A comparison of native state of casein micelles of buffalo and cow milk and its molecular changes under different physico-chemical conditions

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Introduction-Buffalo milk

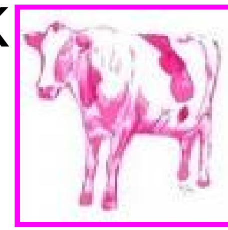
World production (Billion L)	2009
Cow milk	580.5
Buffalo milk	90.3
Others	25.8
Total	696.6

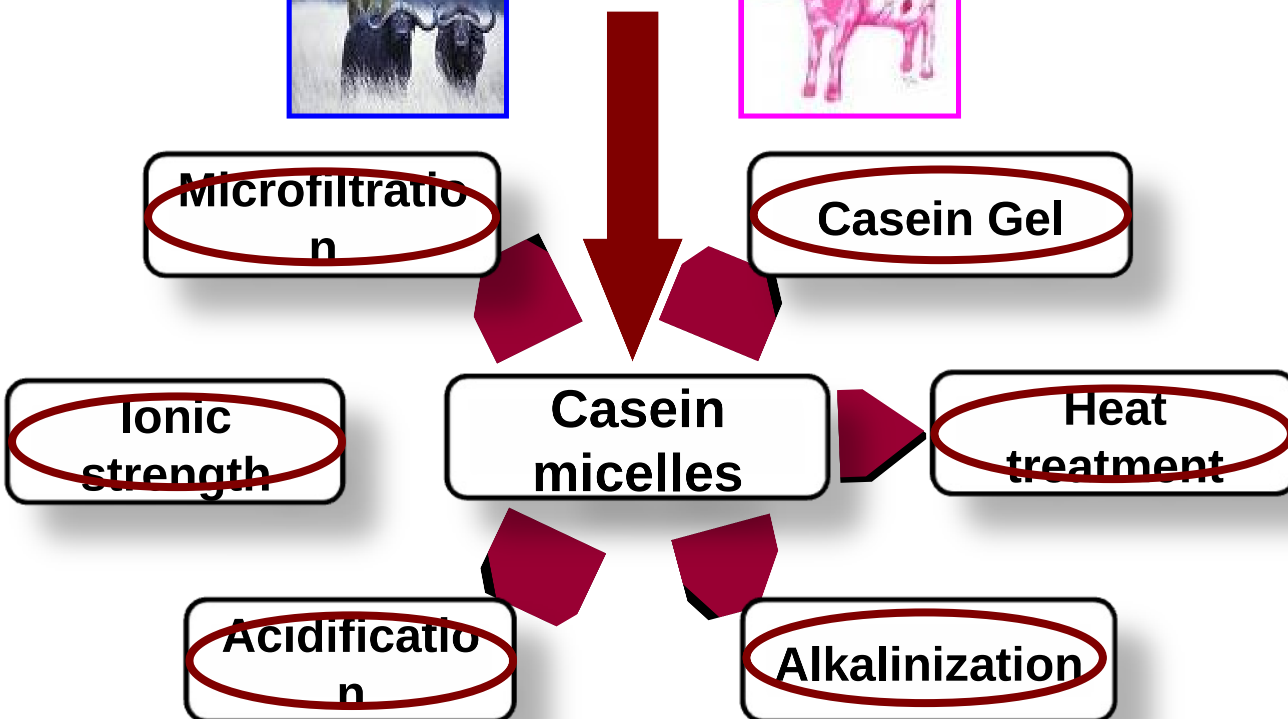
Source: F.A.O, Food and Agriculture Organization

- Buffalo milk is the world's second most produced milk (13%)
- > 92% of this production is from India (68% with 70.0 Billion L) and Pakistan (24% with 21.6 Billion L)
- Buffalo milk is richer in all major components like protein, fat, lactose & minerals than cow milk
- Technological transformation into dairy products is very little in both major buffalo milk producing countries (Pakistan=3% and India=15%)
- A vast knowledge exists on technological transformation of cow milk but a very limited information on the effects of processing on buffalo

