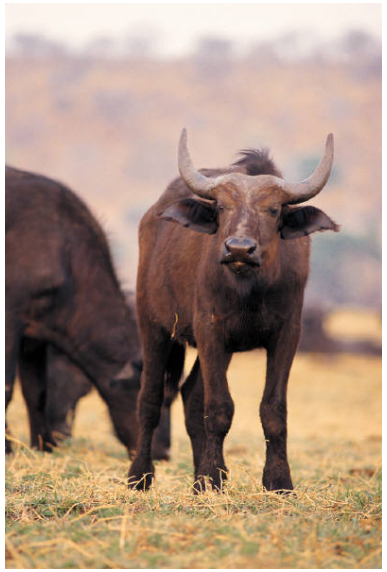
Leatherhead Food International

An evaluation of Lectin Chip Array Technology to identify the species of origin of milk used in the production of mozzarella cheese

FSA Project Q01110



In Collaboration with NutriCognia Ltd.

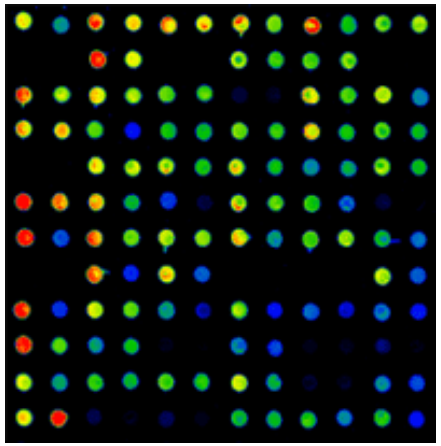
What is chip array technology ?

A miniaturised technique that allows several analytes to be measured at one time and in the same sample

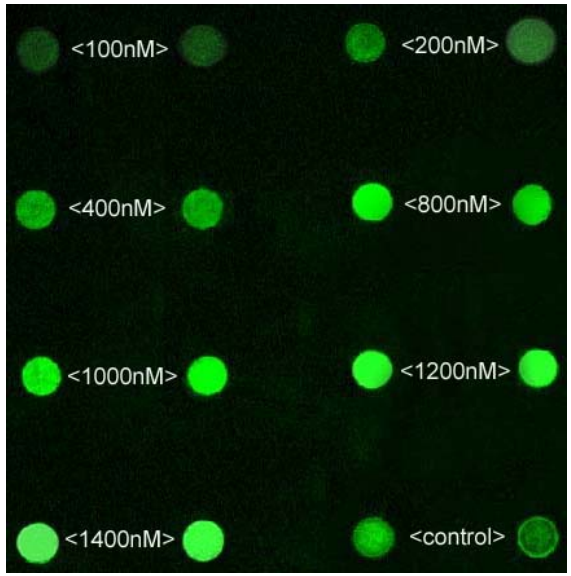
A slide is printed with tiny dots of the capture reagent – antibody or lectin

Sample is added to the surface of the slide

Fluorescently labelled detection reagent is added



The slide is read using a laser scanner and the information analysed by computer



Fluorescence is dependant on concentration

Lectin Chip Arrays

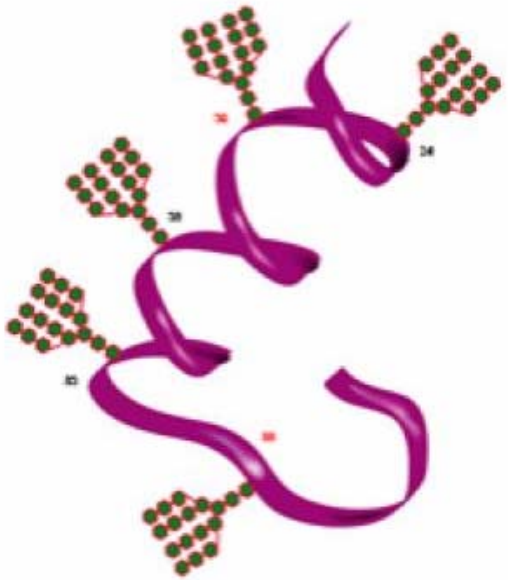
NutriCognia technology analyses carbohydrates present in a sample using a range of naturally occurring carbohydrate binding proteins:

