design and development of nutritionally informed fruit-based food products for cardiovascular benefit. This project has successfully produced a novel polyphenol-enriched blackberry beverage and a polyphenol-enriched blackberry extract for use as a food ingredient. This project has also generated a considerable amount of novel technical and analytical data to inform on the formulation and stability of berry fruit-based beverages. A prototype of this beverage was subsequently tested for physiological efficacy and consumer acceptance in a dietary intervention study.

Furthermore, a novel membrane filtration-based process has been developed for the first time for the selective enrichment of polyphenols from berry puree using ceramic membrane technology. This study demonstrated that beverages with high blackberry content stored at ambient temperature are suitable for the delivery of polyphenols. Pectinase treatment, combined with centrifugation, is an effective clarification process for blackberry purée prior to membrane filtration, and NF using a 450 Da cut-off membrane successfully produced a retentate which was enriched in polyphenols. While our products are pre-commercial, the comprehensive technical and analytical data generated and published provide a notable underpinning evidence-base for relevant industry stakeholders to adopt within their current practices so as to generate polyphenol-enriched functional foods.

- (iii) Outcomes with economic potential

Adopting an integrated approach connecting the disciplines of food science and nutrition; the CardioRubus project has generated outcomes with economic potential in terms of developing novel foods enriched with fruit-derived polyphenols to benefit health.

The CardioRubus project has generated key data to support the development of future polyphenol enriched functional foods including a systematic review to inform on evidence linking berry fruit consumption and the maintenance of cardiovascular and metabolic health, and additionally, we have generated for the first time data on polyphenol intakes in a representative sample of Irish adults, teens and children which is critical for informing on new product formulation and the selection of relevant polyphenol doses to support health.

From a food science perspective, a novel membrane filtration-based process has been developed for the selective enrichment of polyphenols from blackberry puree using ceramic membrane technology, this advancement will assist Irish fruit processors and beverage product formulators in developing polyphenol-enriched beverages. Similarly, data generated on the stability of polyphenols

(to include a comprehensive characterisation of their polyphenol profile and antioxidant capacity) within these beverages and how they are affected by storage conditions is valuable to formulators and processors in the development of new fruit-based beverages. The fruit beverage industry now has important information on the stability of these components in commercially relevant beverage formats under typical storage conditions. The research generated from this project also assists in narrowing the gap between the fruit beverage industry and the more technologically-advanced dairy processing sectors.

(iv) Outcomes with national/ policy/social/environmental potential

This project was conducted using a well-managed consortium of academic researchers, technology developers and a private commercial fruit juice manufacturer, with national funding support, to generate a value-added new product using a raw material that grows well in Ireland. Project findings are of relevance in terms of improving health and quality of life of citizens and potential cost-saving to the Irish health-care budget. This project provides evidence to further support the blackberry fruit as a widely available native source of polyphenols for the Irish population with potential health benefits. Data generated from this project reinforce the importance of berry fruit cultivation and horticultural practices in Ireland and will assist in the development of future consumer -orientated functional foods which could be sourced and produced within Ireland.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Acceptable Format: Walsh, D.R., Murphy, O., Cosgrave, J. (2008). Echinococcosis - an international public health issue. Research in Veterinary Science 774, 891-902.

Heneghan C, Hennessey A, Lyons J, Singh T, Flynn A, Walton J, Kiely M, and Lucey AJ. Dietary intakes of polyphenols and food sources in Ireland. *Manuscript in preparation for submission to the British Journal of Nutrition.*

Heneghan C, Manning E, Dahly DL, Kelly N, Rai DK, O'Mahony JA, Kiely ME, and Lucey AJ. A polyphenol-enriched blackberry beverage does not lower blood pressure or modify cardiovascular risk: results from a randomized controlled crossover trial. *Manuscript under review at the American Journal of Clinical Nutrition.*

Heneghan C, Kiely M, Lyons J and Lucey A. (2018). Effect of Berry-based food interventions on markers of cardiovascular and metabolic health: a systematic review of randomised controlled trials. *Journal of Molecular Nutrition & Food Research*, doi: 10.1002/mnfr.201700645.

