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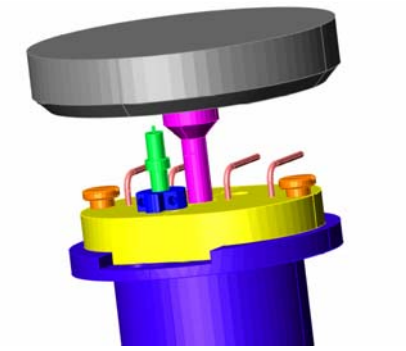
Development of food ingredients for modulation of glycemia



Key external stakeholders:

Ingredient suppliers, nutritional beverage manufacturers

Practical implications for stakeholders:

This research provides scientifically validated knowledge on how to combine dairy proteins and carbohydrates for controlled structure development and glucose release in foods. This included studying the effect of the interaction between carbohydrates and dairy proteins on viscosity development and susceptibility to enzymatic hydrolysis and explored the possible modulating effects of dairy proteins, i.e., alpha(α)-casein, βeta(β)–casein, βeta(β)-Lactoglobulin & alpha(α)-Lactalbumin on the gelatinisation characteristics and related functional behaviour of starch (waxy maize) in food formulations.

We found that it is possible to develop different physical properties in solution due to the interactive effects of varying combinations of carbohydrates (konjac glucomannon, starch, maltodextrin and inulin) and proteins (alpha(α)-casein, beta(β)-casein, alpha(α)-lactalbumin and beta(β)-lactoglobulin). Rheological analysis demonstrated that under suitable gelling conditions;

- Inulin had little effect on the gel-strength of β-lactoglobulin compared to konjac glucomannon
- Konjac glucomannon enhances gelling properties
- Adding maltodextrin to starch in solution results in higher viscosity than starch alone during pasting and the gelatinisation profiles of starch alter when maltodextrin is present.

Main results:

- Inulin had little effect on the gel-strength of β-lactoglobulin compared to konjac glucomannon which enhances gelling properties.
- The gelatinisation profiles of starch change when maltodextrin is present, e.g. the addition of maltodextrin to starch in solution results in higher viscosity than starch alone during pasting
- It is hypothesised that gelatinisation of starch in structured casein networks provides a method for decreasing the digestion rate of the starch and can thus contribute to modulation of postprandial glucose fluctuations.
- Different proteins, in particular α- casein and β- casein, have different abilities to alter the viscosity and subsequent glucose release of food systems.
- Caseins reinforce the structure of starch granules during gelatinisation.

Opportunity / Benefit:

The combination of different proteins and selected carbohydrates creates new opportunities for developing functionality in dairy based beverages. The project can contribute to the development of nutritional formulations designed for sports and/or medical applications such as patients with Type 2 diabetes and/or glucose intolerance. Expressions of interest in accessing or furthering this research are welcome.

Collaborating Institutions:

UCC

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1. Project background:

The rate of glucose absorption in the gastrointestinal tract governs the rate of appearance of glucose in the blood and consequently the hormonal balance such as insulin levels which effect satiation (resulting in increased consumption of food), fat metabolism, and ultimately weight gain. Postprandial increases in blood glucose are dependent upon factors which include physiological status of the body and also the structure of the food consumed. This project explored interactions between four dairy proteins (i.e., α-casein, ß–casein, ß-Lactoglobulin & α–Lactalbumin) and four different carbohydrates (i.e., inulin, konjac glucomannon, maltodextrin and waxy maize starch) to determine how ingredient combinations may be formulated with a view to modulating glycemia (i.e., blood glucose levels). The effect of the different proteins and carbohydrates on viscosity development and in particular the swelling characteristics of starch were determined.

2. Questions addressed by the project:

- What range of structures / functionality can be achieved by combining proteins (i.e., α-casein, ß–casein, ß-Lactoglobulin & α–Lactalbumin) and carbohydrates (i.e., inulin, konjac glucomannon, maltodextrin and waxy maize starch) in solution on heating.
- Can the physical properties of selected protein-hydrocolloid systems be altered for controlled viscosity development in foods. What is the understanding of how such mixed polymer systems breakdown in the course of simulated digestion Is it possible to modulate glucose release from starch by formulation with different dairy proteins, i.e., to control the behavior of mixed polymer systems (proteins and carbohydrates) in foods with a view to ingredient development.
- Can an *in-vitro* system be developed for simultaneous measurement of viscosity and enzymatic hydrolysis. What is the relationship between laboratory studies and follow-up in-vivo studies. Can these methodologies be combined to establish glucose absorption patterns from foods.
- What are the effects of different protein-carbohydrate mixtures on gastrointestinal gene expression *in-vitro* and glycemic index, metabolites and gastrointestinal hormones *in-vivo.*

3. The experimental studies:

Experiments were designed to study the interactions between selected proteins (α-casein, ß–casein, ß-Lactoglobulin & α–Lactalbumin) and carbohydrates (maltodextrin, starch, konjac glucomannon and inulin) with the objective of identifying synergistic effects which can be exploited for the development of new food ingredients for use in foods with glucose regulating functionally.

