

Project number: 6610
Funding source: DAFM/FIRM

Date: June 2019
Project dates: Dec 2013 - May 2019

Whey beverages for the older adult



Key external stakeholders:

Dairy industry, Nutritional Beverage producers, Older Consumer

Practical implications for stakeholders:

Whey proteins can boost cellular antioxidant levels which will delay the ageing process

- Commercial whey proteins and products increased their antioxidant activity during gastrointestinal digestion.
- During digestion, whey peptides cross the intestinal barrier
- These bioavailable whey peptides boost the antioxidant status of muscle cells
- Whey beverages with marine and plant antioxidants were produced at pilot plant scale and maintained their antioxidant activities over a shelf life study

Main results:

- Whey products, individual whey proteins and hydrolysates exhibited antioxidant activity *in vitro*. Alpha-lactalbumin, subjected to simulated gastrointestinal digestion, exhibited the highest antioxidant activity.
- Peptides from whey protein digesta were capable of crossing the intestinal barrier and providing an antioxidant boost to muscle and liver cells.
- Whey protein-based beverages were formulated with a novel combination of plant and marine antioxidants at pilot plant scale using ultra high-temperature, pasteurization and spray drying processing technologies.

Opportunity / Benefit:

Whey protein-based antioxidant beverages could benefit the older consumer to meet their increased protein requirements and boost dietary antioxidant intake.

Collaborating Institutions:

UCD, Università degli Studi di Milano

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1. Project background:

Within each cell of our body, metabolic processes produce damaging free radicals. Cellular antioxidants mop up these free radicals to limit their damage. However as our body's age, our cell's ability to sequester free radicals is reduced so it is important our diet is rich in antioxidants. Whey is not a potent antioxidant but it is a high quality protein that can be added in large quantities to food products to meet the increased protein requirements of the older consumer. Bovine whey provides a complete protein source and is rich in branch chain amino acids. For several years, we and others have noted modest antioxidant activities of individual bovine whey proteins; β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin and immunoglobulins. The antioxidant activity of whey is attributed to the presence of sulphur containing amino acids and the ability to bind iron. However, whey bioactivity is not only the sum of its individual amino acids. In this study we identified several whey peptides, post-gastrointestinal digestion, capable of crossing the intestinal barrier. A selection of these whey peptides could reduce radical damage in cells representing skeletal muscle and liver, the metabolic powerhouse. Therefore addition of whey to food products would not only meet the extra protein demand of the older consumer but would also synergistically boost dietary antioxidant intake. We formulated, at pilot plant scale, whey protein-based beverages rich in marine and plant antioxidants for the ageing consumer and assessed the effect of thermal processing on the antioxidant activities of these beverages.

2. Questions addressed by the project:

Can whey proteins continue to exhibit antioxidant activity once consumed?

Can whey derived peptides transit the gastrointestinal barrier?

Can bioavailable whey peptides boost antioxidant defenses in muscle cells and in liver cells?

Does thermal processing damage antioxidant activity of whey protein-based beverages?

