Spray or Freeze-Drying for Lactoferrin?

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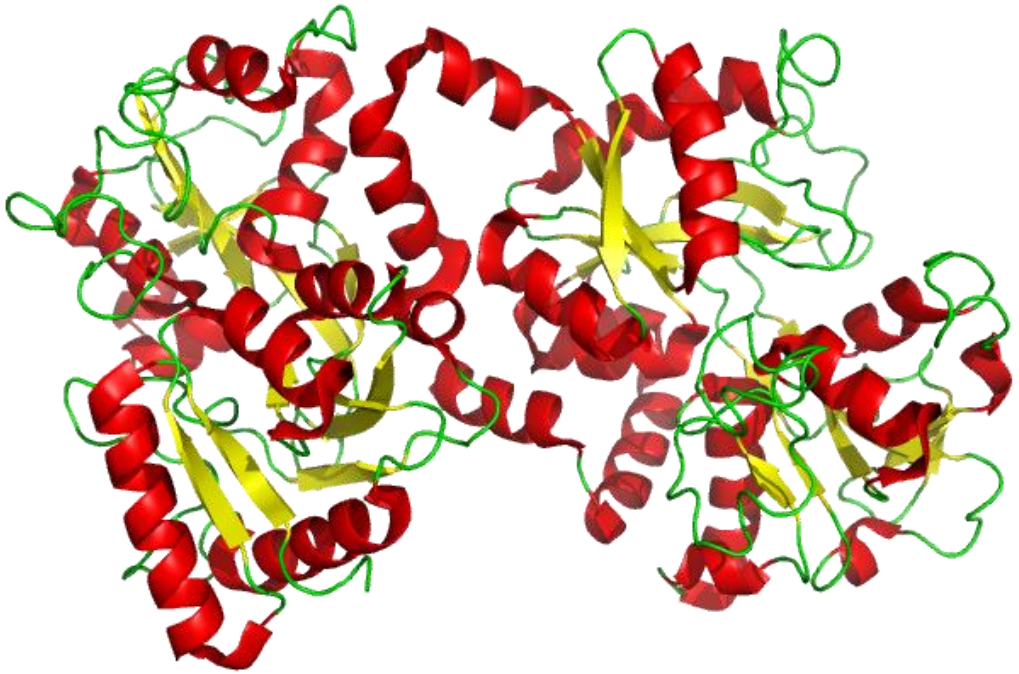
Lactoferrin

- Globular Glycoprotein- 80kDa
- Found in: Saliva, tears, amniotic fluid. **Milk.**
- Iron binding capacity: specific iron binding sites in the N and C lobes of the protein that reversibly bind to ionic iron (Levay & Viljoen, 1995)
- Has antimicrobial, antioxidant and anticancer properties
- **Is heat labile at typical pasteurisation and drying temperatures.**



Lactoferrin Industry- New Zealand

- Four producers in NZ
- Tatua was the pioneer

Tatua

Lactoferrin Industry-Global

- Global capacity ~500-600 T/annum and growing
- Sale price peaked around USD 3000 kg, dropped back
- Key global standard: China's GB1903.17-2016

Tatua

Lactoferrin is purchased for its bioactivity

Should be paying for activity, not mass!

Denaturation of lactoferrin

- Lactoferrin is found in 2 forms:
  - Holo-lactoferrin: Fully iron saturated
  - Apo-lactoferrin: unsaturated.
  - Typically~ 30% of bovine lactoferrin is in the Holo form (Marnila &Korhonen 2009)
- Iron saturated Holo-lactoferrin is more compact and more heat stable (Franco, Pérez, Conesa, Calvo, & Sánchez, 2018).
  - Holo-lactoferrin denatures above 79°C
  - Apo-lactoferrin denatures 52-62°C(Iafisco, Foltran, Di Foggia, Bonora, & Roveri, 2011)
  - Changing pH conditions changes the denaturation temperatures.

- **~70% is in a heat-labile form- can be denatured by typical pasteurization and drying temperatures.**

Prior studies:

- There have been previous studies comparing spray and freeze drying

- Wang,Timilsena, Blanch, & Adhikari, 2017 b, found no significant difference between the functionality of spray dried and freeze dried.
  - ***However, they used a lab scale freeze-dryer and a lab scale spray drier.***

- Lactoferrin is heat labile (Wang et al., 2017 a)- Spray drying, though gentler than other high-temperature drying methods still involves temperatures above denaturation temperature

- Proteins are still sensitive to denaturation due to some mechanisms when frozen, such as ice-induced denaturation (Bhatnagar, Bogner, & Pikal, 2007), protein dehydration (Carpenter, Prestrelski, & Arakawa, 1993), and cold denaturation (Ascolese & Graziano, 2008). These are typically orders of magnitude slower than high-temperature denaturation processes

Difference in scale- Freeze drying

Labconco

VS

Cuddon

Difference in scale- Freeze drying

- Typical lab equipment is different to industrial
  - Product geometry- eg, flasks vs shelves
- Cycle times and temperatures differ significantly between lab and industrial
  - Industrial cycles often higher temperatures than laboratory
  - Throughput is more important for industry
- However, Pilot scale equipment (such as Cuddon FD-18 or FD-80) is designed to mimic industrial geometries and heating cycles to allow direct scaling.