LECTINS

Lectin Chip Arrays can be used to analyse

- Glycoproteins
- Glycolipids
- Polysacharides

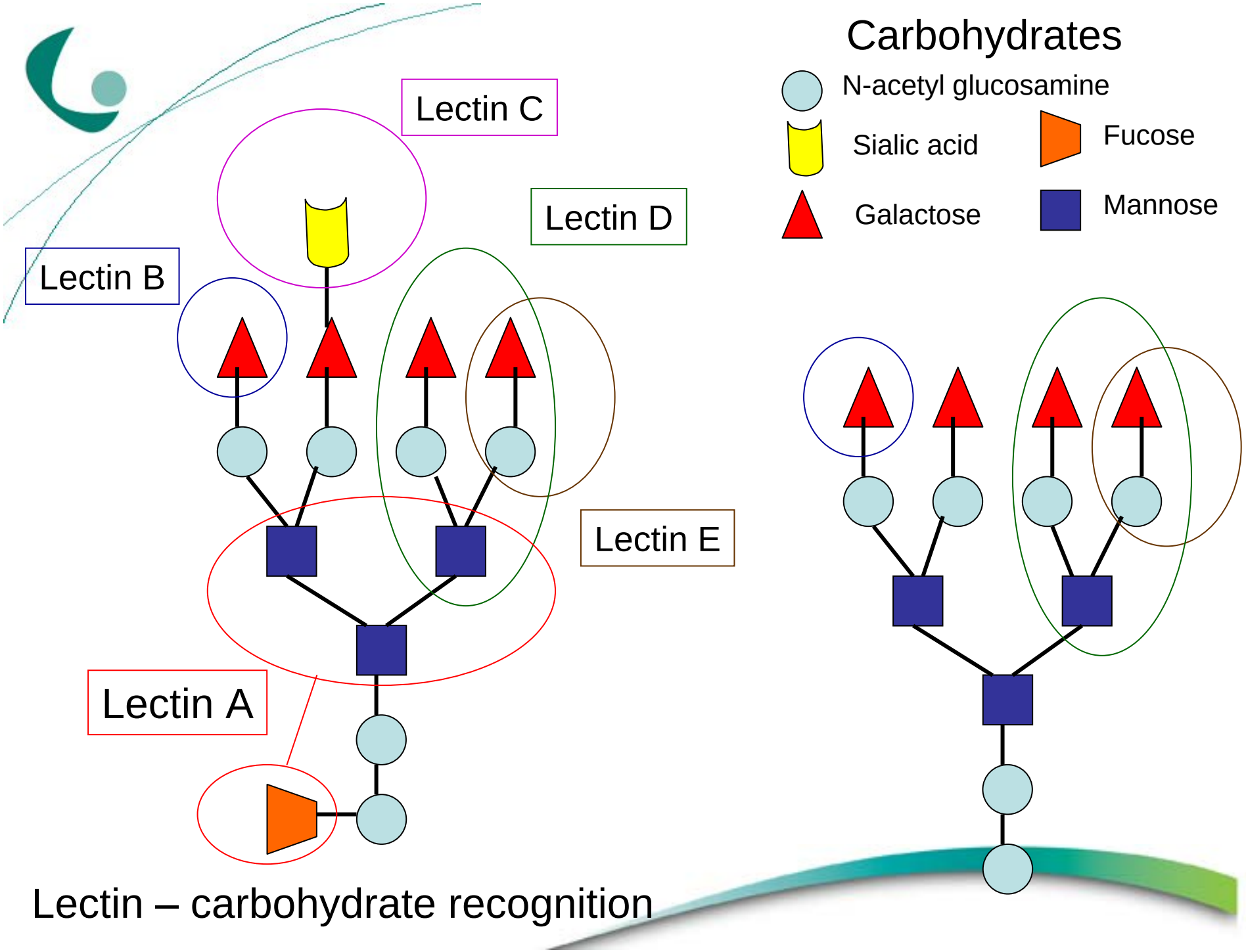
Glycoproteins



- Proteins are ‘strings’ of amino acids

- They become glycoproteins if one or more amino acids has a carbohydrate chemically bound to it

- The carbohydrate may be a single sugar but is more often a polymer of several sugars and may be branched



Lectin – carbohydrate recognition

Why will Glyco ‘fingerprints’ aid cheese speciation?

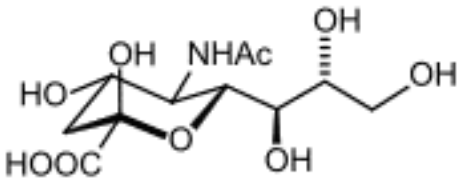
Lectin binding profile of glyco ‘fingerprint’ is likely to differ between species

kappa-casein (milk protein)

- percentage glycosylation varies between species

50% bovine	(cows)
30% ovine	(sheep)
36% caprine	(goats)

- N-acetyl-neuramic acid is present in caprine k-casein but not in ovine k-casein



Sialic acid

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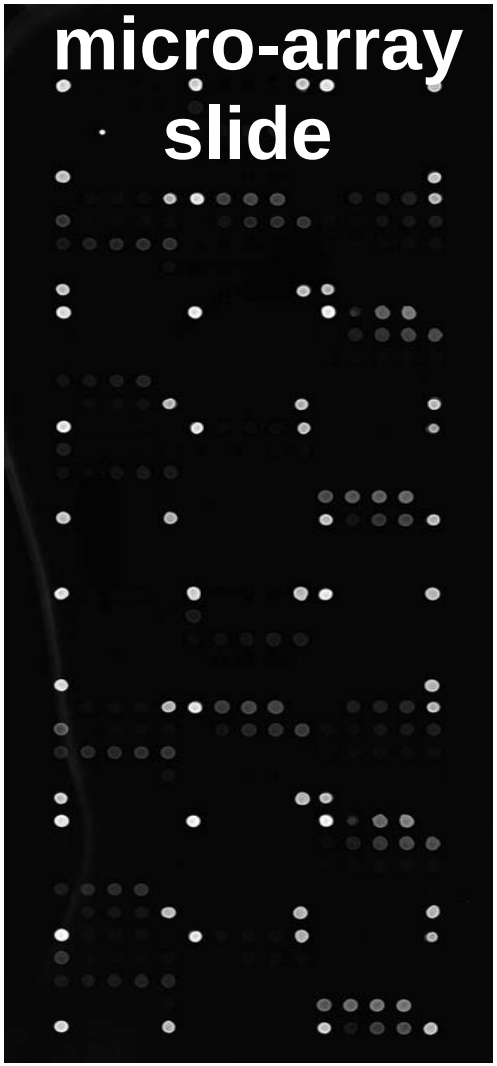
Lectin Chip Array Technology

