Egg processors; mineral and biologicals, opportunities for waste valorisation

Graham J. Hickman, Andrew Stacey, Carole C. Perry*

Nottingham Trent University

Biomolecular Materials and Interfaces Research Group, Department of Chemistry and Interdisciplinary Biomedical Research Centre, Clifton Lane, Nottingham, NG11 8NS

Carole.Perry@ntu.ac.uk

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Waste in the food and egg industry.

- The UK food industry is striving to better manage and reduce waste (~4.2% of production).[1]
- UK industry produces some 2.4 million tonnes of waste and surplus per annum. [1]
  - **Significant costs associated with disposal**
    - Problem symptomatic of deeper inefficiencies in industrial processes.
  - **Corporate social responsibility**
    - unsustainable to send this amount of waste to landfill.
- The industry generates at least 15,000 tonnes of waste per factory per annum.
  - ***Potentially valuable materials in food waste which can be extracted.***

[1] WRAP, Quantification of food surplus, waste and related materials in the grocery supply chain, 2016

UK egg processors.

In 2017 UK households were predicted to consume **12.9 billion** eggs.[1]

Major products of egg processors include:

- Boiled egg – ~30,000 tonnes for 2017[1]
- Liquid egg – ~60,000 tonnes for 2017[1]
- Other e.g. mayonnaise.

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Margaret Thatcher's victory diet: 28 eggs a week

As Baroness Thatcher plotted her victorious election campaign of 1979, one key part of her strategy was so closely guarded that it has never before been revealed: her crash diet.

By Gordon Rayner
12:48PM GMT 30 Jan 2010

Margaret Thatcher
News »
How about that? »

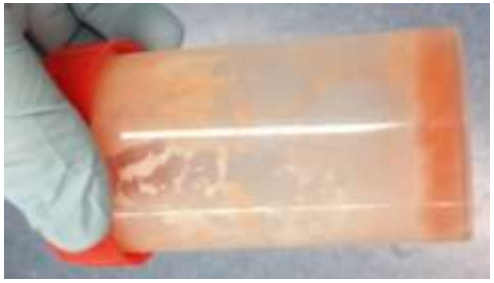
The industry generates at least 15,000 tonnes of waste per factory per annum.

[1] https://www.gov.uk/government/statistics/egg-statistics
[2] https://www.telegraph.co.uk/news/politics/margaret-thatcher/7111052/Margaret-Thatchers-victory-diet-28-eggs-a-week.html

Egg processor waste streams … the problem.

Each production process generates unique waste.

- Boiled egg production generates
  - **shell** from shelling operations and whole **boiled/raw egg** waste from breakage – tonnes per day – mostly solid.
- Liquid egg production generates
  - **shell** from cracking and liquid egg (**white and yolk**) from processing inefficiencies (~4-5%) – tonnes per day – solids and liquids.



Waste currently disposed into landfill or onto farmland.

- Care taken to control protein level and, as an animal by-product waste (category III) is controlled by law.

The business challenge.

How can you find find someone to use a recycled product?

- Finding problems in other industries that can use the food co-products.
- Considerations of potential customers:
  - Low carbon miles.
  - Heavy metal contamination.
  - Inconsistency of supply.
  - Importing.
  - Vegetarian sourcing.
- Key considerations:
  - Size of market?
  - Sustainable pricing?
  - Need?
  - Desire to change?

The academic challenge for industrial projects.

- Applying research to a specific (often limited) industrial problem generates issues. Endless **conflict of I.P vs. disclosure**.

Traditional materials from new sources – conflicts in analytical methods for calcium carbonate.

Short title (<70 char.): Biogenic calcium carbonate – conflicts in analytical methods.

David J. Belton, Graham J. Hickman and Carole C. Perry*

Biomolecular and Materials Interface Research Group, School of Science and Technology,

Nottingham Trent University, Clifton lane, Nottingham, NG11 8NS, the United Kingdom

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Abstract

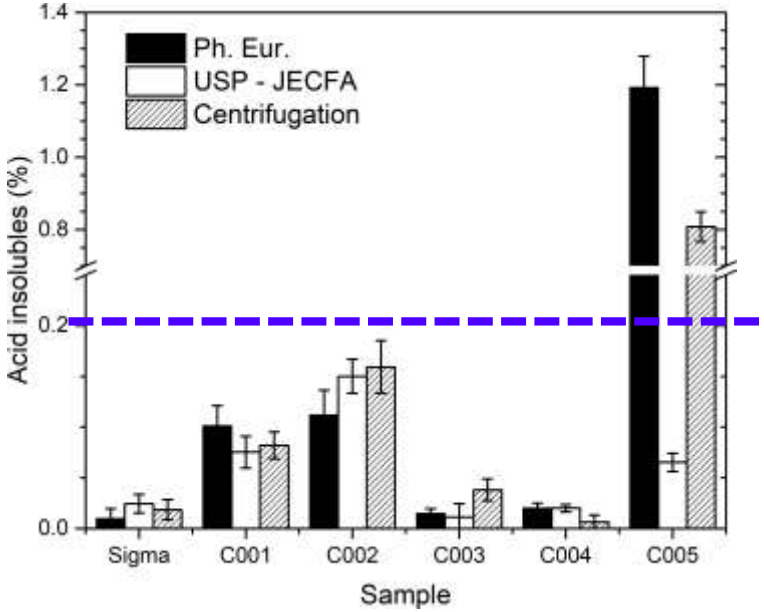
Calcium carbonate or E170 is a common additive in food and pharmaceutical products. In addition to acting as a source of calcium, the carbonate has a range of uses including as a colour, regulator of acidity and bulking agent, among others. Globally, a range of regulatory agencies and pharmacopoeia control the analyses and specification of additives in food, supplements, pharmaceutical substances and excipients. Accordingly, a range of specifications and analyses exist for calcium carbonate depending on the application and target market of the product. In this contribution, we analyse calcium carbonates from geological, synthetic and biogenic sources, focussing on acid insoluble impurities. We conclude that analysis of calcium carbonate from different origins may require the modification of existing tests to comply with various regulatory bodies, due to the variation of impurities specific to the source of the material. We suggest an analytical approach involving centrifugation that improves the analytical efficiency, especially for calcium carbonated of biological origin.

Keywords: calcium carbonate, E170, FCC, USP, pharmacopeia

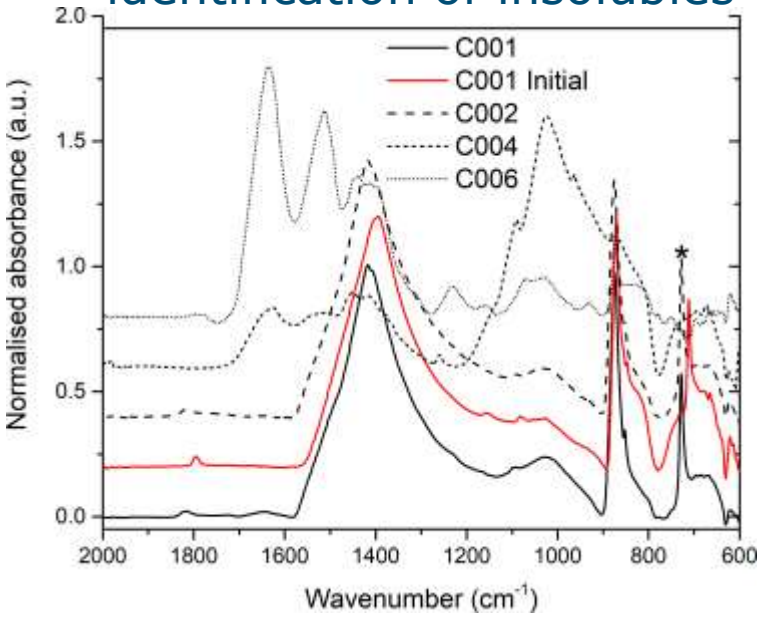
Conflicts in assays for calcium carbonate.