Difference in scale- Spray drying

Armfield

VS

Difference in scale- Spray drying

- Residence time small scale
  - Laboratory scale **~6 s** (Schmitz-Schug, Foerst and Kulozik 2013)
- Residence time large scale
  - Pilot scale operating co-current **~1min** (Kieviet and Kerkhof 1995)
  - Pilot scale counter-current **~2-3 min** (Gianfrancesco 2009)
  - Pilot scale with fine return and IFB **~9+ minutes** (Jeantet et al. 2008)
- ***Therefore:***
  - Thermal histories differ
  - Particle sizes differ
  - ***Degrees of denaturation will differ***

Our aim

- Help those choosing between the two drying methods for Lactoferrin:

- Use industrially relevant equipment:
  - Freeze-dryer that replicates industrial heating geometries, tray materials and dimensions, and heating programmes
  - Pilot-scale production spray dryer.

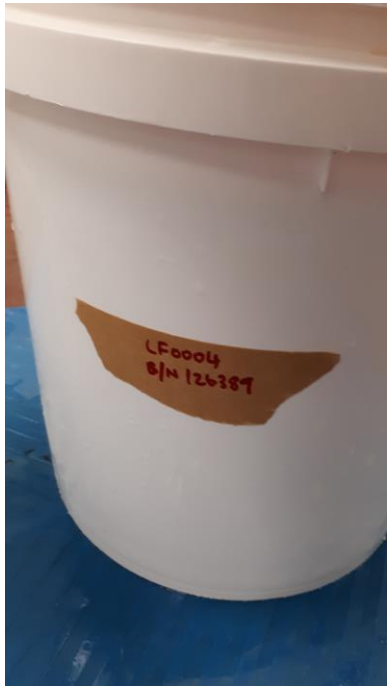
- Evaluate properties of the dried Lactoferrin:
  - Denaturation by DSC studies
  - Iron binding by incubation in an iron rich solution
  - Antimicrobial activity by incubation of bacteria against serial dilution of lactoferrin.

Methods- Lactoferrin

- Cost of LF normally makes larger studies prohibitively expensive

- Approximately 30-40L total solution kindly provided by Tatua

- 16-18% TS solution. >95% LF
- Commercially extracted from pasteurised skim milk using an agarose based strong cation exchanger



Methods- Freeze drying

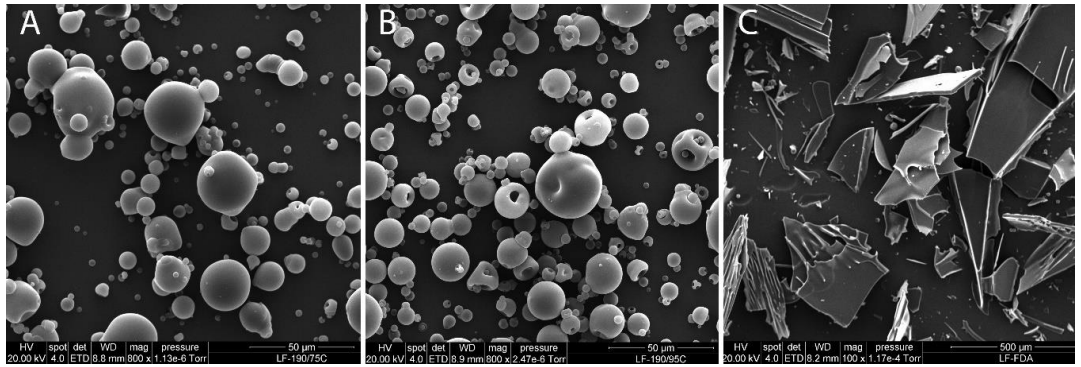
- Cuddon FD-18 located at Massey University FoodPilot.
- Same heading geometries, tray geometries as industrial scale Cuddon driers.
- LF-solution loaded on LF trays to 12-15mm depth, frozen in place at -40°C.
- Temperature-time profile identical to industrial production was used.

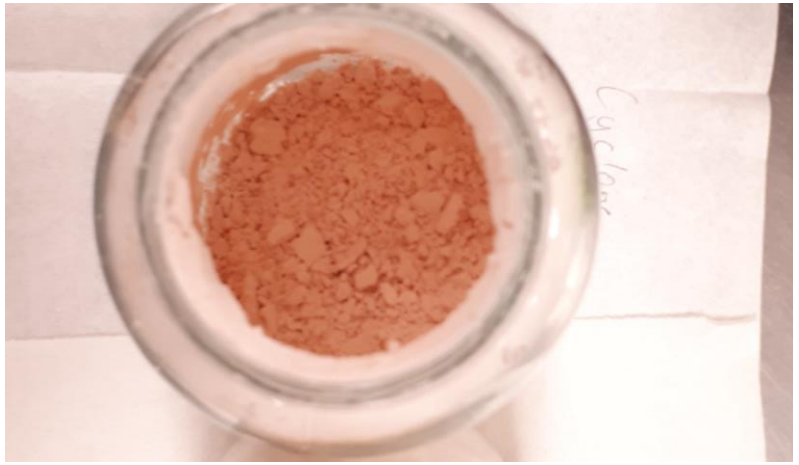
Methods- Spray drying

- GEA-Niro Mobile Minor spray-drier, located at Massey University FoodPilot
- Inlet temperature of 190°C for all runs
- Outlet temperature controlled at 75°C or 95°C