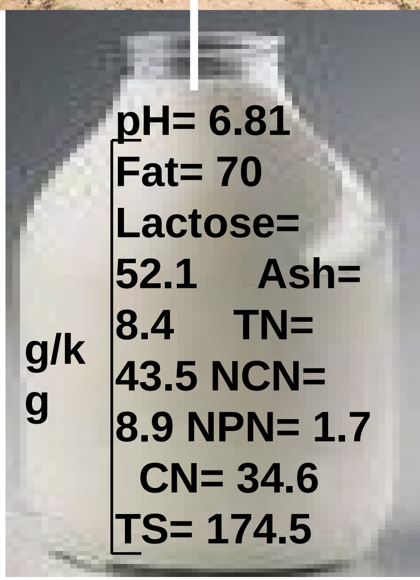
Objective

- To gain knowledge on casein micelles of buffalo milk:
  - native state
  - molecular changes under different physico-chemical conditions
- Casein micelles of cow milk used as a reference



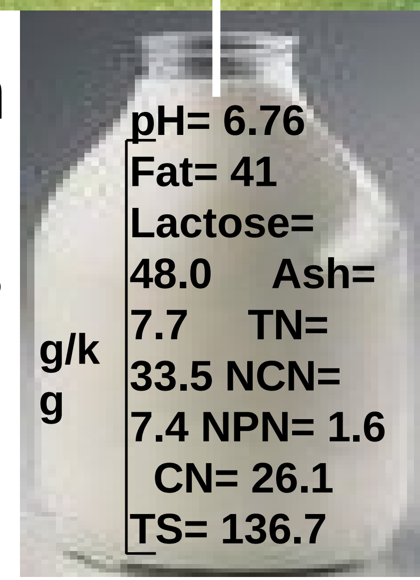


General Composition

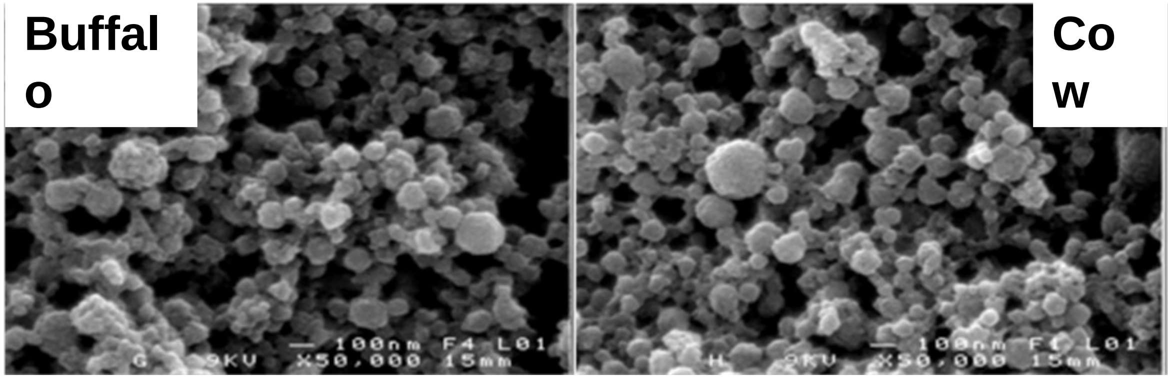


- Buffalo milk was richer in all major components particularly casein contents than that of cow milk

TN: Total nitrogen; NCN: Non casein nitrogen; NPN: Non protein nitrogen; CN: Casein; TS: Total solids