- Regulation has large impact on valorisation, especially applications in food and healthcare. – Not easy applications for waste!

acid insolubles



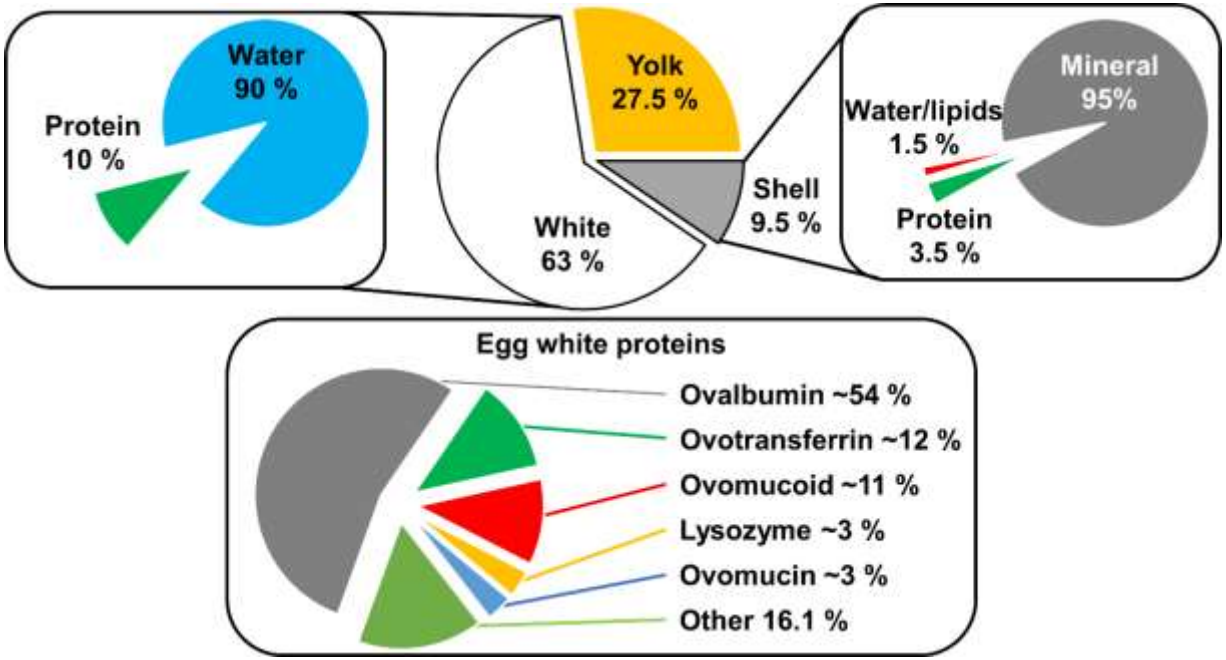
identification of insolubles



Even existing regulation (e.g. E170) may struggle with novel products from waste streams.

Egg co-products as a resource?

A breakdown of the composition of the egg shows that the waste contains many potentially valuable materials both organic and inorganic.



E. D. N. S. Abeyrathne, H. Y. Lee, D. U. Ahn; Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review, *Poultry Science*, Volume 92, Issue 12, 1 December **2013**, Pages 3292–3299, https://doi.org/10.3382/ps.2013-03391
Cordeiro, C. M., & Hincke, M. T. (**2011**). Recent patents on eggshell: shell and membrane applications. *Recent Pat. Food Nutr*. Agric., 3, 1.
Kovacs-Nolan, J., Phillips, M., & Mine, Y. (**2005**). Advances in the value of eggs and egg components for human health. *J. Agric. Food Chem*., 53, 8421.

Long journey for our solution …

- **IUK - Proof of Concept – Eggshells.**

Viability (literature and laboratory) of recovery from the shell co-product. Chemistry was viable, recovery matched that indicated in the literature. Needed greater depth.

- **IUK - 131471 – Nutrition for Life – "Eggshell waste – Investigating the chemistry of the isolation processes of high value materials"**

Collaborative 1 year PoC defining a recovery process and developed methodologies. Focus was difficult until customer discussions lead to a real need – $CaCO_3$.

- **IUK - 102402 – Recovering valuable materials from waste - "Recovery and processing of high value food and pharmaceutical ingredients from waste egg shells"**

Scale up (laboratory to < 1 tonne) and integration down-stream.

Composition of the eggshell co-product.

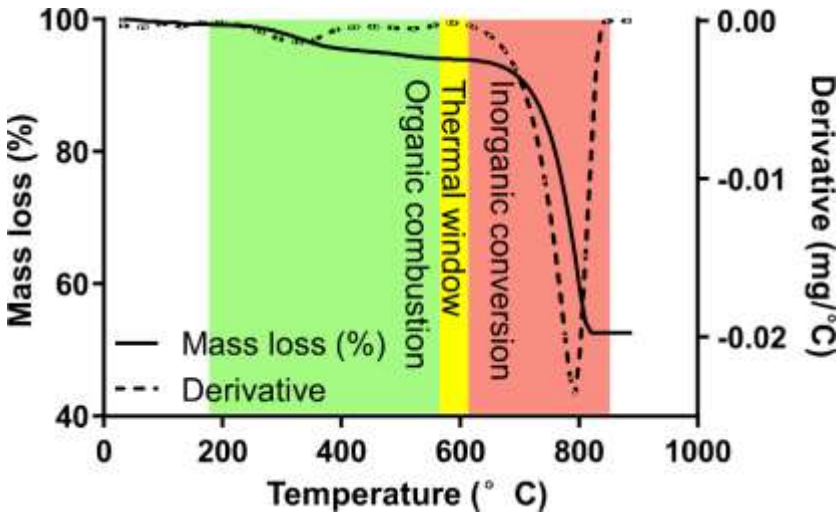
Composition by mass of milled eggshell co-product (freeze dried)		
% Organic	**% $CaCO_3$**	**% Other (water)**
3.89 ± 0.11	92.98 ± 0.11	3.13 ± 0.22

The co-product matched what was expected of the composition of eggshell.

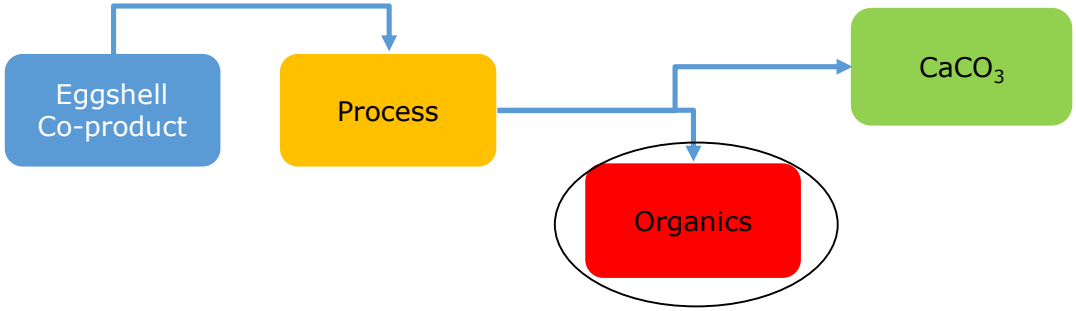
Methods were explored to separate the two major components (organic & inorganic).