Results- Drying

	Volume-weighted Mean Diameter (µm)		
	This study	*(B. Wang et al., 2017)*	*Commercially Spray Dried*
FD	N/A	N/A	N/A
190/75	30.2	12.9	57.1
190/95	17	12.8	





- Mean diameters are greater than those reported in previous literature (B. Wang, Y. P. Timilsena, E. Blanch, & B. Adhikari, 2017) as a result of the larger drier used for this study

- Would have experienced harsher thermal histories (Walton, 2000)

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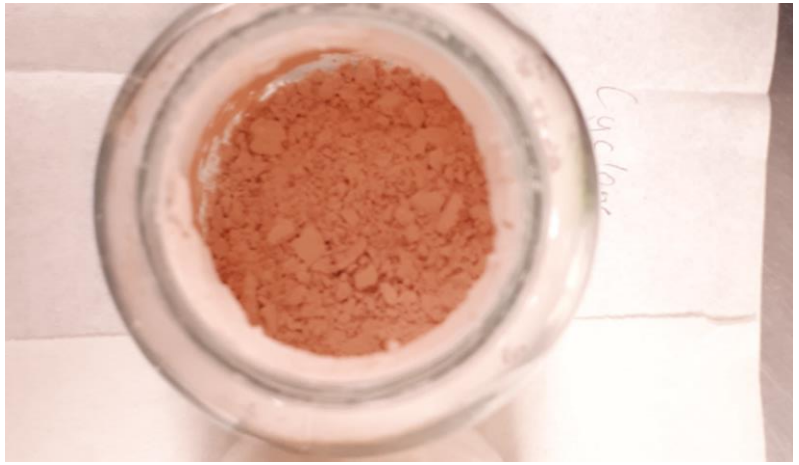
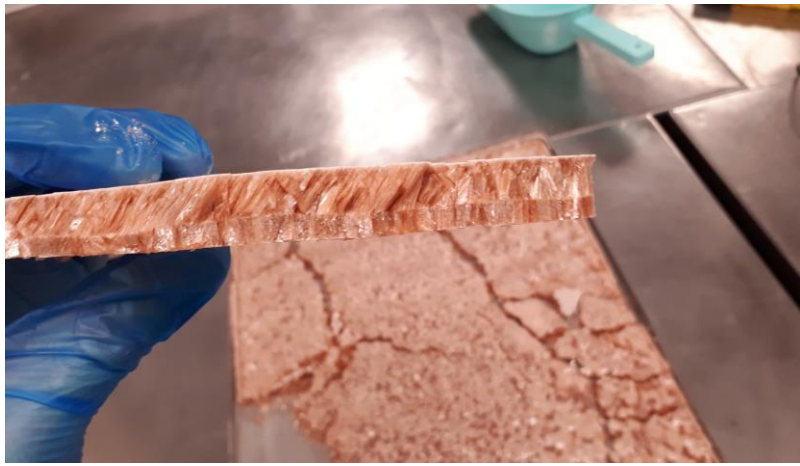
New Zealand's Innovation Agency

Results- Drying



	Moisture (%)	
	This study	*(B. Wang et al., 2017)*
FD	0.55	2.7
190/75	6.39	8.6
190/95	3.92	5.2