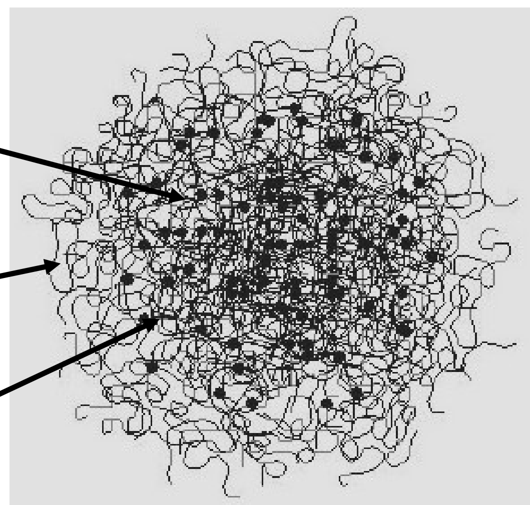
Native Casein Micelles



Size: 200 nm
Charge: -20 mV
Hydration: 1.9 g of $H_2O.g^{-1}$ dry pellet

Size: 180 nm
Charge: -20 mV
Hydration: 2.2 g of $H_2O.g^{-1}$ dry pellet

Model of Carl Holt

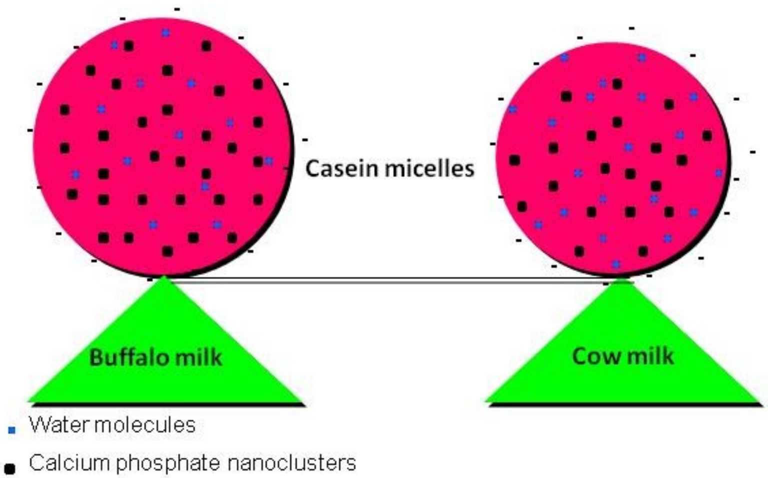


Association of α_{s1}-, α_{s2} & β-casein

κ-casein

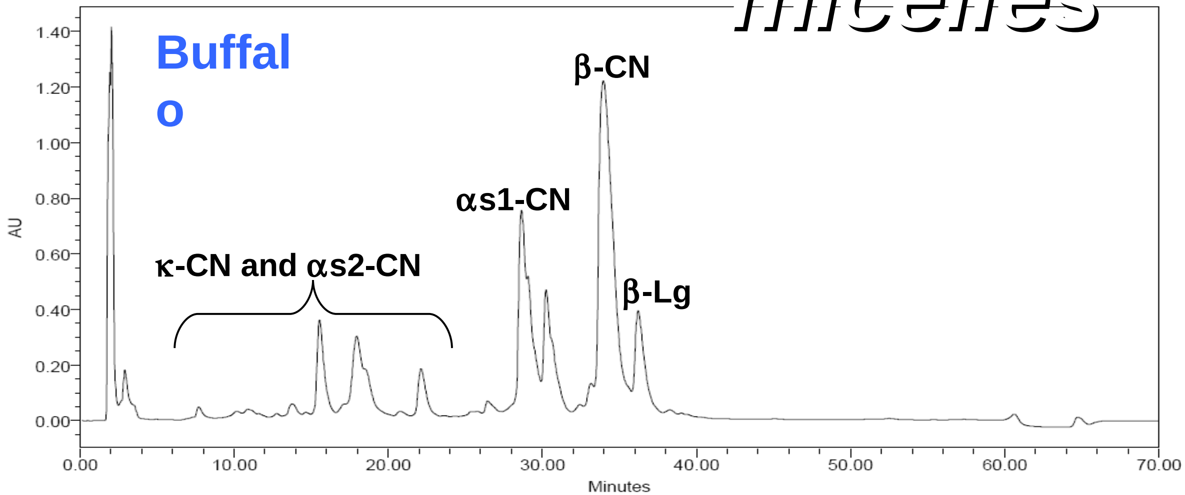
Nanoclusters

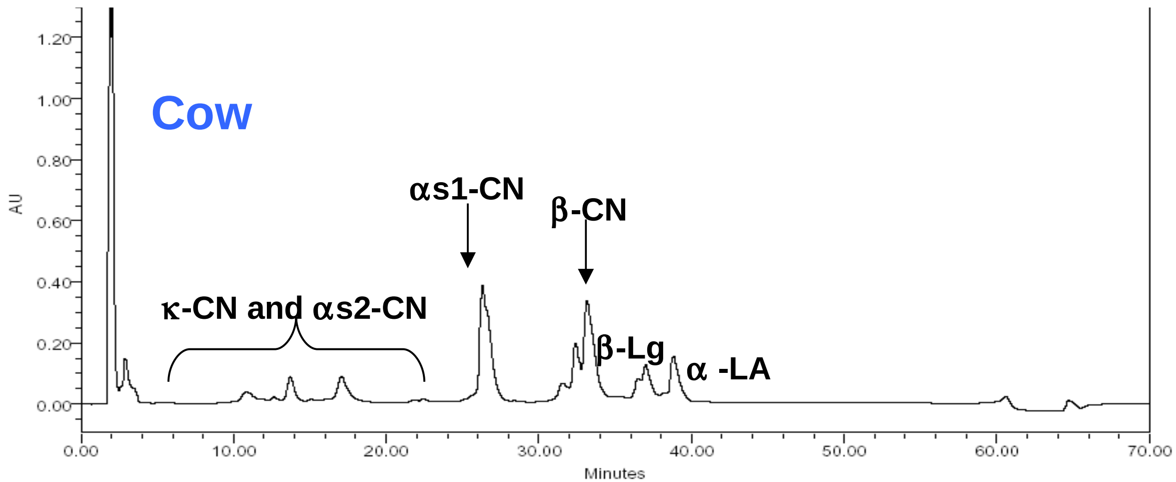
- **Casein concentration:** Buffalo milk > cow milk
- **Homology in amino acid sequencing:** 95 %
- **Size:** Buffalo milk > cow milk
- **Colloidal minerals:** Buffalo milk > cow milk
- **Hydration:** Buffalo milk < cow milk