- A microscope slide is printed with tiny dots of different lectins (carbohydrate binding proteins)
- Where a sample has bound to a lectin, it can be detected using a fluorescence probe
- A micro array scanner is used to photograph the intensity of the fluorescence on the slide
- NutriCognia's proprietary software CarboDeep is used to produce a fingerprint for the sample



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Project Outline (1)

- 1st major objective is to show differences between glycoproteins from cows milk compared with buffalo milk.

- A database with a ‘fingerprint’ for cows milk and a ‘fingerprint’ for buffalo milk will be produced. The technology will be tested using milk mixed to contain different amounts of cow and buffalo milk

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Project Outline (2)

- 2nd stage of the project will concentrate on mozzarella, fingerprints of glycoproteins isolated from cheese made from 100% buffalo milk will be compared with those from cheese made with 100% cow's milk

- Mozzarella adulterated with 5%, 10% and 30% cow's milk will be used to assess the method for its suitability for use as an enforcement tool

Fresh mozzarella di Bufala Campana

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Project Progress

Collection of authentic milk samples

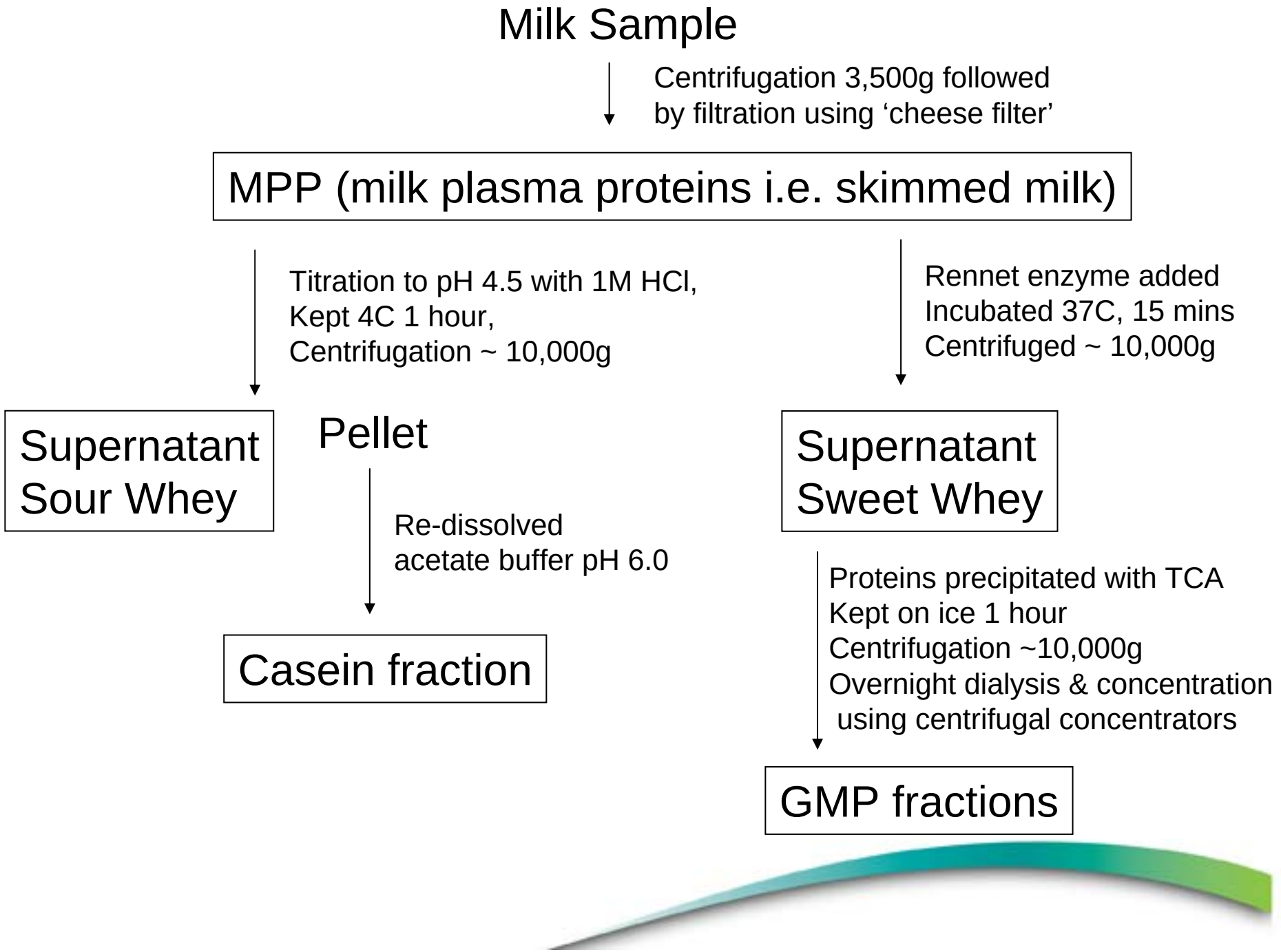
Cows milk

Two UK dairies provided milk samples before & after pasteurisation.