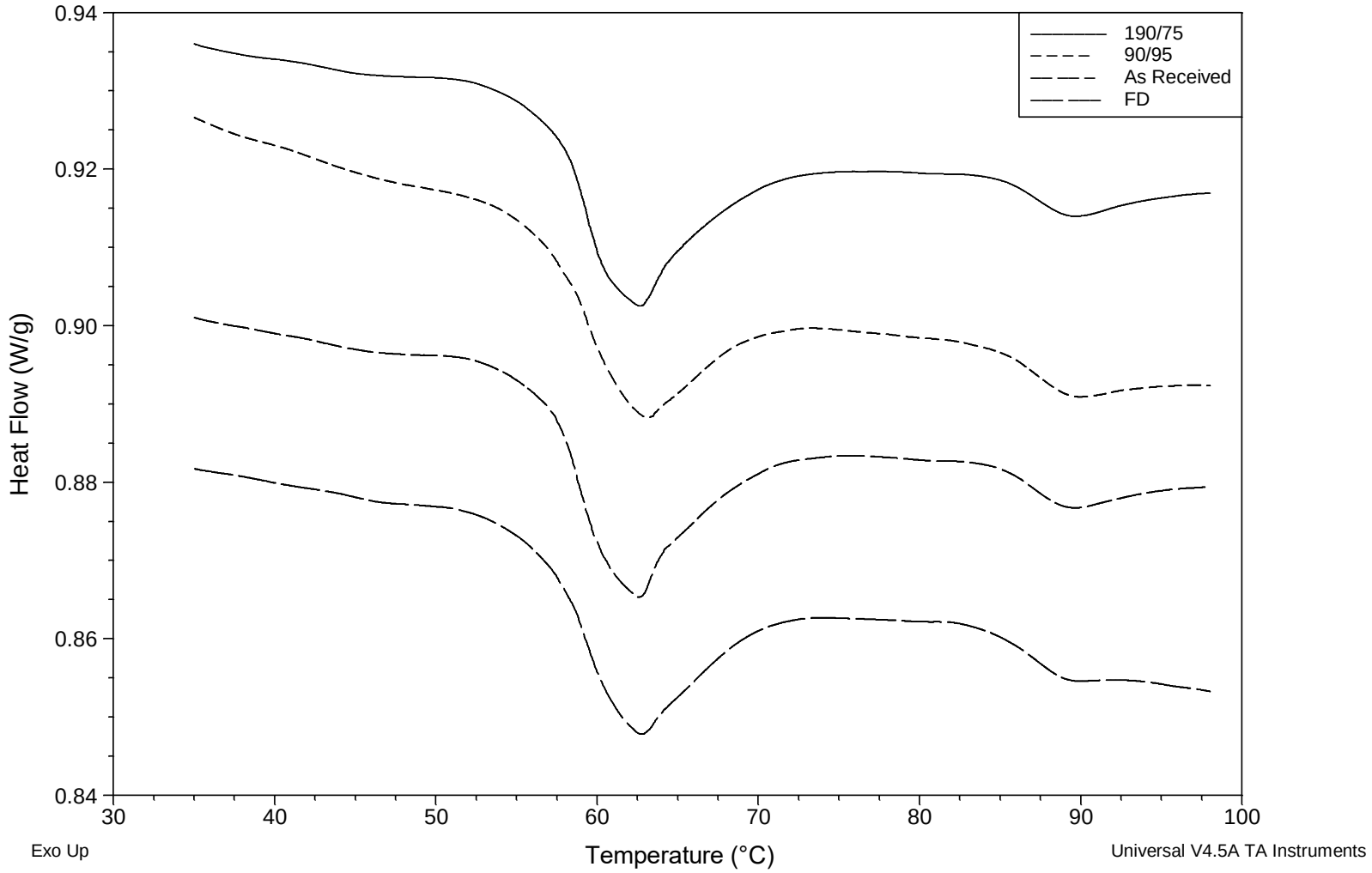
- We produced drier lactoferrin than reported in previous comparison studies
- Previous studies had moisture levels above what would be expected for commercially produced lactoferrin, and higher than allowable under China GB1903.17-2016 :
  - ***Less than 4.5% LOD***



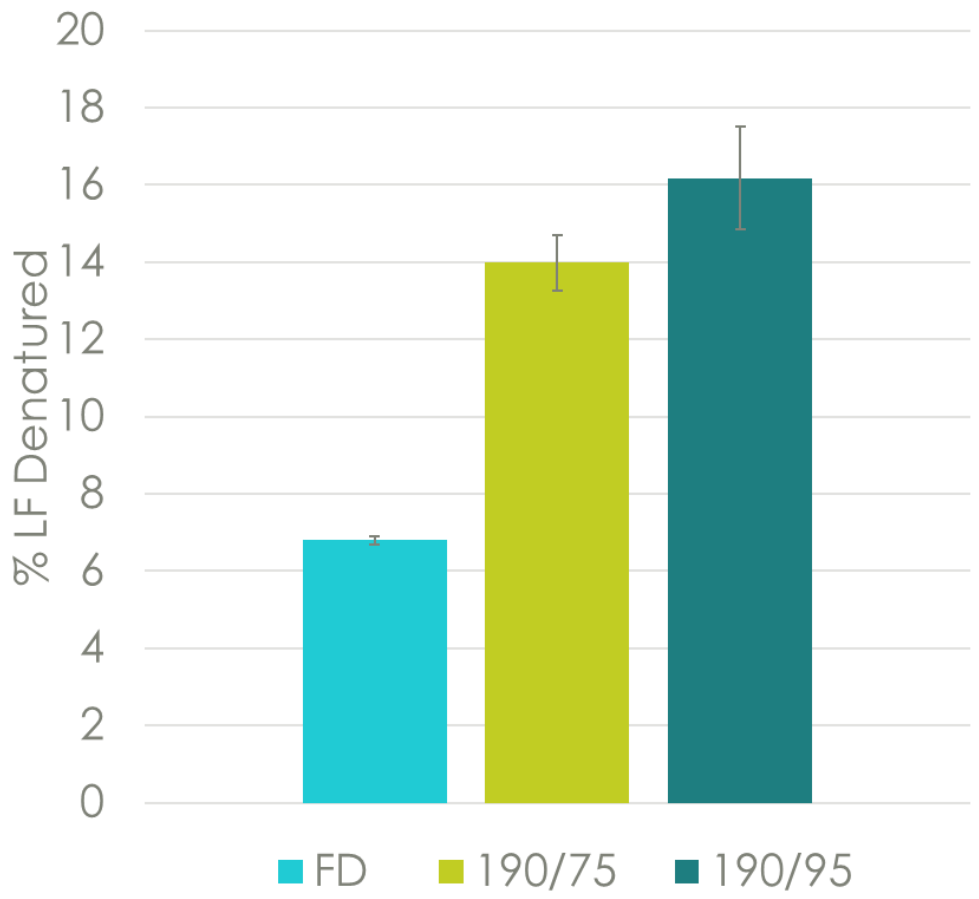
Method- DSC

1. Lactoferrin samples reconstituted to 15% in MilliQ water
2. 10-15mg samples sealed in DSC pans
3. Heated 20 °C -100°C and 5 °C/ min
4. Denaturation determined relative to native, from enthalpy of first peak:
5. $$\%Denatured = \left(1 - \frac{\Delta H_{sample}}{\Delta H_{as-received}}\right) * 100$$

