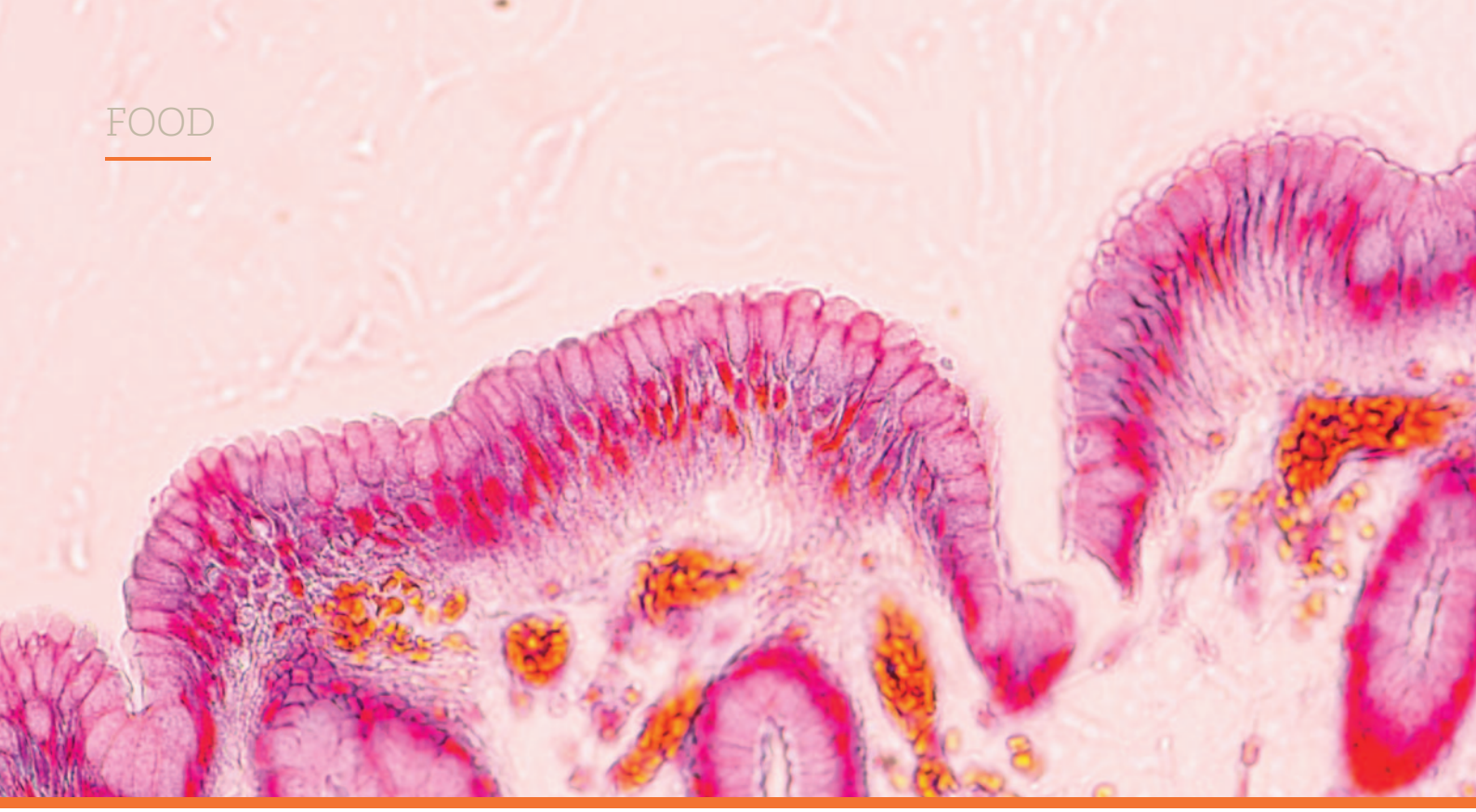

The project investigates gastro-intestinal digestion in the infant gut using in vitro *models.*

A gentler approach to making infant formula

TEAGASC and Cork Institute of Technology researchers are investigating ways to improve infant formula manufacture through gentle processing.

At present, 10-12 % of the entire global infant formula (IF) market is manufactured in Ireland. This is an important export product. IF usually undergoes high heat treatment to ensure safety and long shelf life, but there is scope to improve its nutritional quality. For example, heat treatment denatures proteins, and causes proteins to aggregate and interact with sugars to form various by-products.