Buffalo milk

One UK buffalo farmer provided milk samples before & after pasteurisation. The original herd imported from Eastern Europe.

NutriCognia provided some protein subfractions prepared from Israeli Holstein cow and buffalo herds.


Milk Sample
Centrifugation 3,500g followed
by filtration using 'cheese filter'
MPP (milk plasma proteins i.e. skimmed milk)
Titration to pH 4.5 with 1M HCl,
Kept 4C 1 hour,
Centrifugation ~ 10,000g
Rennet enzyme added
Incubated 37C, 15 mins
Centrifuged ~ 10,000g
Supernatant
Sour Whey
Pellet
Supernatant
Sweet Whey
Re-dissolved
acetate buffer pH 6.0
Proteins precipitated with TCA
Kept on ice 1 hour
Centrifugation ~10,000g
Overnight dialysis & concentration
using centrifugal concentrators
Casein fraction
GMP fractions

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Project Progress

The following fractions prepared from the milk samples:-

MPP
Sour Whey
Casein
Sweet Whey
GMP

The glycoprofile of each sample was analysed using lectin chip array assays and CarboDeep technology.

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Project Progress

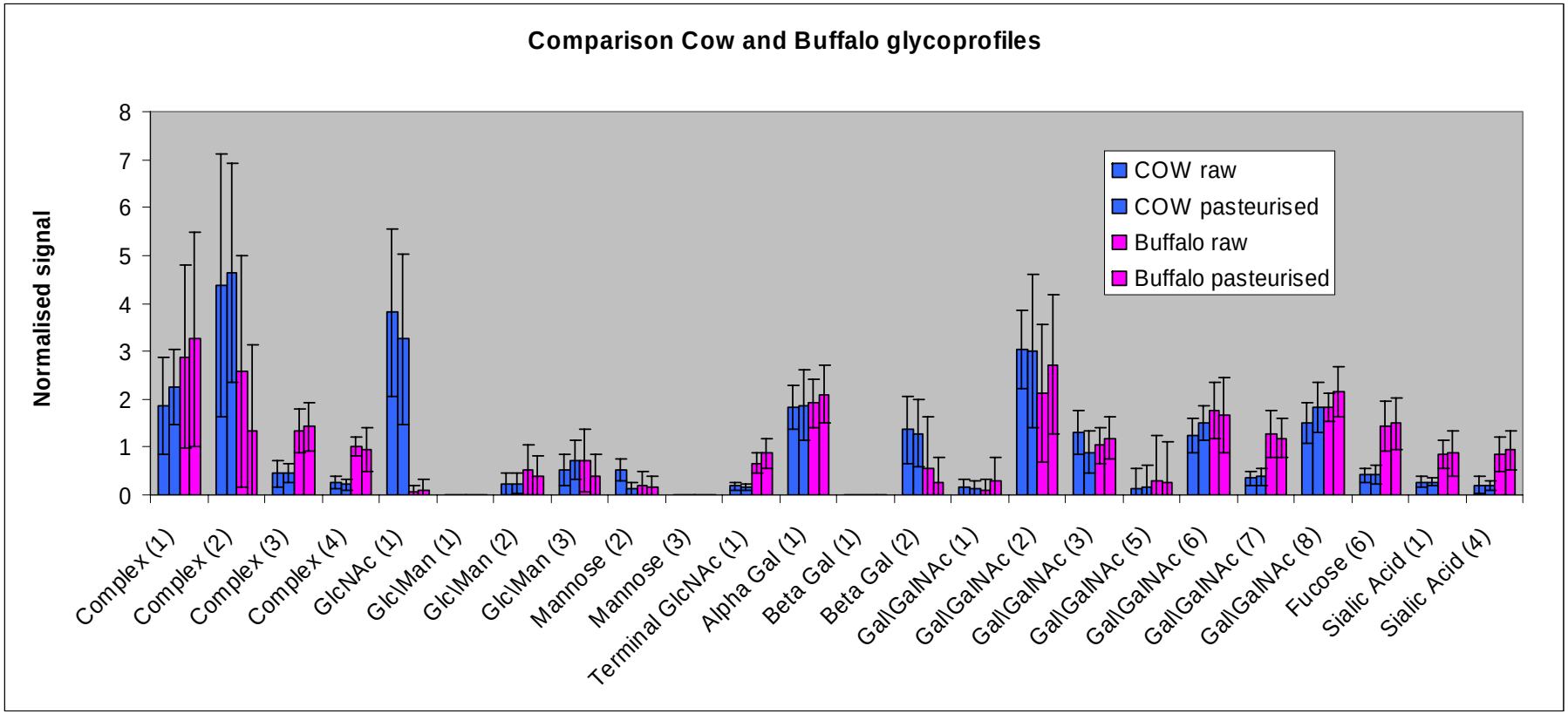
There were differences in glycoprofile between cow and buffalo samples for the following protein fractions:

MPP
Sour Whey
Sweet Whey

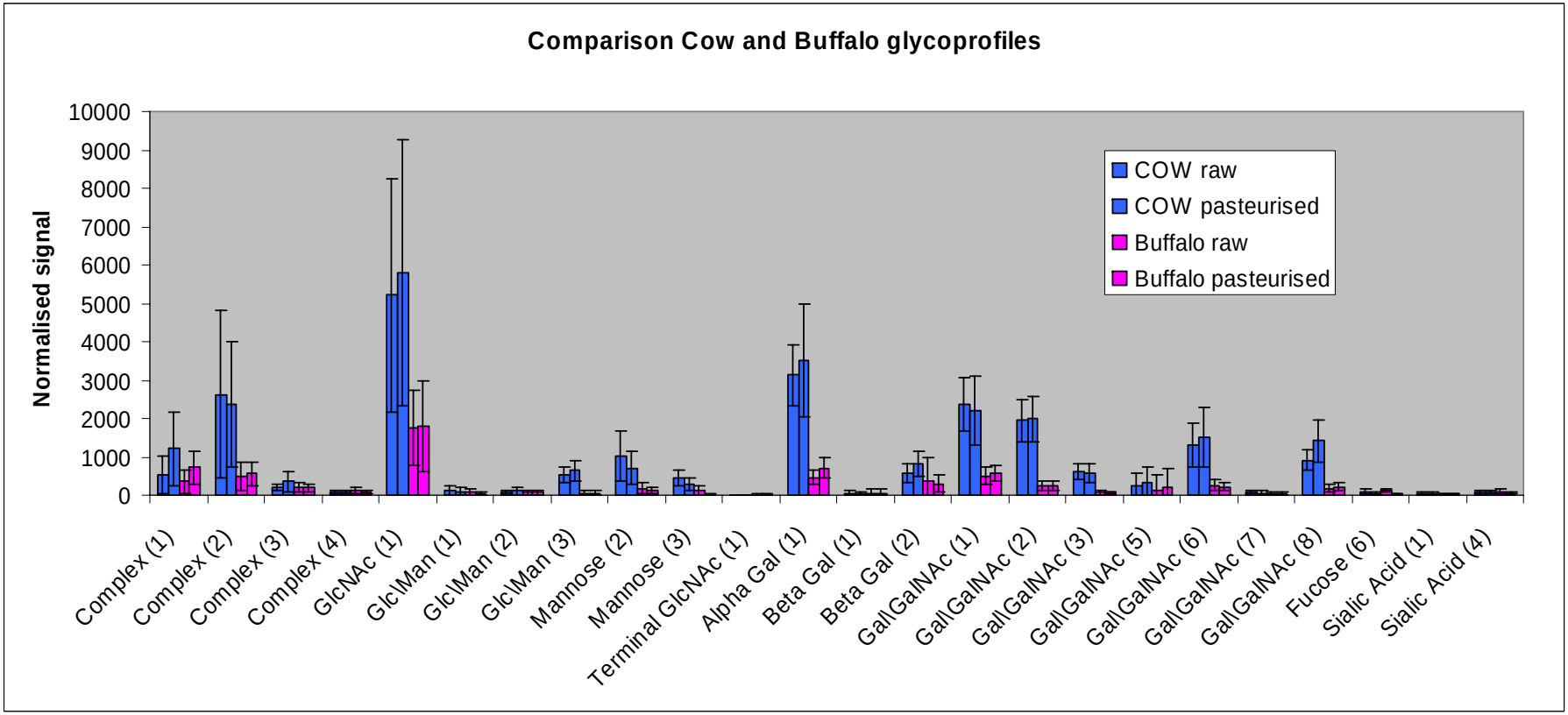
The following fractions showed no differences between cow and buffalo samples