Results- DSC


0.94
0.92
0.90
0.88
0.86
0.84
Heat Flow (W/g)
30
40
50
60
70
80
90
100
Temperature (°C)
190/75
90/95
As Received
FD
Exo Up
Universal V4.5A TA Instruments

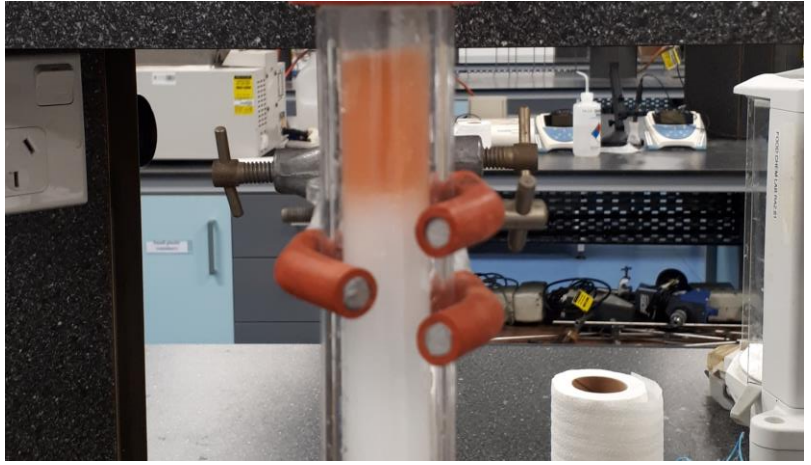
Spray-dried was significantly more denatured



- Freeze dried 6.8%
- Spray Dried- 75°C exit-14%
- Spray Dried- 95°C exit-16.2%
- Lactoferrin which was acceptably dry, had 2.4x the denaturation.

Methods- Iron binding

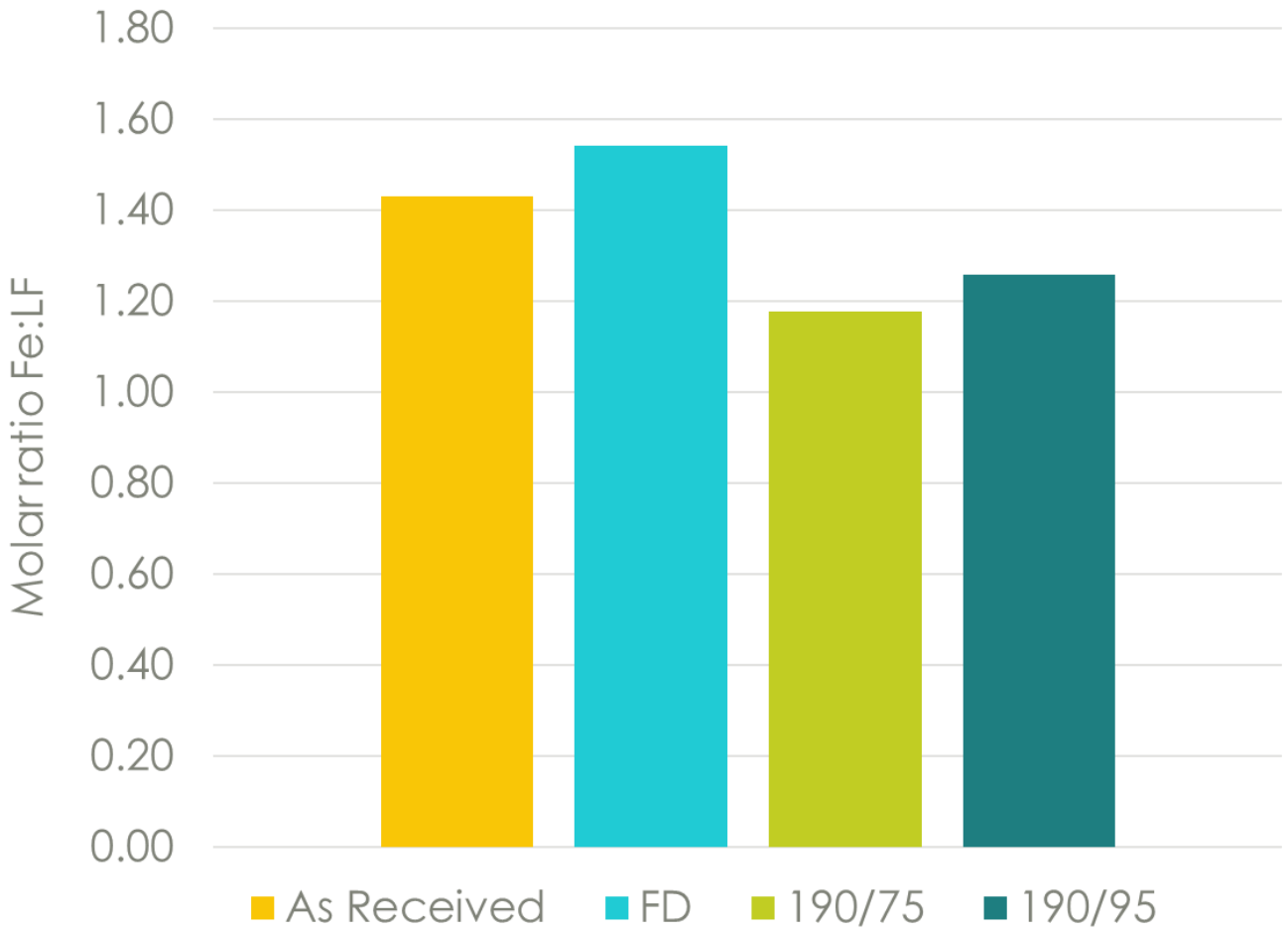
1. Incubated LF in a solution of Fe(NO3)3 and Nitrilotriacetic acid (NTA), in a LF:Fe:NTA ratio of 1:2:5
   - NTA is a chelating agent, which wants to absorb free iron ions- LF must compete against it.
2. Unbound iron removed by size-exclusion chromatography though Sephadex G25-150 (Mata, Sánchez, Headon, & Calvo, 1998).
3. Protein in final isolate measured by Bradford assay.
4. Samples microwave digested according to AOAC 2015.06.
5. A PinAAcle 900Z graphite-furnace atomic absorption spectrometer was used to determine Fe content according to Bass and Bosnak (2011).