Our project, entitled Thermal Or Membrane processing for Infant formula (TOMI), aims to produce IF by cascade membrane filtration. This will reduce heat treatments to harness the health benefit of dairy proteins in their natural state. The new formula will be tested for microbial and chemical safety. We will also follow the fate of proteins during gastro-intestinal digestion in the infant gut using *in vitro* models, allowing us to compare the health benefits of our new formulation to standard IF. TOMI has the potential to radically improve the quality of IF produced in Ireland.

Manufacture of cascade membrane infant formula

Cascade membrane IF (C-IF) production differs from standard IF manufacturing, in that it involves a split stream processing step. The protein component is processed as a parallel but separate stream to the other components (lactose, fat, minerals). The protein stream is processed using membrane filtration, while the remaining components are processed using standard heat treatment, and the two streams are recombined to give the final product. At the Teagasc Moorepark pilot plant (**Figure 1**), C-IF was manufactured and the product compared to a standard IF manufactured in the same facility. The removal of a heat step and the characteristics of the membrane-filtered protein stream resulted in a different product to standard formula. These characteristics included a different protein profile, more native proteins, and reduced potential for thermally induced degradation products.

Proteins in cascade membrane infant formula

When we compared C-IF powder to standard heat-treated IF, C-IF powder contained eight-fold more native whey proteins. The state of protein aggregation also differs, where the more gentle membrane filtration results in fewer aggregated proteins compared to heat treatment. In addition, it was shown that low-heat treatment can retain the native structure and activity of some indigenous enzymes, which may exhibit added health benefits. To investigate the potential health benefits of the altered protein profile, the standard and C-IF powders were subjected to a simulated gastro-intestinal digestion mimicking the infant gut. The rate of digestion of the proteins appeared to differ between C-IF and standard IF, with intact whey proteins taking longer to digest in the gastric phase in C-IF powder. At Teagasc, human intestinal barrier models (Caco-2 monolayers) were cultured and differentiated in transwell plates over 21 days. Exposing these monolayers to digested IF samples reduced barrier integrity. In

particular, standard IF post digestion significantly decreased transepithelial electrical resistance (TEER) values compared to C-IF ($P < 0.05$). This may indicate that IF produced by cascade membrane filtration can promote a healthier gut barrier.

The new formula will be tested for microbial and chemical safety. We will also follow the fate of proteins during gastro-intestinal digestion in the infant gut using *in vitro* models, allowing us to compare the health benefits of our new formulation to standard IF.

Microbial and chemical safety of cascade membrane infant formula

In Cork Institute of Technology, the microbial safety of the pilot scale process was assessed by standard plating methods. No significant differences in the microbial quality of the final powder products produced by standard and membrane processing were identified. However, additional work detailing the microbial profile of C-IF using state-of-the-art molecular analysis is ongoing. Advanced glycation end products (AGEs) are non-enzymatic by-products formed when sugars and proteins are heated together. Scientific studies are inconclusive regarding negative effects of AGEs formed in milk products, but the general impression is that AGEs are not naturally present in milks and should not, therefore, be in infant formula. Recent microbial contamination issues have pushed IF manufacturers to resort to more extreme heat treatments, increasing the potential for AGE formation. The levels of a characteristic AGE, carboxymethyl lysine, were determined in standard IF and C-IF, using an ELISA kit. Significantly lower levels of carboxymethyl lysine were observed for the C-IF compared to standard IF. These results are encouraging, but to increase data accuracy and investigate other AGEs, high-performance liquid chromatography and mass spectrometry (HPLC MS/MS) instrumentation is required. As such, we have initiated a collaboration with Prof. Mike Davies at the University of Copenhagen, Denmark, to quantify levels of these compounds on day one of manufacture, three months later (shelf life), and to investigate what happens to these compounds during gastro-intestinal digestion in the infant gut model.

Innovation in IF

IF constitutes a particularly sensitive food product given the vulnerability of the target population. Therefore, the benefits of any innovation must clearly outweigh any risk involved in change management. The TOMI project has demonstrated that C-IF will provide a product with an alternative native protein profile, reduced AGEs and indicated positive effects on the intestinal cells. Additional work is required to quantify these benefits and confirm that the alternative manufacturing process will provide a significantly improved IF.

The Teagasc Moorepark pilot plant.

Acknowledgements

This work was funded by the Department of Agriculture, Food and the Marine (FIRM Grant No. 15/F/604 TOMI). Yihong Chen is in receipt of a Teagasc Walsh Fellowship. The experimental work was undertaken by postdoctoral scientist Simona Bavaro (Teagasc) and PhD students Colm Shanahan (CIT) and Yihong Chen (Teagasc). Future work will include a preclinical (pig) trial at the Pig Development Department (Teagasc) under the direction of Peadar Lawlor.

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