- Recovery of inorganics by thermal treatment.
- Recover of organics by acid dissolution of the carbonates.

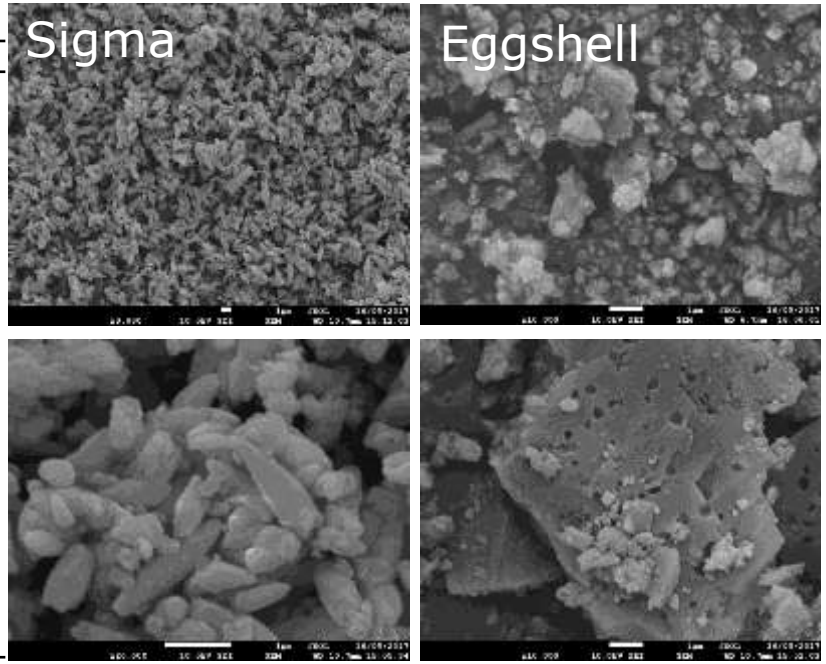
Ideally recover both the organics and the inorganics in the process!



The process & the product.



Test	EP (PhEur)	E170	Sigma	Eggshell
Identity	PASS	PASS	PASS	PASS
Assay (%)	>98.5-100.5	>98.0-100.0	100.03	>95.00
Loss on drying (%)	<2.0	<2.0	0.20	<2.0
Acid-insoluble substances (%)	<0.2	<0.2	0.02	0.2-0.3
Free alkali (%)	-	<0.05	<0.05	<0.05
Magnesium & alkali salts (%)	<1.5	<1.0	0.65	3.0 - 4.0
Fluoride (mg/kg)	-	<50 mg/kg	13.4	<4.0
Antimony (mg/kg)	-	<100	0.05 (ppb)	<2.0
Barium (mg/kg)	PASS	<100	5.49	<20.0
Copper (mg/kg)	-	<100	NR	<2.0
Chromium (mg/kg)	-	<100	0.20	<1.0
Zinc (mg/kg)	-	<100	1.72	<3.0
Arsenic (mg/kg)	<4.0	<3.0	2.7 (ppb)	2.0 (ppb)
Cadmium (mg/kg)	-	<1.0	0.05 (ppb)	0.03 (ppb)
Lead (mg/kg)	<20	<3.0	0.27 (ppb)	0.1 (ppb)
Mercury (mg/kg)	-	-	0.14 (ppb)	0.15 (ppb)
Chlorides (mg/kg)	<330	-	PASS	PASS
Sulphides (%)	<0.25	-	PASS	PASS
Iron (mg/kg)	<200 mg/kg	-	95.44	<5.0



FoodWasteNet (PoC 15_6) "Viability of obtaining biopharmaceuticals from eggshell waste"

Goals:

- Determine extractable components and deliver optimised processes for recovery of different biomolecules from the waste stream.
- Define customer specification for the different components isolated from the waste stream i.e. purity.
- Assess both economic viability to determine value of the biomolecules and extraction viability. To what extent can further treatment can be conducted?
- Examine different routes to process extracted biologicals to customers specification, considering industrial scalability.

FoodWasteNet (PoC 15_6) - Method

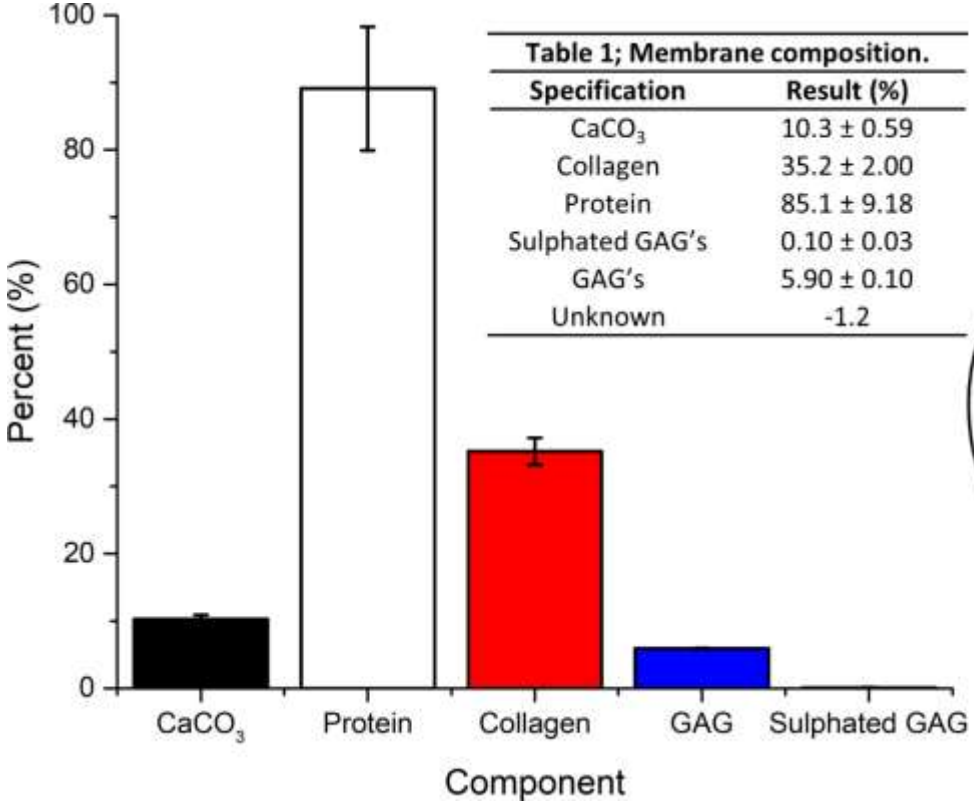
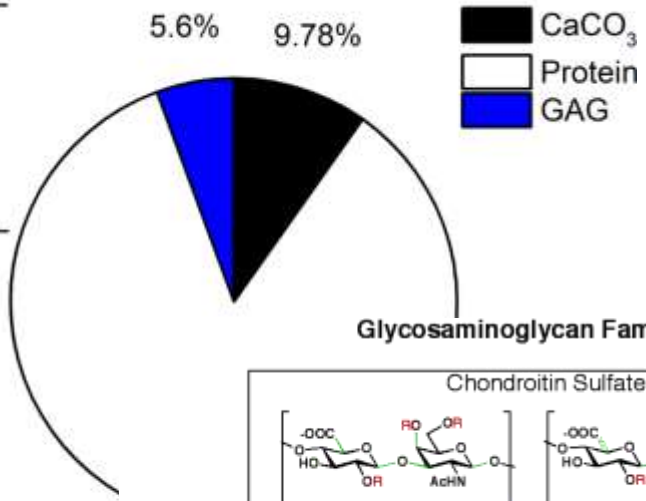
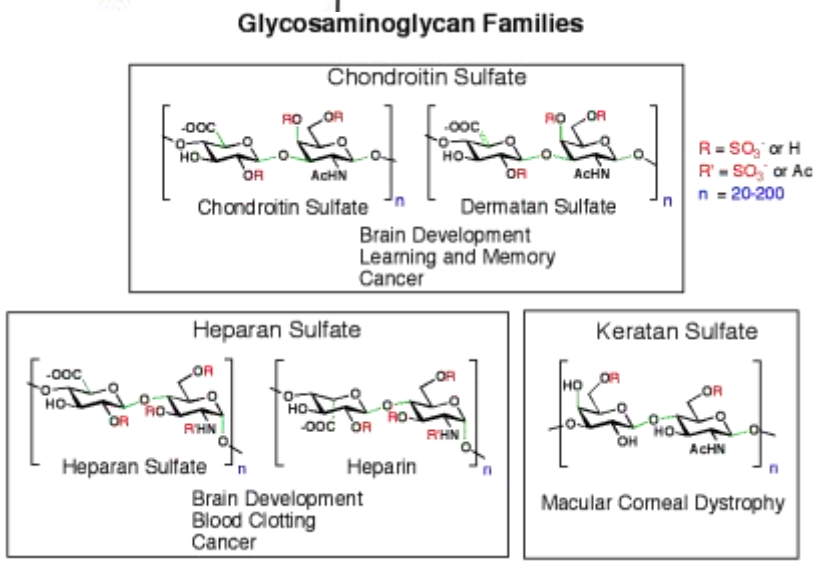