Anton Paar

Perkin Elmer

Results- Iron binding: Spray-dried bound less iron.



Spray Dried lactoferrin bound 18-24% less iron than freeze-dried

Methods- Antimicrobial activity

1. Serial dilutions of LF samples prepared with MilliQ water and sterilised by 0.22µm filrtration- 0.01-3 mg/mL

2. Innocula of *Escherichia coli* NCTC 8196, *Cronobacter sakazakii* ASQ 5, and *Salmonella typhimurium* prepared in 0.1% peptone water

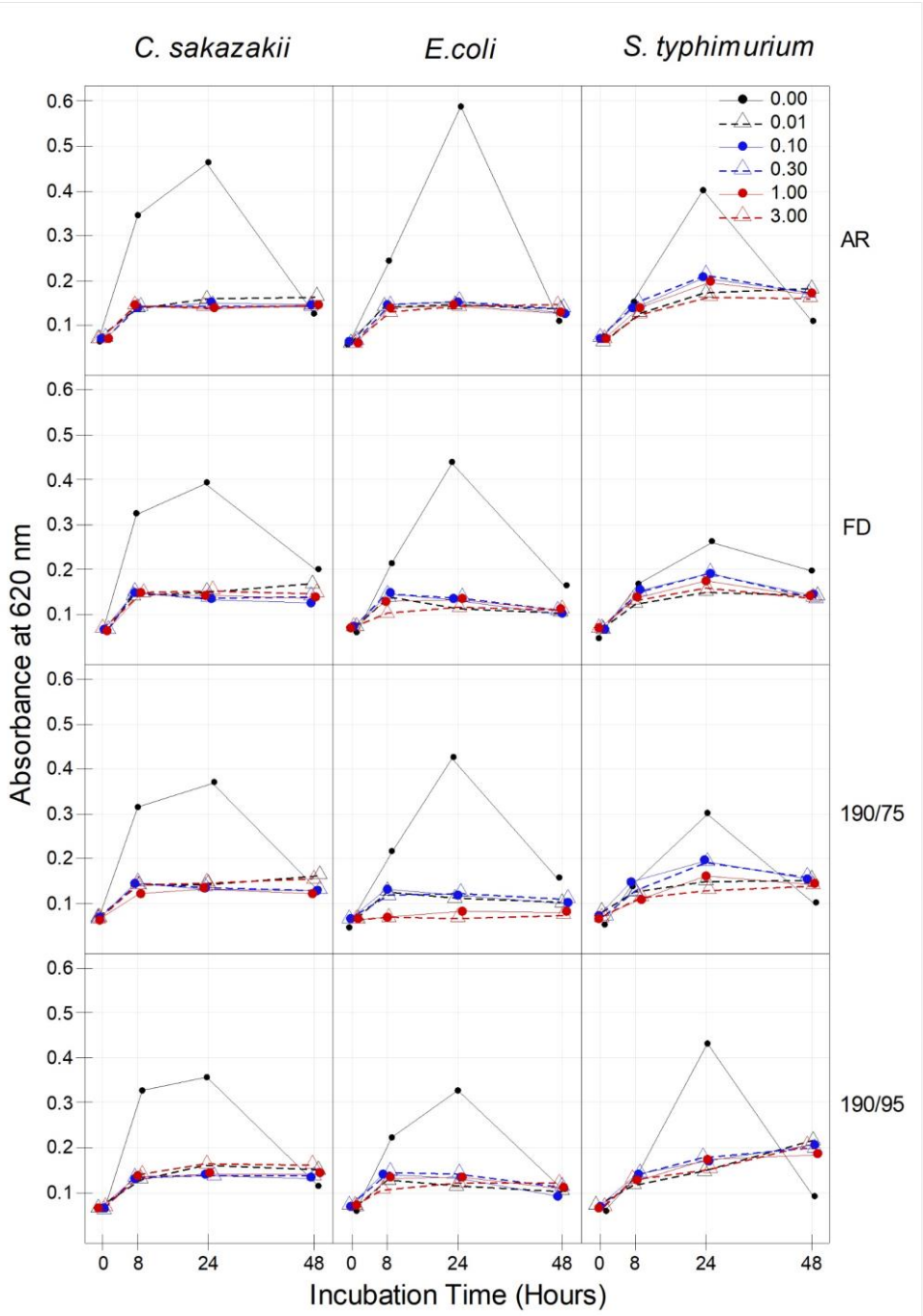
3. 100µL of each pipetted into wells of 96 well plates