- Ternary phase diagrams and HPLC were used to study primary interactions of different protein-carbohydrate systems. Inulin and glucomannon were initially selected because of their low and high viscosities whereas maltodextrin and starch because of differences in their resistance to hydrolysis by α-amylase.
- A novel rheological stomach was engineered and used to study the effect of protein / carbohydrate interactions on structural properties and the ensuing influence on enzyme kinetic parameters (i.e., hydrolysis of carbohydrate). A method for *in-vitro* digestion was developed and optimised taking into account enzyme concentration and other specific physiological considerations including buffer type, pH, dilution effects that occur in the small intestine and speed of mixing.
- Bioassays were developed using gastrointestinal cell lines (for example Caco-2, HT-29) to measure gene response to selected protein-carbohydrate complexes.
- A real-time PCR assay for the satiety signal, peptide YY (PYY) was established. Experiments were performed to investigate which fractions of the casein hydrolysates were responsible for the differences in incretin responses - fractions of α and β casein hydrolysates were generated via

reverse-phase HPLC. Four fractions in total were generated for each protein and placed on an STC-1 cell line for 4 hours at a concentration of 3.8mg/ml. Supernatant was collected and RNA was isolated, quality assessed and quantified. cDNA was then synthesised and used for real-time RT-PCR assays using Lightcycler and SyBrG technology. Levels of Proglucagon (GLP-1), GIP, CCK and PYY gene expression were measured in response to the casein fractions. In addition, GLP-1 and GIP ELISAs were preformed on the cellular supernatant in order to determine levels of secreted incretin peptides.

- *In-vitro* studies were used to design isoglucosidic protein-carbohydrate suspensions for evaluation in monogastric animal model system (pig). Postprandial metabolite and gastrointestinal hormone responses were correlated with glucose response and type of protein-carbohydrate system used.
- *In-vivo* studies were completed on human subjects at the University of Surrey in the UK using an analogue cheese matrix.

4. Main results:

- The effect of maltodextrin on viscosity development in protein stabilised colloidal systems was determined. The methodologies and knowhow have been implemented at Teagasc for use by industry.

- The presence of caseins during heating makes gelatinised starch granules smaller and tougher and could be a useful guide in formulation of products where starch and milk (or milk-protein ingredients) are used together. The inability of the more hydrophilic whey proteins to reinforce the starch granules suggests that binding of caseins to the lipid–protein layer occurs predominantly by hydrophobic association. The first evidence demonstrating the ability of protein to penetrate starch during gelatinisation was demonstrated and this was deemed a significant finding.

- New methodologies are in place at Teagasc for measuring the effect of foodstuffs to glycemic response, including *in-vitro* (new reactor cell) and *in-vivo* (catheterising of pigs) tests.

- A number of bioassays have been developed on the project. These methodologies have potential use in the development of new bioactives for use in functional foods, having a significant impact as a new tool for industry to develop foods for the growing anti – diabetic / glucose control sector of the special foods market segment. Findings demonstrated the effect of starch gelatinisation with or without casein on (1) gene expression and peptide secretion levels of the incretin hormones glucagon-like peptide 1 and glucose-independent insulinotropic polypeptide and (2) gene expression of the sodium–glucose co-transporter and GLUT-2 in intestinal cell culture systems. The intestinal epithelial cell line, STC-1, and the enteroendocrine colonic cell line, Caco-2, were exposed to *in-vitro* digested foods (starch gelatinised with α-casein, starch gelatinised with β-casein and gelatinised starch alone). The encapsulation of starch with casein before *in-vitro* digestion lowered levels of incretin hormone secretion. Digestion of starch gelatinised with casein also released less glucose than starch alone as indicated by significantly ($p<0{\cdot}05$) lower levels of glucose transporter mRNA transcripts. Some subtle cellular response differences were observed following exposure to starch gelatinised with α-casein compared to β-casein.

5. Opportunity/ Benefit:

The project has shown that different proteins, in particular, α- and β- casein have different abilities to alter the viscosity and subsequent glucose release of food systems. The effect of the different proteins on the rheological properties of foods through manipulation of the swelling properties of starch provides mechanisms for modulation of food structures for the industry. Since nearly all foods contain both proteins and starches, the difference between dairy proteins, i.e., casein and whey proteins, in their ability to effect viscosity and texture development is an important finding for industrial applications.

6. Dissemination:

Outcomes from the project have been disseminated through peer-reviewed publications, technical publications, conference presentations and posters, RELAY workshops and directly to industry. Findings were presented at the International Hydrocolloids Conference 2008 in Singapore, the 5th International Symposium on Food Rheology and Structure - Swiss Federal Institute of Technology in Zurich 2009, Food Science and Technology Conference, University College Cork 2008/2009/2010 and 2011, Functional Foods Research Conference 2010 and the International Congress on Obesity, Stockholm, Sweden 2010.

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Main publications:

- Bruen, C.M, Kett, A.P, O Halloran, F, Chaurin, V, Fenelon, M.A, Cashman, K.A and Giblin, L. (2012). Effect of gelatinisation of starch with casein proteins on incretin hormones and glucose transporters in-vitro. British Journal of Nutrition. Jun 27: 107, Issue 2, 155-163
- Kett, A.P, Bruen, C.M, O Halloran, F, Chaurin, V, Lawlor, P.G, O Mahony, J.A, Giblin, L, Fenelon, M.A. (2011) The effect of α- or β- casein addition to waxy maize starch on post prandial levels of glucose, insulin and incretin hormones in pigs. In press: Food and Nutrition Research.
- Kett, A. P, Chaurin, V, Fitzsimons, S.M, Morris, E.R, O'Mahony, J.A and Fenelon, M.A. (2012). Influence of milk proteins on the pasting behaviour and microstructural characteristics of waxy maize starch. Submitted to Food Hydrocolloids.

7. **Compiled by:** Dr. Mark Fenelon

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