Table 1; Membrane composition.

Specification	Result (%)
$CaCO_3$	10.3 ± 0.59
Collagen	35.2 ± 2.00
Protein	85.1 ± 9.18
Sulphated GAG's	0.10 ± 0.03
GAG's	5.90 ± 0.10
Unknown	-1.2





Calculated as percentage of dry organic. Calcium carbonate calculated from calcium concentration determined by ICP-OES. Protein determined by the Lowry assay using albumin standard. Collagen calculated as a percentage of protein content based on hydroxyproline content. Glycosaminoglycans (GAGs) determined using the carbazole assay with sulphated glycosaminoglycans determined using dimethylmethylene blue.

http://chemistry.caltech.edu/groups/hsieh/hsieh1/research.htm

FoodWasteNet (PoC 15_6) Approach for upscaling recovery - Ultrafiltration.

Scalable technology for separation and concentration of biomolecules depending on the Mol. Wgt. Cut Off of a semi-permeable membrane.

Analysis of the retentate showed successful recovery with minimal irreversible fouling.

FoodWasteNet (PoC 15_6) Ultrafiltration-results.

	GAG, Collagen and Total Protein content of permeate (P) and retentate (C).				
	Sample	**GAG (mg/mL)**	**GAG sulphated (mg/mL)**	**Collagen (mg/mL)**	**Total protein (mg/mL)**
Membrane 1	4P1	N/A	0.02 ± 0.00	N/A	0.34 ± 0.01
	4P2	0.41 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	1.87 ± 0.01
	4P3	0.03 ± 0.01	0.01 ± 0.00	0.05 ± 0.01	3.96 ± 0.51
	4P4	0.63 ± 0.10	0.01 ± 0.01	0.10 ± 0.20	6.82 ± 0.13
	4C1	0.29 ± 0.06	0.04 ± 0.01	1.90 ± 0.90	4.43 ± 0.11
	4C2	0.08 ± 0.15	0.01 ± 0.00	5.89 ± 0.76	1.70 ± 0.15
	4C3	**8.23 ± 1.41**	**2.68 ± 0.59**	**9.02 ± 0.78**	**22.50 ± 2.75**
	4C4	**14.4 ± 1.43**	**6.90 ± 0.84**	**11.2 ± 0.68**	**31.32 ± 2.70**
Membrane 2	5P1	0.00 ± 0.06	0.00 ± 0.00	0.02 ± 0.01	1.71 ± 0.01
	5P2	0.30 ± 0.09	0.00 ± 0.00	0.02 ± 0.01	2.56 ± 0.14
	5P3	0.18 ± 0.06	0.01 ± 0.01	0.05 ± 0.01	5.85 ± 0.33
	5C1	0.49 ± 0.04	0.03 ± 0.01	2.93 ± 0.38	3.98 ± 0.30
	5C2	0.43 ± 0.08	0.13 ± 0.06	4.42 ± 0.32	7.66 ± 0.34
	5C3	5.52 ± 0.58	2.48 ± 0.37	9.02 ± 1.52	21.3 ± 3.32

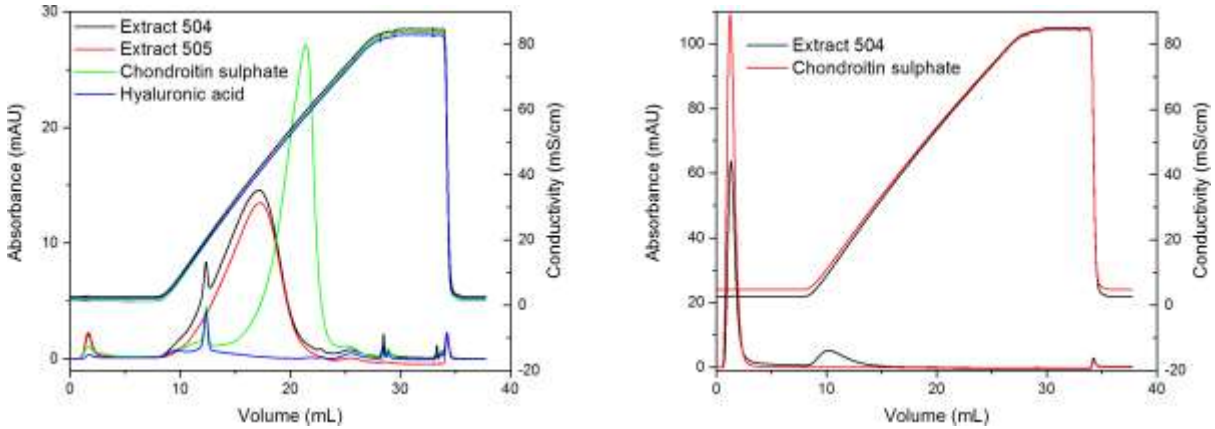
Successful recovery and concentration with minimal irreversible fouling,

Membrane 1 = 10 kDa spiral membrane (Koch). Membrane 2 = 100 kDa tubular membrane (Koch).

FoodWasteNet (PoC 15_6) Separation of mixtures: Ion exchange.

Using the retentate from ultrafiltration examined the potential for :

- strong anion (Q Sepharose Fast Flow $-N^{+}-(CH_3)_3$) and
- cation exchangers (SP Sepharose Fast Flow $-(CH_2)_3SO_3^{-}$) for purification.