4. Incubated under aerobic conditions at 37 °C.

5. Activity measured by absorbance at 620 nm at 0, 8, 24, and 48 h using SPECTROstar Nano plate reader

Results- Antimicrobial activity

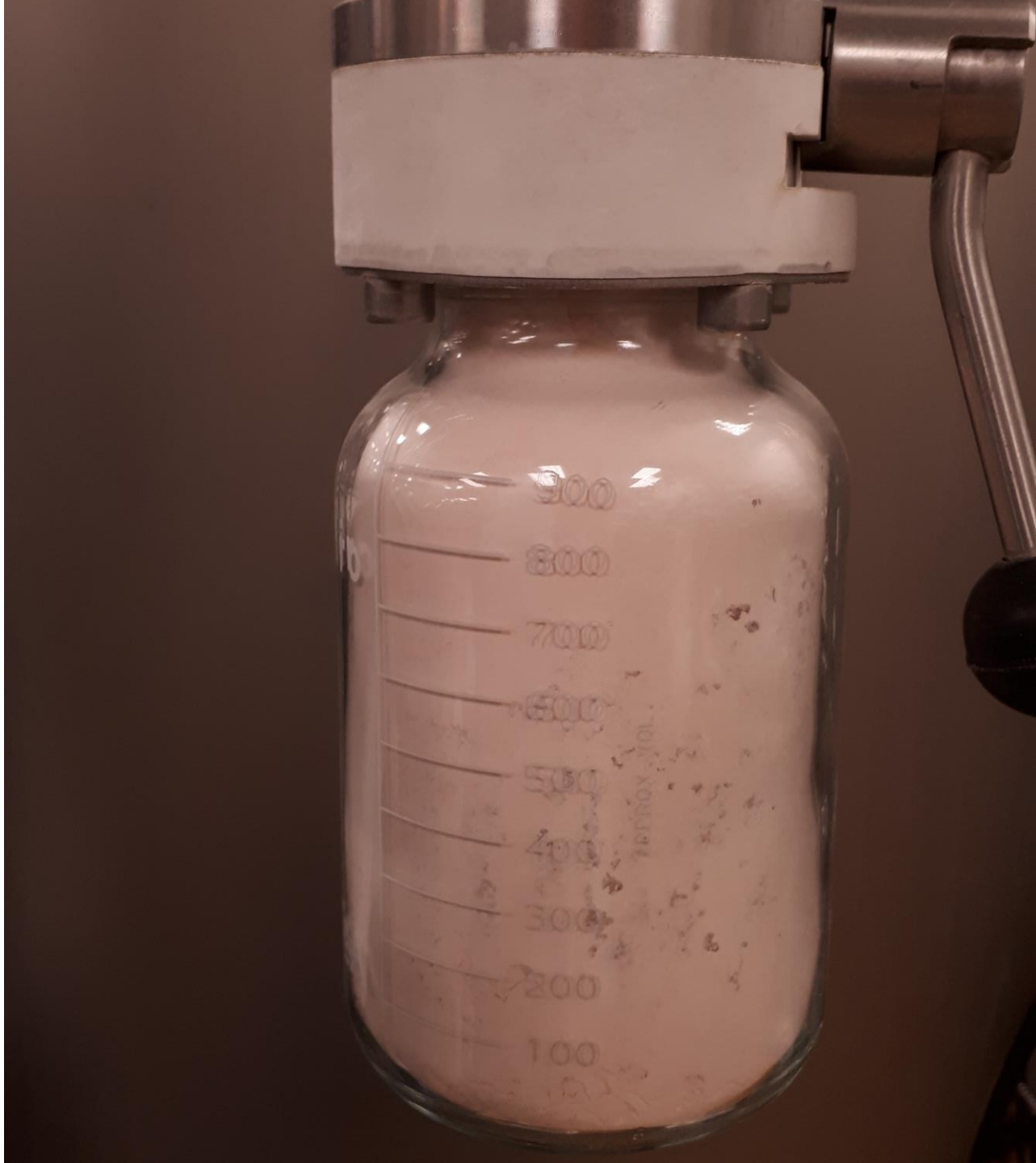
- All samples showed antimicrobial activity- Bacteriostatic, not bactericidal
- Lactoferrin antimicrobial activities have a range of causes
  - Iron binding
  - Lactoferricin
  - Cell membrane interactions.