Kelly NP, Kelly AL, and O'Mahony JA. (2017). Strategies for enrichment and purification of polyphenols of fruit-based origin. *Trends in Food Science & Technology*, 83, 248-258.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

Conference abstracts

Heneghan C, M. Kiely M, Manning E and Lucey AJ (2017). Effect of a blackberry-derived polyphenol enriched beverage on blood pressure: A randomized controlled crossover trial. *Proceedings of the Nutrition Society 76(OCE3) E57.*

Heneghan C, Lyons, J, Lucey A and Kiely M (2016). Dietary exposure of polyphenol-containing foods within the Irish population. Proceedings of the Nutrition Society 75(OCE3) E127.

Heneghan C, Lyons, J, Lucey A and Kiely M (2015). Food-based berry intervention studies and blood pressure: a systematic review of randomised controlled trials. Proceedings of the Nutrition Society 74 (OCE4), E227.

(iii) National Report - Nil

(iv) Workshops/seminars at which results were presented

Alice Lucey (UCC) highlighted the projects' activities in the following presentations at National and International meetings/conferences during the lifetime of the CardioRubus project:

June 21st, 2017: An oral presentation titled "Effect of a blackberry-derived polyphenol enriched beverage on blood pressure: A randomized controlled crossover trial." was presented by A Lucey at the 11th World Congress on Polyphenol Applications, hosted at the University of Vienna, Austria.

September 13-15th, 2016: A scientific poster entitled "Dietary exposure of polyphenol-containing foods within the Irish population" was presented by A Lucey at the 1st International Food Bioactives & Health Conference, Institute of Food Research, Norwich, UK, Sept 13-15th 2016.

Clara Heneghan (UCC) highlighted the projects' activities in the following presentations at National and International meetings/conferences during the lifetime of the CardioRubus project:

December 7th, 2017: An oral presentation titled "Can a polyphenol-enriched beverage increase polyphenol intakes in the Irish Population? Results of a scenario modelling assessment" was presented by C Heneghan at the New Horizons in Medical Research Conference hosted by UCC, Dec 7th, 2017.

June 21-23rd, 2017: An oral presentation titled "Effect of a blackberry-derived polyphenol enriched beverage on blood pressure: A randomized controlled crossover trial." was presented by C Heneghan at the Nutrition Society Irish Section Summer Conference, Queens University, Belfast, Northern Ireland.

To Note: Clara received the "Best Oral Presentation" Award as part of the Nutrition Society Student Competition at this conference.

December 8th, 2016: A scientific poster entitled "Dietary exposure of polyphenol-containing foods within the Irish population" was presented by C Heneghan at the New Horizons in Medical Research Conference hosted by UCC.

July 7-10th, 2016: An oral presentation entitled "Dietary exposure of polyphenol-containing foods within the Irish population" was presented by C Heneghan at the Nutrition Society Irish Section Summer Conference, hosted at UCD, Dublin Ireland.

February 2016: An oral presentation entitled "Berry-based food interventions on markers of cardiovascular and metabolic health: a systematic review of randomized controlled trials" was presented by C Heneghan at the Nutrition Society Postgraduate Conference, hosted by UCC.

Nov 24th, 2015, on oral presentation "A Summary update of Blackberry studies added to the eBASIS database" was presented by C Heneghan during the 'Extending eBASIS to study habitual intakes of bioactive compounds in the diet' Workshop, at the 4th BACCHUS Project Consortium Meeting, Bratislava, Slovakia.

Oct 27-30th, 2015, a scientific poster entitled "Dietary exposure of polyphenol-containing foods within the Irish population" was presented by C Heneghan at the International Conference on Polyphenols and Health (www.icph2015.com) held at the Congress Center Tours, France.

June 17th, 2015, a scientific poster entitled "Food-based berry intervention studies and blood pressure: a systematic review of randomised controlled trials" was presented by C Heneghan at the Nutrition Society Irish Section Summer Conference which was hosted by UCC.

Noirin Kelly (UCC) highlighted the projects' activities in the following presentations at national and International meetings/conferences during the lifetime of the CardioRubus project:

Dec 14th, 2015, an oral presentation entitled "Development of a polyphenol-enriched blackberry extract" was presented by N Kelly at the 44th Annual Food Research Conference organised by Teagasc, Moorepark.