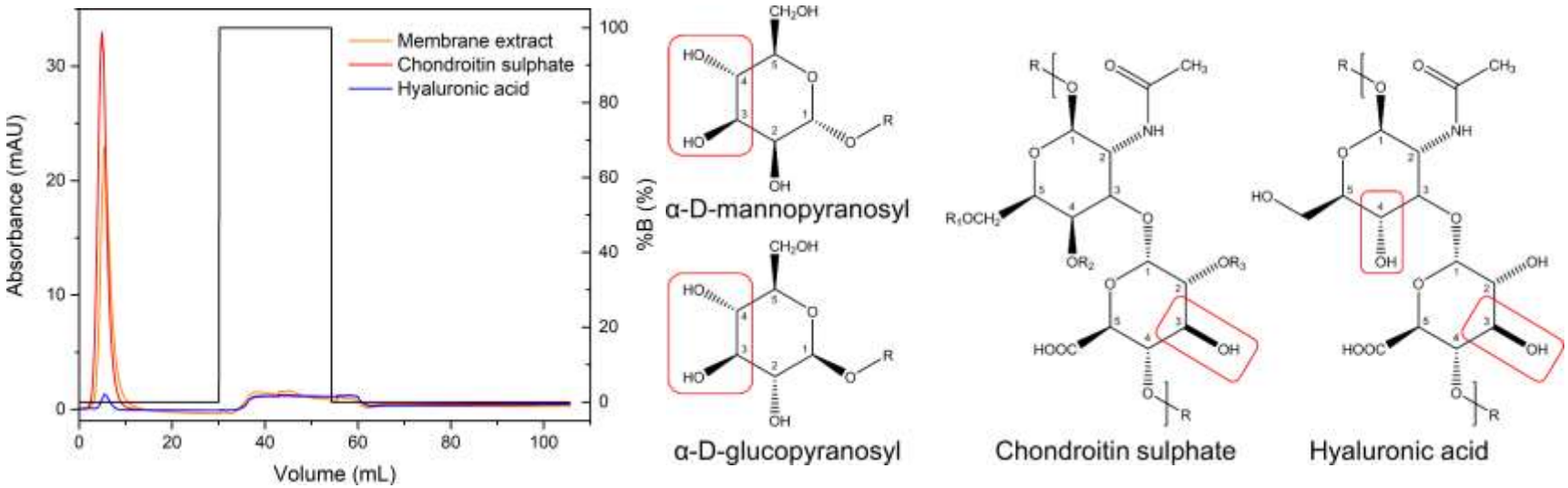


- anion exchange retained chondroitin sulphate and hyaluronic acid standards.
- extracts 504 (10 kDa) and 505 (100 kDa) gave a broad peak across the gradient, 504 included an additional peak with a retention volume matching hyaluronic acid.
- Variety of other components that could bind (e.g. collagen).

Require specificity to isolate the components of interest.

FoodWasteNet (PoC 15_6) – Recovery of GAGs by Affinity chromatography.

Identified the potential for using lectins in an affinity approach.



- Off-the-shelf ConA column not effective. Limited lectin solutions on the market – principally Concanavalin A and Lentil lectin (mannose binders).
- More time – more choices; Galab technologies peanut or wheat germ agglutinins would have more potential.

FoodWasteNet (PoC 15_6) – Fragmentation problem.

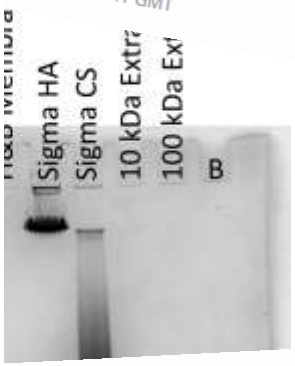
Despite positive resolution by s … presence of GAGs, the presence of thes … l electrophoresis.

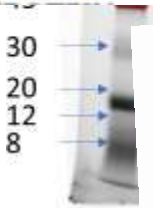
Chondroitin remains one of 'most adulterated supplements in the market'

By Elaine Watson

18-Apr-2011 - Last updated on 19 Apr-2011 at 16:41 GMT

Acceptance criteria: The electropherogram from the *Sample solution* exhibits a major band that is identical in position to the band from *Standard solution A*. The band from *Standard solution B* is clearly visible at a mobility similar to the band from *Standard solution A*. Any secondary band in the electropherogram of the *Sample solution* is not more intense than the band from *Standard solution B*. NMT 2% of any individual impurity is found. [NOTE—Document the results by taking a picture within 15 min of completion of destaining.]





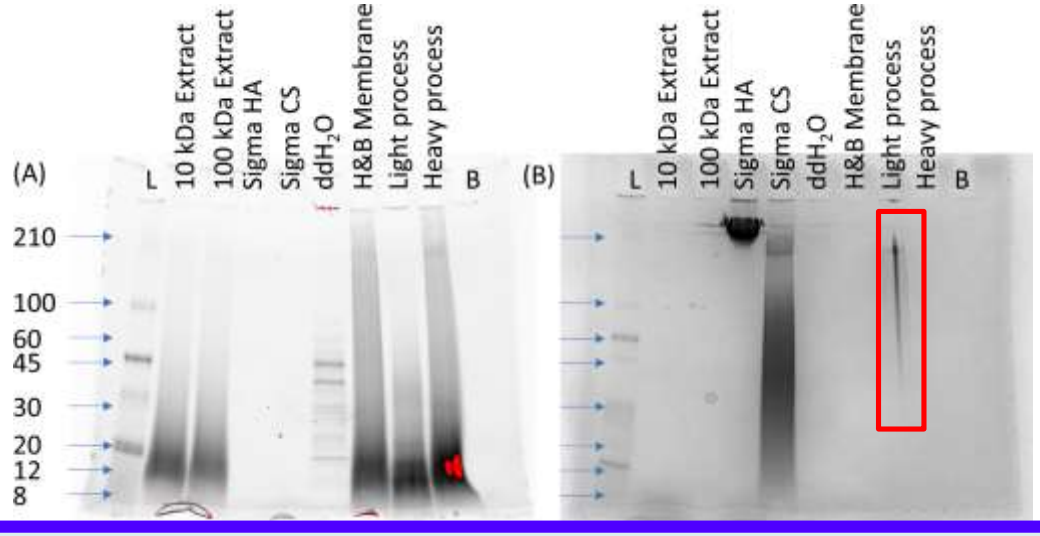
Recommendations

For chondroitin to be described as of pharmacopeial standard it must pass all the tests prescribed in the appropriate monograph. As a food supplement, manufacturers may consider pharmacopeial quality to be excessive. Nevertheless, in view of the well-documented cases of serious adulteration of

Gel (A) was imaged u … e same gel imaged after

FoodWasteNet (PoC 15_6) – Fragmentation solution.

… back to the process and with some modification we were able to identify staining suggestive of high molecular weight glycosaminoglycan extraction.



- Gaining high purity calcium carbonate and high purity biologicals is a contradiction in terms of process development.

Complementary staining of SDS-PAGE with (A) a trihalo in-situ stain (protein) and (B) Alcian blue (glycosaminoglycan) shows degree of fragmentation.

FoodWasteNet (PoC 15_6) – Outcomes

- Evaluated mass balance and potential of recovery for glycosaminoglycans, collagen and total protein.
- Information translated into potential economic value of extraction for each component.
- Detailed specifications (specifically chondroitin sulphate) identified for potential products (USP, Ph. Eur. etc.).
- Appropriate processing techniques were identified and partial costs for the manufacturing process identified.
- **Evidence obtained that high molecular weight GAGs can be isolated using a modification of our existing process.**

FoodWasteNet (PoC 15_6) – Follow-on

- Future work identified as:
  - Explore how processing conditions influence the specification of commercial grade chondroitin sulphate, hyaluronic acid and collagen starting with a known grade of material.
  - Progress enzymatic processing to understand the merits of such an approach and how it could be incorporated. Gain data to assess the economic viability.
  - Further trials at a scale of 1000 L or greater to understand the operational costs of UF membrane technologies.
  - Explore scaling anion exchange and size exclusion chromatography to production levels.
  - Explore a wider range of different lectin molecules for an affinity approach.
- Subsequent IUK application to answer some of the above questions, was well received technically but unsuccessful as of mid 2017 despite excellent review response.