3. The experimental studies:

Commercial whey protein isolate, individual whey protein fractions and whey protein hydrolysates were exposed to simulated *in vitro* gastrointestinal digestion conditions (INFOGEST protocol). The antioxidant activity pre and post *in vitro* gastrointestinal digestion was determined by means of oxygen radical absorbance capacity (ORAC), radical cation decolourisation assay (ABTS) and ferric reducing ability of plasma (FRAP). Individual peptides generated during gastrointestinal digestion were identified by ultraperformance liquid chromatography/electrospray ionisation-high resolution tandem mass spectrometry (UPLC/ESI-HR-MS/MS). Intestinal cells, Caco-2 and HT-29, were exposed to digested whey proteins (0.5 mg/mL) to determine their oxidative status and to quantify mRNA transcripts of antioxidant genes SOD1, catalase and glutathione peroxidase 2. To study the transport of whey peptides after gastrointestinal digestion, a simulated model of the intestinal barrier was generated using co-cultures of differentiated intestinal epithelial human cells (Caco-2 and HT-29). After differentiation, cells were exposed to digested whey proteins (250 $\mu\text{g}/\text{cm}^2$) for 2 hours and peptides were identified in the apical and basolateral (bioavailable) compartments. Selected bioavailable peptides were synthesized to study their antioxidant activity in C2C12 murine muscle cells and HepG2 human liver cells, and their immune activity in THP-1 human macrophage cells. Finally at pilot plant scale, 200 mL single serving beverages were formulated using ultra-high temperature (UHT) or pasteurization. Spray dried powders were also produced to consider products for the export market. Beverage composition included WPI (4.4%), α -lactalbumin (0.1%), vitamin B12, astraxanthin, EGCG and marjoram extract. A shelf life study was performed on final products.

4. Main results:

The exposure of intact whey proteins to simulated *in vitro* gastrointestinal digestion conditions significantly increased their antioxidant activity ($p < 0.05$). Alpha-lactalbumin exhibited the highest antioxidant properties post digestion (determined by ORAC, ABTS and FRAP) which may be a result of its high degree of hydrolysis (57.59%), release of the antioxidant amino acid, tryptophan (6.955 μmol of tryptophan/g of protein) and release of several bioactive peptides. Intestinal cells treated with 0.25 mg/mL of digested α -lactalbumin significantly reduced ($p < 0.05$) radical formation and significantly increased ($p < 0.05$) mRNA transcripts of the antioxidant biomarkers, catalase and glutathione peroxidase 2. Intestinal barrier exposed to whey protein isolate found that of the 169 peptides identified in the digesta, only 31 were capable of crossing the barrier. From this bioavailable list, 6 peptides were synthesized for bioactivity testing: ALPM, GDLE,

VEELKPT, AVEGPK, TKIPA and VGIN. Peptides ALPM, GDLE, VGIN and AVEGPK (5 mM) significantly reduced ($p < 0.05$) radical formation in muscle and liver cells. In human macrophages all peptides, except TKIPA, elicited an immune response with secretion of the cytokine, IL-1 β , significantly increased ($p < 0.05$). This project successfully produced antioxidant rich (astraxanthin, EGCG and marjoram extract) whey protein based beverages at pilot scale. Ultra high-temperature processing produced beverages with greater clarity and stability than pasteurization without comprising antioxidant activity. Shelf life study indicated antioxidant activity was maintained over 24 weeks for UHT and spray dried products.

5. Opportunity/Benefit:

Whey proteins can be used in beverage formulations not only to provide protein but also boost dietary antioxidant intake of the older consumer. Thermal processing and spray drying at pilot plant scale does not reduce antioxidant activity. Whey proteins once consumed release antioxidant amino acids and peptides, as they transit the gut. Some whey peptides are capable of crossing the intestinal barrier to delay ageing processes in muscle. Whey based antioxidant rich formulations should therefore be considered for the older adult to combat muscle loss and delay or prevent the onset of sarcopenia.

6. Dissemination:

Main publications:

- Corrochano, A.R., Buckin, V., Kelly, P.M. and Giblin, L. (2018) 'Invited review: Whey proteins as antioxidants and promoters of cellular antioxidant pathways' *Journal of Dairy Science* 101: 4747 – 4761.
- Corrochano, A.R., Sariçay, Y., Arranz, E., Kelly, P.M., Buckin, V. and Giblin, L. (2018) 'Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion' *Journal of Dairy Science* 102: 54 - 67.
- Corrochano, A.R., Arranz, E., De Noni, I., Stuknytė, M., Ferraretto, A., Kelly, P.M., Buckin, V. and Giblin, L. (2018) 'Intestinal health benefits of bovine whey proteins after simulated gastrointestinal digestion. *Journal of Functional Foods* 49: 526 - 535.
- Arranz, E., Corrochano, A.R., Shanahan, C., Villalva, M., Jaime, L., Santoyo, S., Callanan, M.J., Murphy, E. and 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* 57: 102209.
- Corrochano, A.R., Ferraretto, A., Arranz, E., Stuknytė, M., Bottani, M., O'Connor, P.M., Kelly, P.M., De Noni, I., Buckin, V. and Giblin, L. (2019) 'Bovine whey peptides transit the intestinal barrier to reduce oxidative stress in muscle cells' *Food Chemistry* 288: 306 - 314.
- 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* 15: 5634 – 5646.
- Giblin, L., Yalcin, A., Bicim, G., Kramer, A., Chen, Z., Callanan, M.J., Arranz, E. and Davies, M. (2019) 'Whey proteins: targets of oxidation, or mediators of redox protection' *Free Radical Research* 53: 1136 – 1152.

Conferences:

- Corrochano, A.R., Sariçay, Y., Brodkorb, A., Buckin, V., Kelly, P.M. and Giblin, L. (2015) 'Antioxidant activity of whey pre and post gastrointestinal digestion in vitro' *44th Annual Food Research Conference*, December, Fermoy, Ireland.
- Corrochano, A.R., Sariçay, Y., Buckin, V., Kelly, P.M. and Giblin, L. (2016) 'Gastrointestinal digestion increases the antioxidant activity of whey protein hydrolysates. *13th IDF Dairy Science & Technology Symposia*, April, Dublin, Ireland.
- Corrochano, A.R., Sariçay, Y., Brodkorb, A., Buckin, V., Kelly, P.M. and Giblin, L. (2016) 'Is antioxidant activity of whey protein maintained during upper gastric transit?' *17th International Conference on Oxidative Stress Reduction, Redox Homeostasis and Antioxidants*, June, Paris, France.
- Kelly, P.M. (2016) 'Membrane technology developments' *13th IDF Dairy Science & Technology Symposia*, April, Dublin, Ireland.
- Corrochano, A.R., Arranz, E., Ferraretto, A., Bottani, M., Stuknytė, M., Buckin, V., Kelly, P.M., De Noni, I. and Giblin, L. (2017) 'Antioxidant activity of whey protein: survival during gut transit and bioavailable to target cells?'. *NutRedOx COST Action CA16112 Meeting & the 6th NutriOx Atelier*, September, Strasbourg, France.
- Corrochano, A.R., Ferraretto, A., Bottani, M., Stuknytė, M., Cattaneo, S., Arranz, E., Buckin, V., De Noni, I., and Giblin, L. (2017) 'Upper gut transit of whey protein' *Society for Dairy Technology Spring Conference Advances in Nutritional Dairy Products & Ingredients*, April, Cork, Ireland.
- Corrochano, A.R., Ferraretto, A., Bottani, M., Stuknytė, M., Cattaneo, S., Buckin, V., De Noni, I. and Giblin,

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- Arranz, E., Corrochano, A.R., Villalva, M., Jaime, L., Santoyo, S., Murphy, E. and Giblin, L. (2018) 'Antioxidant activity of whey-based beverages: effect of shelf life and gastrointestinal transit on bioactivity' *NutRedOx COST Action CA16112 Meeting*, February, Palma de Mallorca, Spain.
- Arranz, E., Corrochano, A.R., Villalva, M., Jaime, L., Santoyo, S., Murphy, E. and Giblin, L. (2018) 'Efficacy of antioxidant whey-based beverages' *2nd Food Bioactives and Health Conference*, September, Lisbon, Portugal.

Popular publications:

- Giblin, L., Arranz, E. and Corrochano, A.R. (2017) 'The benefits of Whey' *Teagasc T Research* 12:3: 28-29.
- Quinlan, A. (2020) 'Drink to keep people younger by delaying muscle ageing' *Irish examiner* 2 March 2020.

7. Compiled by: Linda Giblin