To Note: Noirin was awarded 2nd prize for her Oral Presentation.

June 3-5th, 2015, a poster entitled "Development of polyphenol-enriched blackberry-based ingredients" was presented by N Kelly at the 9th ISANH Congress on Polyphenol Applications at St. Julian's in Malta.

Dec 10th, 2014, an oral presentation entitled "Development of polyphenol-enriched ingredients from blackberries" was presented by N Kelly at the 43rd Annual Food Research Conference organised by the Institute of Food Science and Technology Ireland (IFSTI) at UCD Dublin.

Dilip Rai (Teagasc Ashtown) highlighted the projects' activities in the following presentations at national and International meetings/conferences during the lifetime of the CardioRubus project:

Oct 27-30th, 2015, Dilip Rai attended the International Conference on Polyphenols and Health (www.icph2015.com) held at the Congress Centre Tours, France. A poster entitled 'Dipeptidyl peptidase-4 Inhibitory polyphenolic fractions from Irish and international blackberries' was presented at this conference.

Dec 14th 2014, a poster entitled "The effect of various drying methods on the antioxidant capacity of blackberries (Rubus sp.)" was presented at the 43rd Annual Food Research Conference at UCD Dublin.

(v) Intellectual Property applications/licences/patents - Nil
An Invention Disclosure Form (IDF) for the Cardio-Rubus beverage and polyphenol rich blackberry extract have been filed with the Office of Technology Transfer, UCC.

(vi) Other

Project feature article: Rai D, Kiely M & Lucey A. "Linking Blackberry polyphenols to heart health", TResearch (Teagasc quarterly magazine) Autumn 2015 Issue, pages 16-17.

5. Scientists trained by Project

Total Number of PhD theses: 1

Clara Heneghan. University College Cork,

PhD thesis title: "Dietary Bioactives and Cardiovascular Disease".

Academic Supervisors: Dr Alice Lucey & Professor Mairead Kiely.

PhD conferred February 2018.

Total Number of Masters theses: 2

Ms. Noirin Kelly. University College Cork, MSc (Food Science & Technology (Research)),

Thesis title: "Formulation of nutritional beverages and development of polyphenol-enriched ingredients from blackberry puree"

Academic Supervisor: Dr Seamus O'Mahony

Thesis submitted: November 2016.

Ms. Shauni Fitzgerald. University College Cork, MSc (Food Science & Technology (Self-funded)),

Thesis title: "Contribution of berries to cardio-protective bioactive intakes"

Academic Supervisors: Professor Mairead Kiely & Dr Jacqueline Lyons.

Thesis Submitted: 2015.

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
University College Cork	2	0.41
Teagasc	1	0.26
Total	3	0.67

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	6	3.85
PhD students	1	3.58
Masters students	1	2.00
Other (Research Nurse)	1	0.25
Total	9	9.68

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
1) Clara Heneghan (PhD student)	Module: "Next Generation Food Formulation", UCC, March 22 nd -24 th 2017.
2) Noirin Kelly (MSc student)	1.Module: "Next generation food formulation" hosted at UCC. 2. Module: "Industrial scale R&D" hosted at Teagasc, Moorepark.

9. Project Expenditure

Total expenditure of the project: €551,371

Total Award by DAFM: €591,178

Other sources of funding including benefit in kind and/or cash contribution(specify): €n/a

Breakdown of Total Expenditure

Category	UCC	Teagasc Ashtown	Total
Contract staff	147,625.61	42,181.63	189,807.24
Post graduates	120,181.91		120,181.91
Consumables	66,403.30	6,022.86	72,426.16
Travel and subsistence	9,323.84	878.79	10,202.63
Sub total	343,534.66	49,083.28	392,617.94
Durable equipment	17,146.54		17,146.54
Other	23,821.60		23,821.60
Overheads	103,060.40	14,724.98	117,785.38
Total	487,563.20	63,808.26	551,371.46