FoodWasteNet (PoC 17_29) – "Sourcing and customising protein from the Cat. 3 waste of liquid egg processing industry."

Came from a lead to discuss traditional and sustainable materials used in paint – initially focused on $CaCO_3$ but developed into polymer materials in paints – egg protein?

- Mixture of egg whites and yolk from multiple sources, e.g. losses as part of the cracking process.
- Due to contact with the shell the egg white *cannot* now enter the food chain (Cat. III).
- Problem for processors but represents an opportunity as the co-product is a rich source of biomolecules.

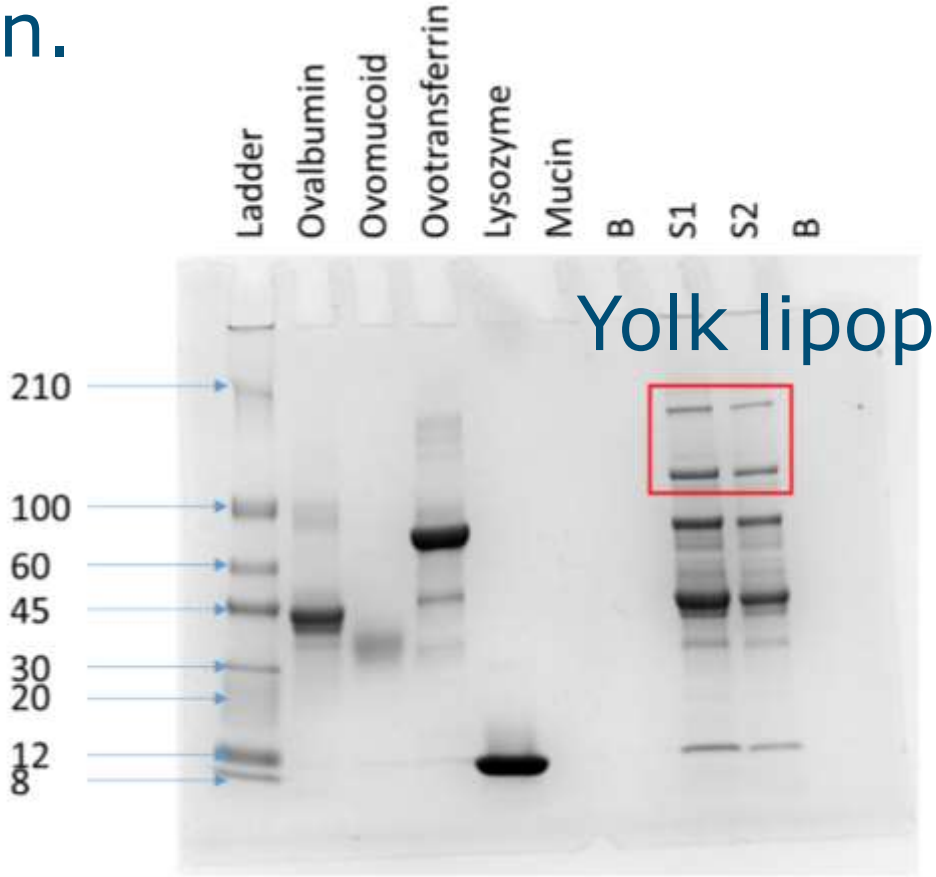
FoodWasteNet (PoC 17_29) – Cat III. waste characteristics.

Samples obtained from a UK production line.

Test	S1	S2	Egg white	Egg yolk	Whole egg
pH	7.7	7.8	9.6	6.6	8.6
Solids 105°C (%)	22.7	22.2	15.6	46.6	22.4
Density (g/mL)	1.0	1.0	1.0	1.0	-
Viscosity 16°C (mPa)	32.0	38.84	3.84	253.05	17.38
Fat (%)	8.8	9.4	-	68.6	12.7
Protein (mg/mL)	155.0	193.0			-

Cat. III waste fats originating from yolk determined using Soxhlet extraction with 2:1 EtAc/EtOH. Protein determined by BCA assay.

FoodWasteNet (PoC 17_29) – Cat. III composition.

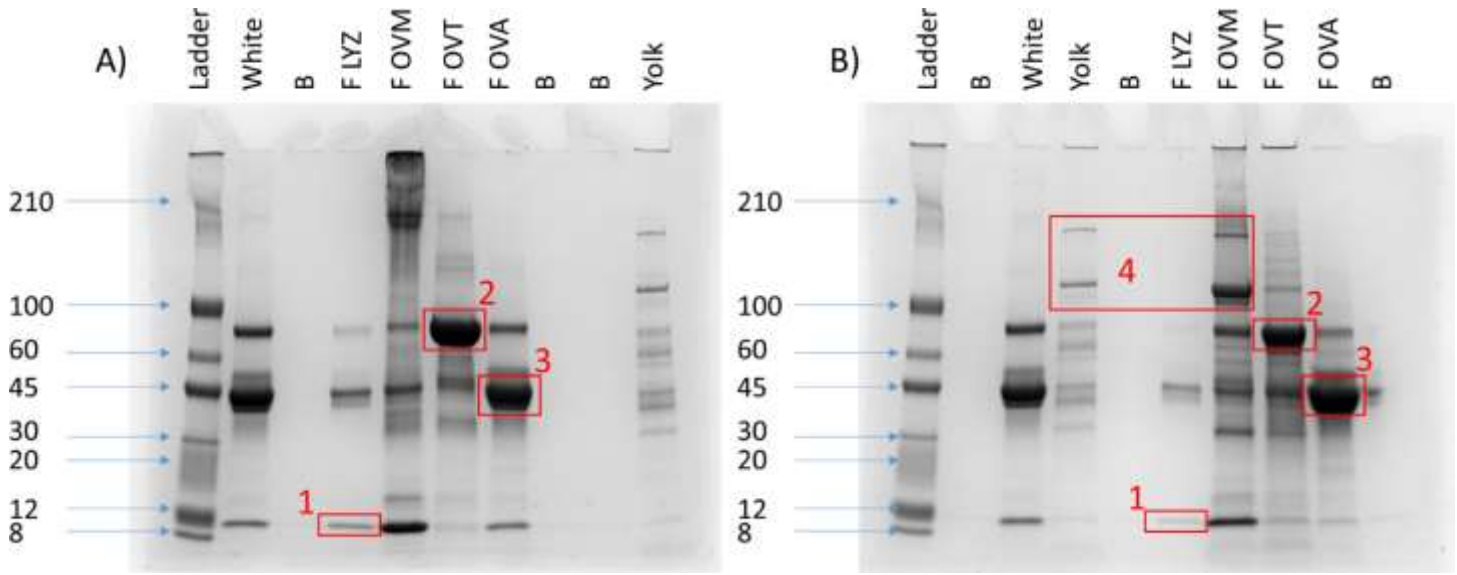


Yolk lipoproteins?

Proteins are commercial standards, S1 & 2 samples of the co-product and B empty wells. ALL expected proteins identified.

FoodWasteNet (PoC 17_29) – Sequential separation of major proteins.