Casein
GMP

MPP fraction

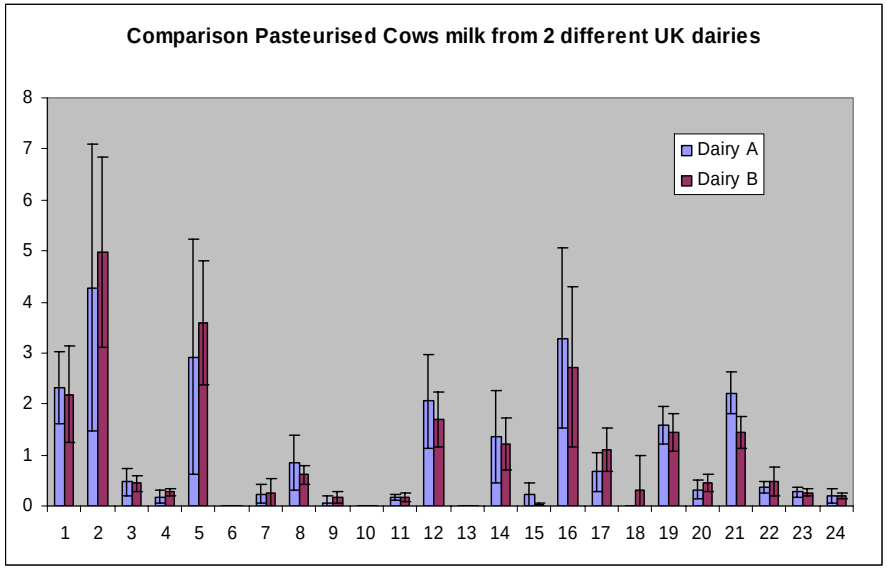


Sweet Whey fraction

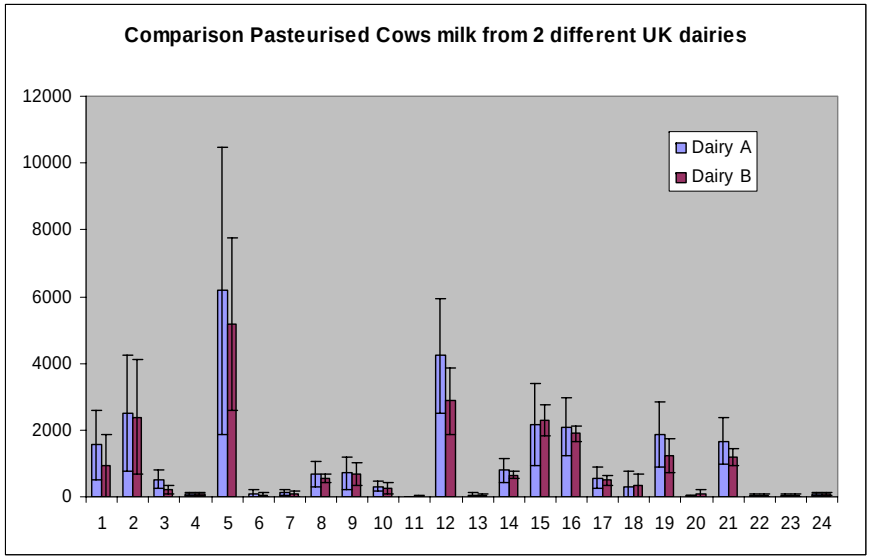


Comparison cows milk from different dairies

MPP fraction

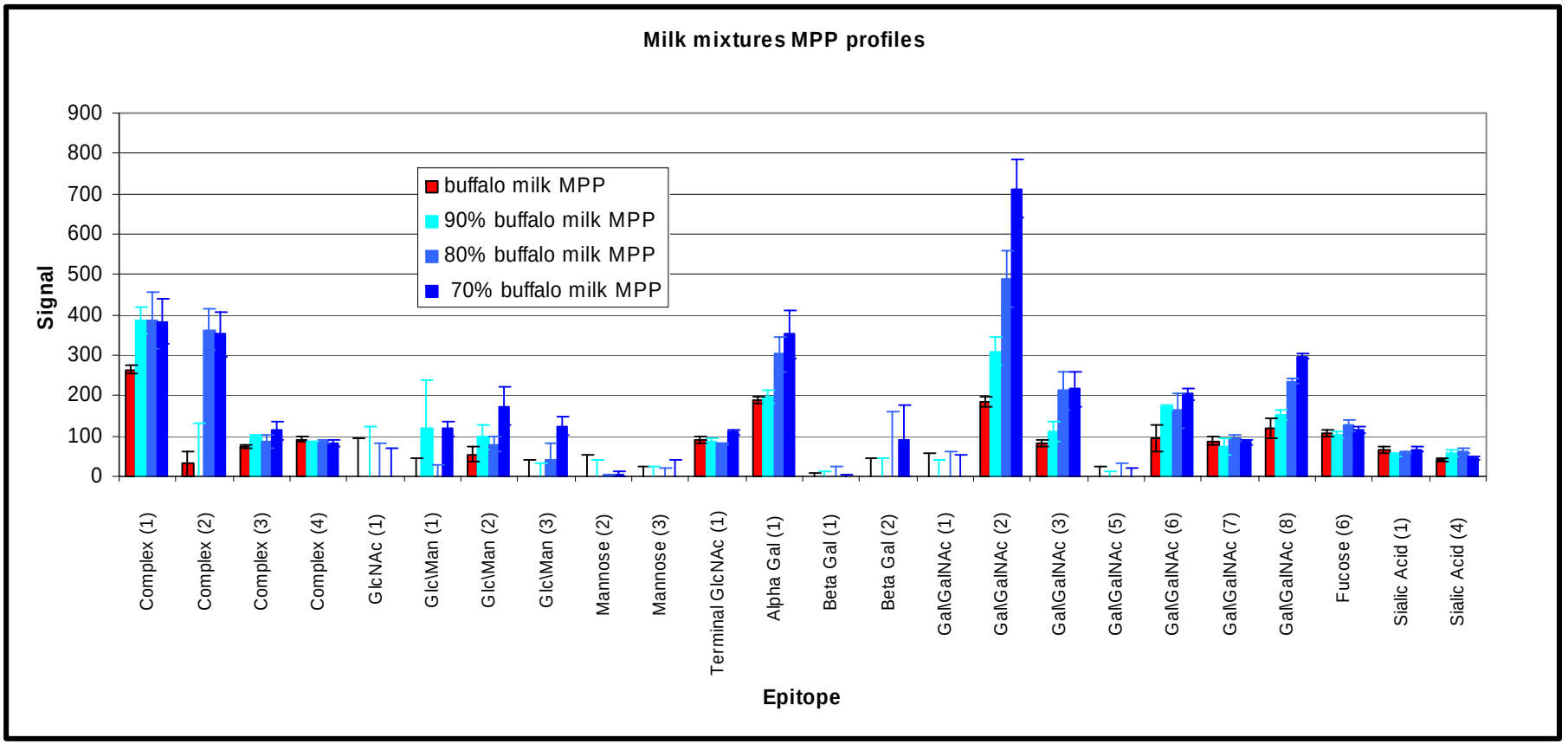


Sweet Whey fraction

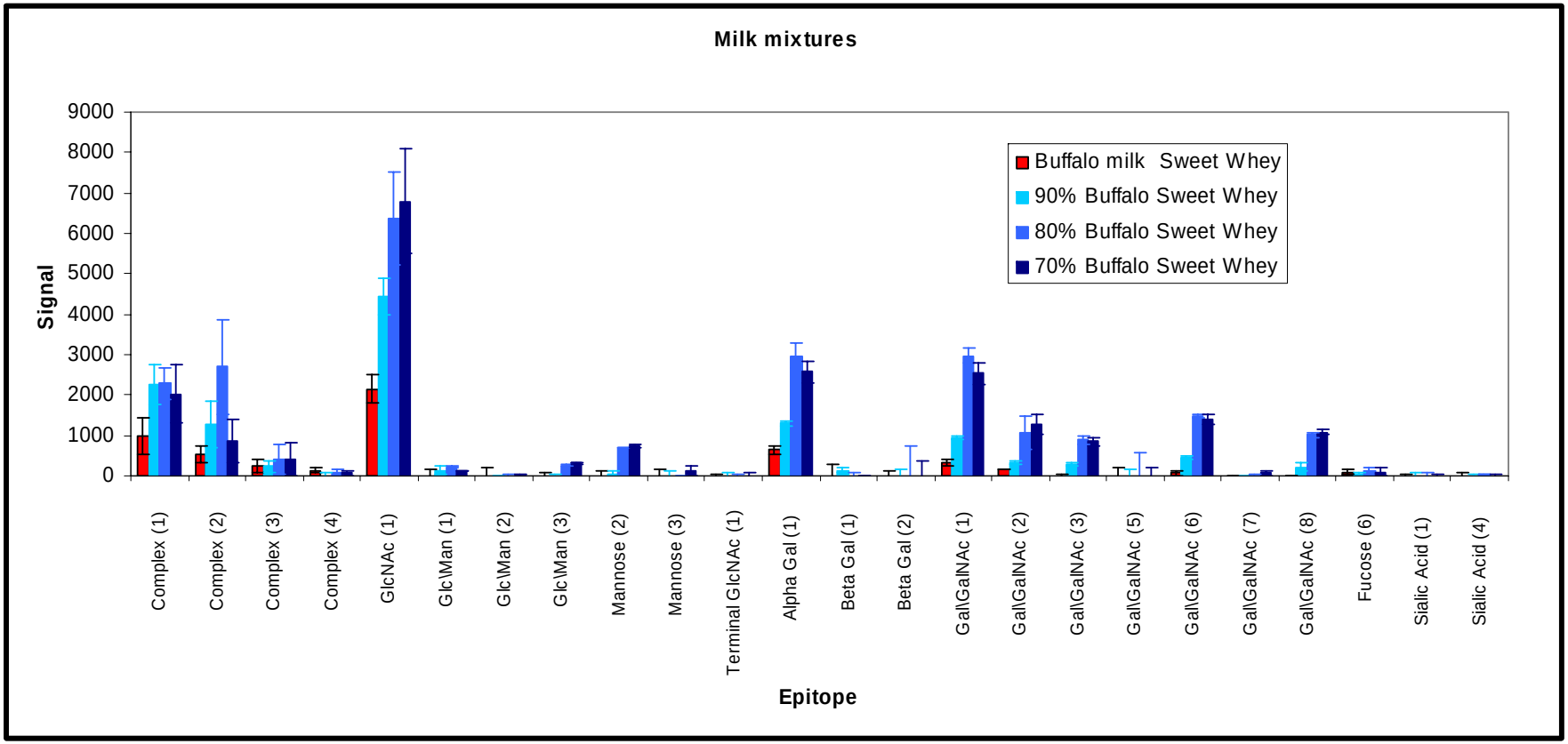


Similar profiles between the English and Israeli herds

Analysis of ‘adulterated’ milk samples **MPP**



Analysis of ‘adulterated’ milk samples
Sweet Whey



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Mozzarella Cheese

Buffalo Mozzarella has PDO status and can only be made with buffalo milk.