10. Leveraging

The output achieved from this project has informed multiple research funding applications. Specifically, two on-going research projects: RubusElite (FIRM 17/F/277) and ProcessPotato (FIRM 17/F/299) both funded by the Irish Department of Agriculture, Food and the Marine and an Enterprise Ireland (EI) -Innovation Partnership (IP 2018 0758E): " Technological innovations to ensure product safety and quality of sprouted seeds and wheatgrass juice" have been directly leveraged from the CardioRubus project. A competitive proposal has also been submitted to EI DTIF fund entitled 'Agricultural utilisation of bio-Refined Macro Algae Derived bio-Actives" (Michael Gaffney and Dilip Rai - TEAGASC). Additionally, to build on the findings of the current project a proposal for Commercialisation Feasibility Funding has been submitted to EI to support the commercialisation of these foods and future fruit-derived polyphenol enriched functional foods.

This project has also provided the opportunity for the conduction of a MSc project titled ""*Contribution of berries to cardio-protective bioactive intakes*"" supervised by the PI. This research augments and supports the CardioRubus project at no economic cost to the project. Collaborations leveraged from this project have also directly supported the participation of a Task Leader (D Rai) in two EU COST Actions, namely COST Action POSITIVE (FA1403) which investigated "Interindividual variation in response to the consumption of plant food bioactives" and CA16112 "NutRedOx: Personalized Nutrition in aging society: redox control of major age-related diseases" which support the advancement of research in the area of plant-based bioactives and health. This participation has facilitated a constructive two-way exchange of knowledge; the research activities of the CardioRubus project were of direct relevance to the objectives of CA POSITIVE where Dr Rai represented our project and has also co-authored extensive meta-analysis which has evaluated the major challenges which currently face phytochemical-based dietary intervention studies with health outcomes, a key outcome of this COST Action. Partaking in these EU COST Actions could potentially lead to pathways linking to successful HORZION 2020 project(s).

11. Future Strategies

This project provides evidence to support berry fruits as a widely available native source of polyphenols for the Irish population with potential health benefits. Data generated reinforces the importance of berry fruit cultivation and horticultural practices in Ireland and will assist in the development of future consumer-orientated functional foods. Continued dissemination of project findings to relevant stakeholders is a priority to ensure findings reach the widest audience possible.

Significant efforts have been undertaken to establish new research links for collaboration in further research which builds upon the objectives and achievements of the CardioRubus project. This has enabled the identification of key strategic directions for the expansion of research activity and our interaction with other RPOs, to include:

- The optimisation of delivery systems for polyphenols within food matrices to support enhanced polyphenol bioavailability and stability, supporting further integration between food science and nutrition. (Collaborations established: UCC and Teagasc). The Food Ingredients Research Group at UCC are continuing to work on the development and application of innovative filtration technology with Inopor and 2e Technical Development for fruit, dairy and cereal applications.
- Investigating potential roles for polyphenols in musculoskeletal health and performance nutrition. (Collaboration established: UCC and Waterford Institute of Technology).

- A better understanding of the metabolism of polyphenolic compounds within food matrices, particularly the role of gut microbiota in polyphenol metabolism (collaboration: APC, UCC and Teagasc).
- To investigate sustainability practices and commercial potential through the recycling of associated polyphenol waste-streams derived from beverage production (Collaboration: UCC and Teagasc).

These links while broad in their endeavours, integrate an array of multi-disciplinary skill-sets which will advance polyphenol research in the areas of optimised food delivery systems for polyphenols, musculoskeletal health and gut health. Many of these overarching objectives will be investigated as part of the on-going DAFM-funded RubusElite and Process Potato projects. The RubusElite project is a multi-disciplinary project which will develop and test for physiological efficacy a novel high-protein dairy-based beverage, tailored for performance nutrition which incorporates the antioxidant-rich blackberry-polyphenol extract developed as part of the CardioRubus project. This research will enable opportunities for future scientifically validated polyphenol-based functional foods and the delivery of out-put with tangible value to stimulate participation in Commercialisation activities, future EU funding initiatives and Cost Actions. This project also provides a constructive platform to expand research activities to investigate optimised delivery systems for other plant-based bioactives and nutrients within functional foods.