Methodology using ammonium sulphate and an ion exchange media adapted to recover fractions enriched for each major protein.[1]

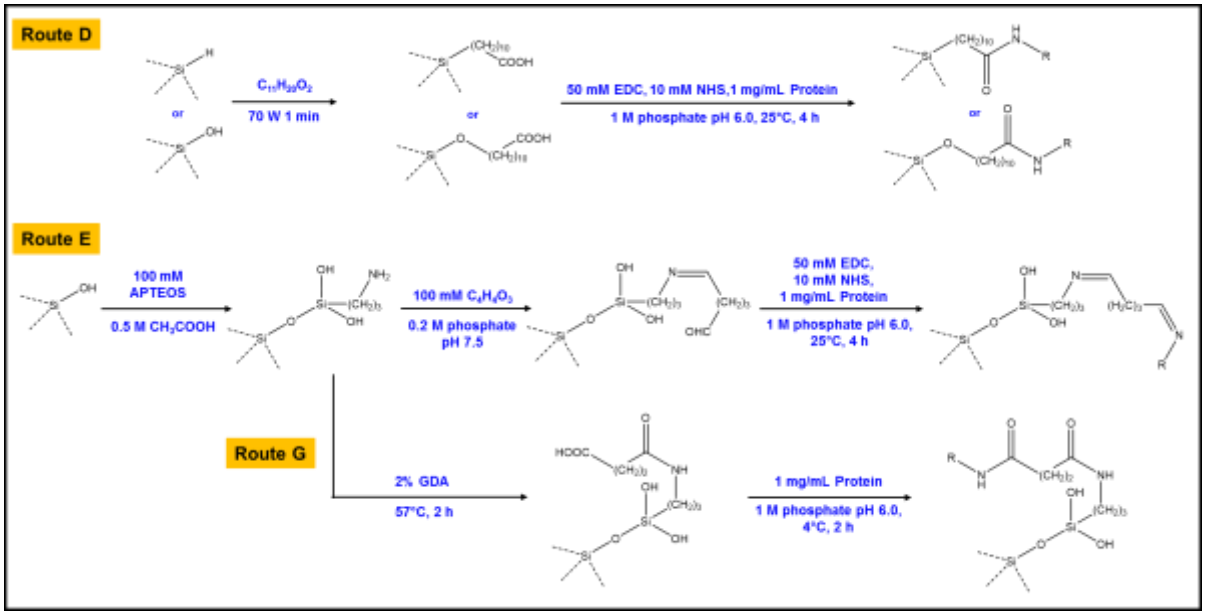


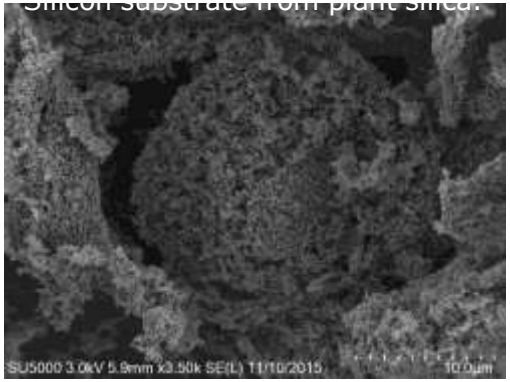
Ovomucin appears to aid in the separation of the yolk proteins.

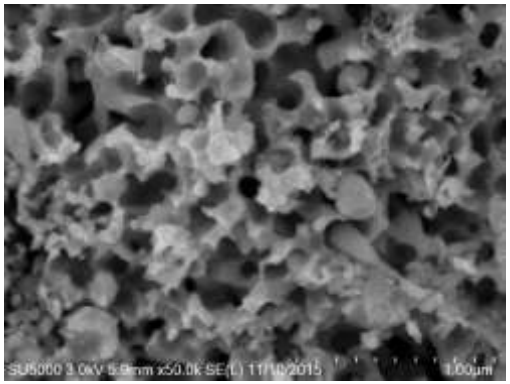
[1] Abeyrathne ED1, Lee HY, Ahn DU. (2014). Sequential separation of lysozyme, ovomucin, ovotransferrin, and ovalbumin from egg white. Poult Sci., 93(4):1001-9. doi: 10.3382/ps.2013-03403.

FoodWasteNet (PoCs 15_6/ 17_29) Proteolytic digestion using immobilized enzymes.

We have explored a variety of enzyme loading strategies for tethering enzymes to inorganic substrates (e.g. SiO_2 and Si).



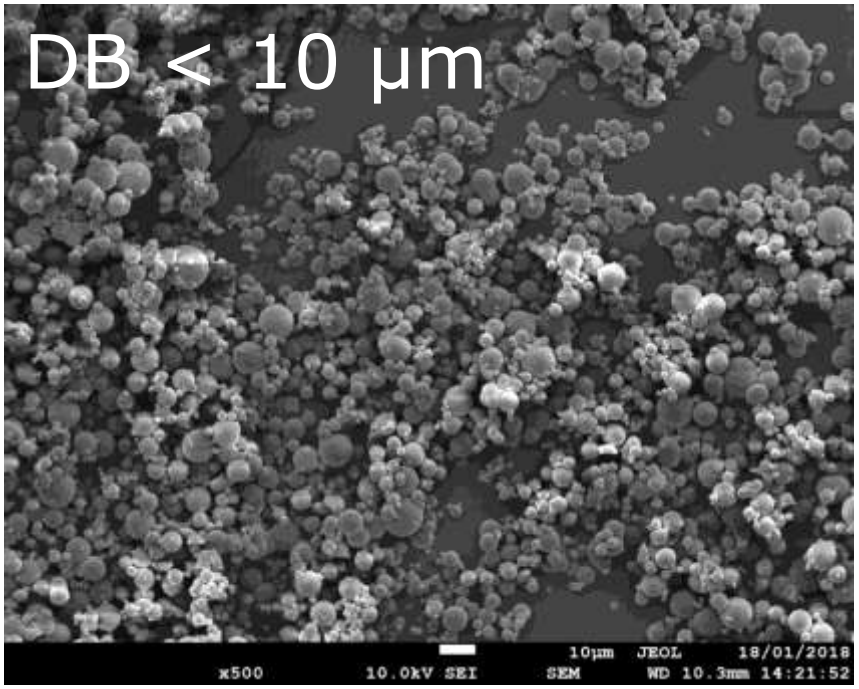


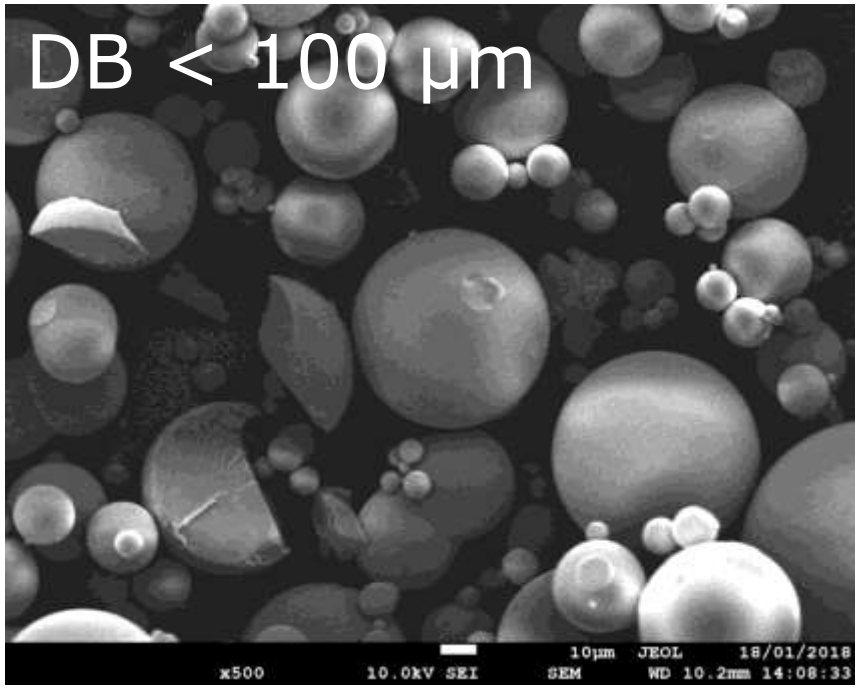


Funded by British Council Newton Fund/ ConaCyt, Mexico
Sola-Rabada et al., 2018 J. Colloids and Interfaces B. in the press

FoodWasteNet (PoCs 15_6/ 17_29) – Enzyme reactor substrates.