Fresh mozzarella di Bufala Campana

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Mozzarella Cheese

Test cheeses prepared by AB Cheesemaking

100% Buffalo milk
100% Cows milk

Test ‘adulterated’ samples

30% Cows 70% Buffalo
10% Cows 90% Buffalo
5% Cows 95% Buffalo

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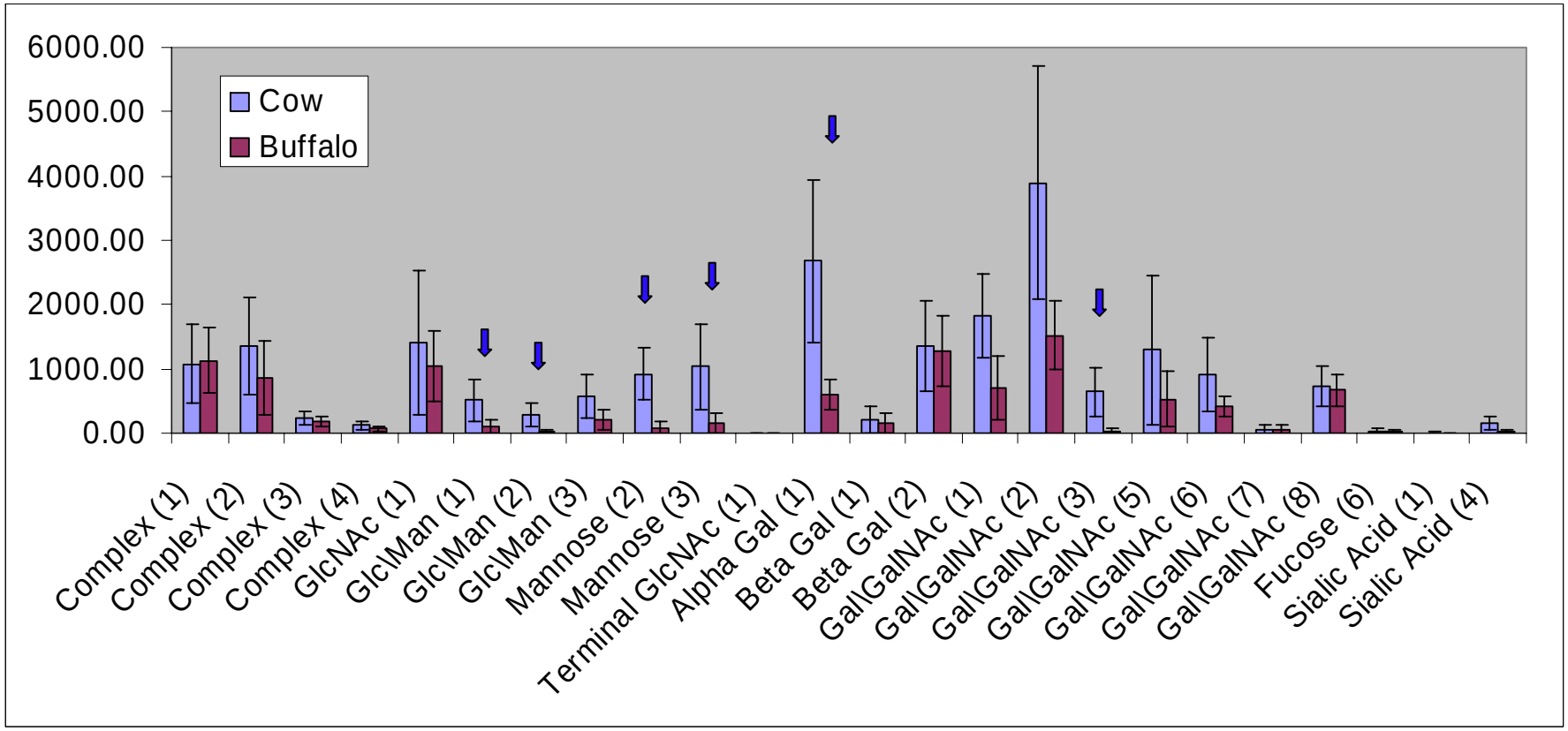
Mozzarella Cheese

Whey protein extract prepared (Mayer, 2005)

- Cheese homogenised in citrate buffer
- Acid precipitation of sample
- Supernatant desalted ready for analysis

Cheese glycoprofiles



Results are from 9 separate protein preparations

Lectins specific for GlcMan 1, GlcMan 2, Mannose 2 and 3, also Alpha Gal 1 and Gal GalNAc3 have shown clearly different signals for both types of samples.

Mozzarella Cheese

Summary

Unique fingerprints established for cow cheese and buffalo cheese

Tests of ‘adulterated’ samples to be completed shortly

Leatherhead Food International:

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Collaborators: NutriCognia
AB Cheesemaking

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