Conclusions

Previous comparisons in literature were at small scales

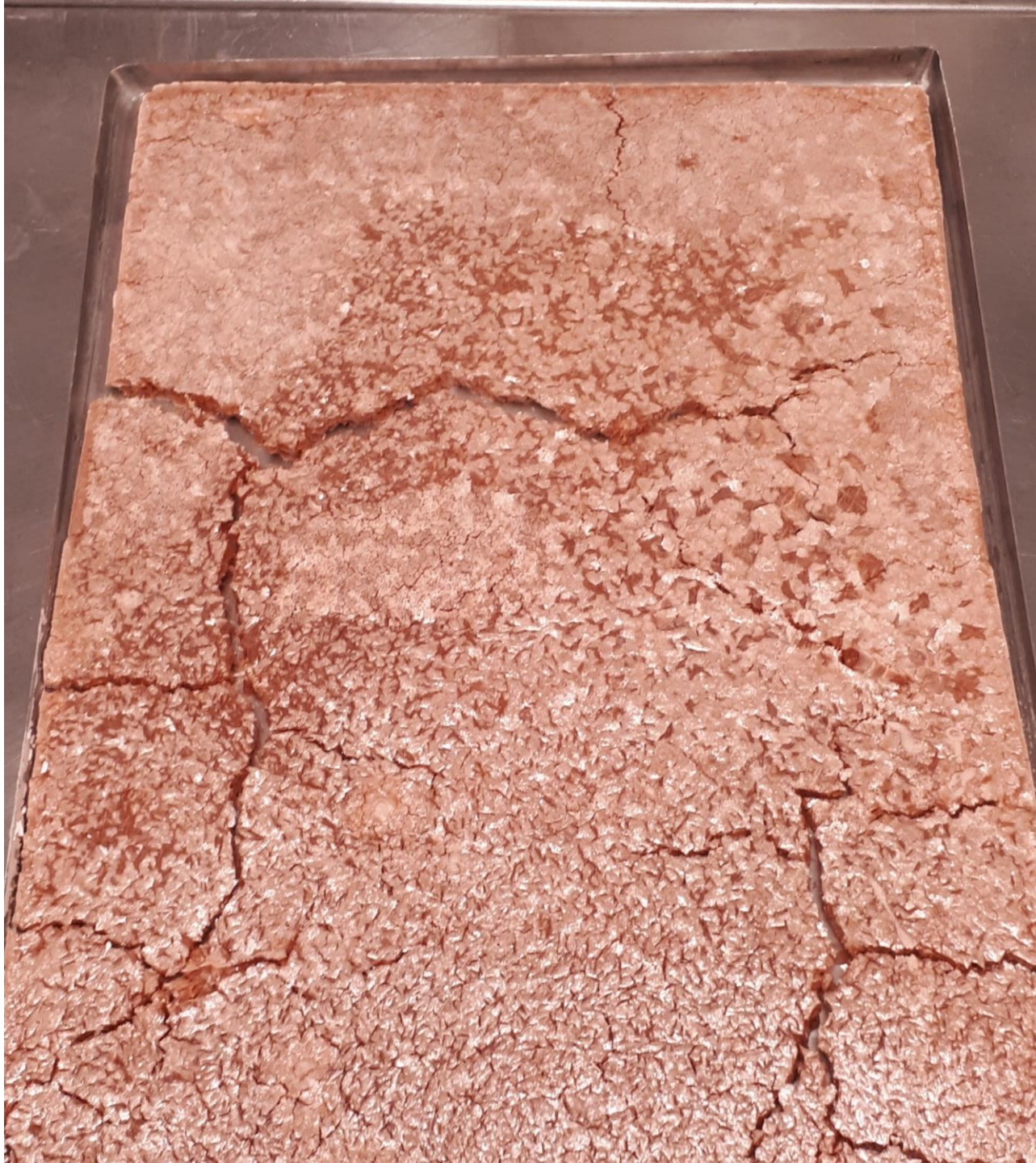
- Lactoferrin produced in previous studies was too moist.
- Particle sizes in dried powders were small.
- Thermal histories were not relevant to industrial scale manufacturing.



Conclusions

Spray dried will have lower biological activities

- In contrast to small-scale trials, some denaturation was seen in all samples.
- Spray-dried lactoferrin had twice the denaturation of freeze-dried, when compared to fresh.
- All lactoferrin had a bacteriostatic effect in the conditions of our trial.
- Iron binding capacities were lower in spray-dried samples.



More information

- Jolin Morel, Siti Norbaizura Md Zain, Richard Archer, *Comparison of drying techniques for bovine lactoferrin: Iron binding and antimicrobial properties of dried lactoferrin*, International Dairy Journal, Volume 124, 2022.

- Jolin Morel, Siti Norbaizura Md Zain, Richard Archer, *Spray, or freeze drying for lactoferrin?,* Food NZ, Volume 21, 2021

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