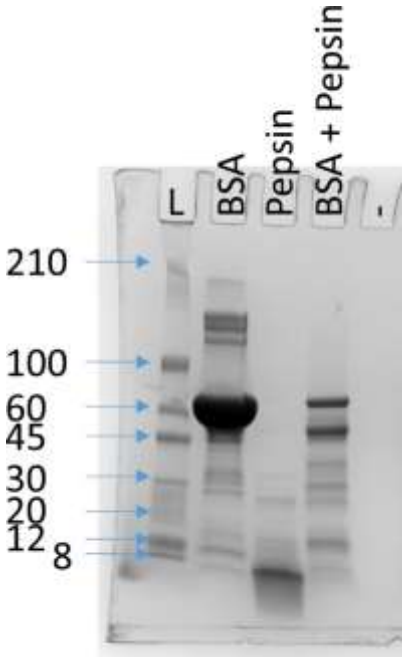
Development of enzymatic reactor using amino functionalised silica substrate (Karmaker) tethered with papain/bromelain using NHS/EDC coupling chemistry.

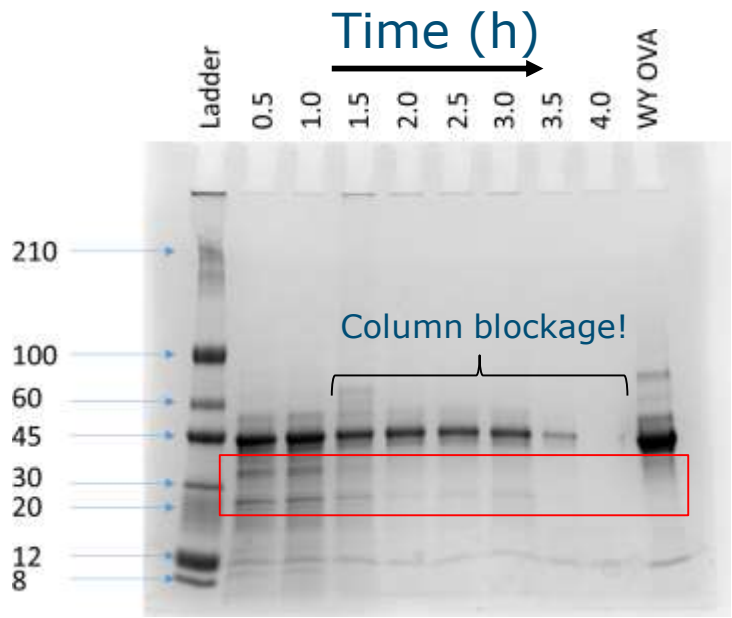




FoodWasteNet (PoC 17_29) Proteolytic digestion.

Our initial reactors worked but were very susceptible to fouling when processing crude protein fractions from 'egg'.





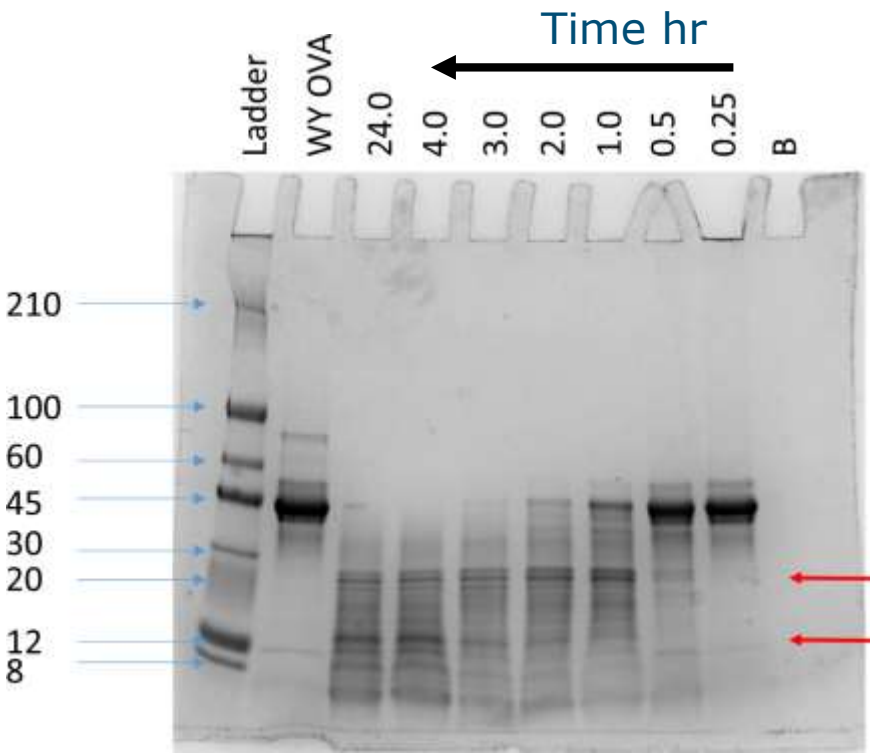
Content by particle size in the Cat. III liquid egg co-product.

Mesh (µm)	S1 mg/mL	% dry mass	S2 mg/mL	% dry mass
250	4.5	2.03	3.2	1.42
125	3.1	1.38	4.0	1.82
50	1.9	0.87	1.0	0.45
25	1.7	0.76	-	-
Sum	11.2	5.04	8.2	3.69

Real world samples are not 0.45 or 0.25 micron filtered, similarly we cannot afford to adopt the bucket approach!

FoodWasteNet (PoC 17_29) Proteolytic digestion

Redesign of reactor with a new substrate capable of dealing with our co-products yielded a controlled digest (complete 3-4 h) with no fouling.



Digest conducted at 50 °C, 100 mM phosphate, 10 mM KCl, 5 mM cyctein, pH 6.0. WY OVA starting material is 1 mg/mL ovalbumin isolated from the co-product.

Summary

- 102402 – A process developed that produces material of marketable quality. This material is available for sampling with prospective customers – in 5 kg and 25 kg.
- Other 'components' of these projects are being discussed with potential customers.
- Publications being developed: "Barriers to adoption of biogenic carbonates in the food & supplements industries."

- A question- are animal by-products OK going back into human use (i.e. medication - chondroitin sulphate)?
  - this already happens around the world.
  - In the UK regulation is a barrier but ultimately it depends on what people want
    - a market for renewable materials with good properties (i.e. low metals) vs. importing which is no guarantee of quality or use of less environmentally sound practices

Acknowledgements

FoodWasteNet

Bumble Hole Foods Ltd
Producers of Quality Egg Products since 1961

Chrysalis health & beauty

axium PROCESS

MPH
Microbix Plant Health Ltd

Innovate UK

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