Native Casein micelles

RP-HPLC Profiles



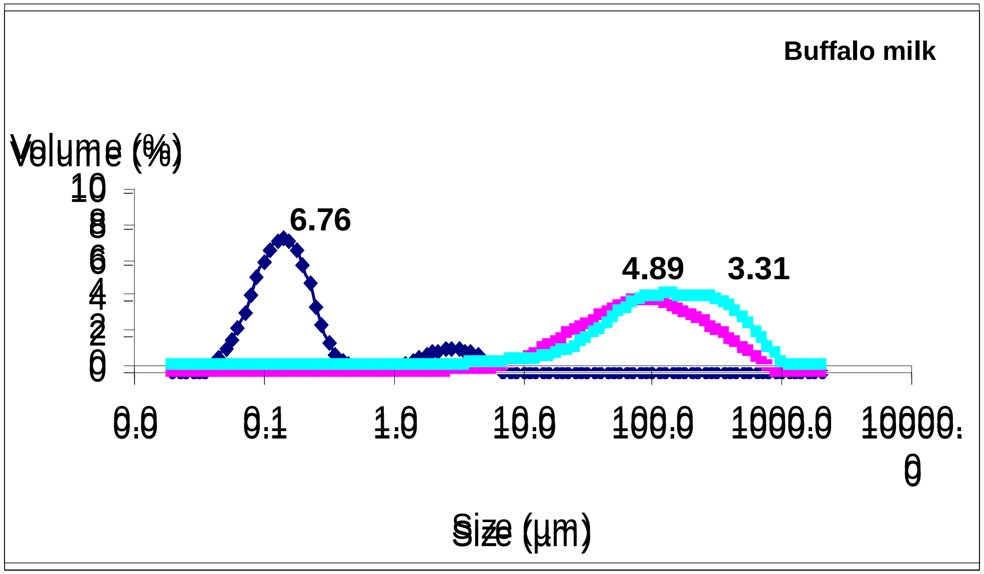


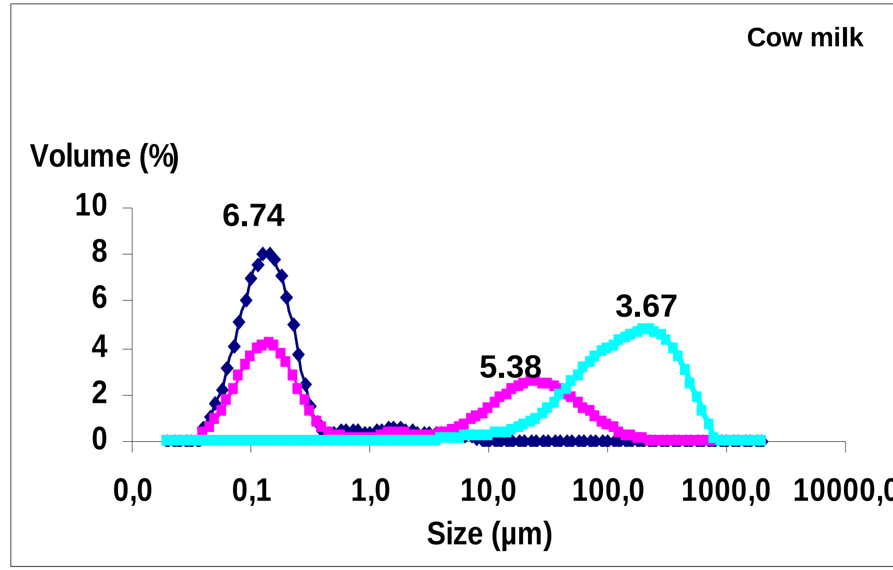
- HPLC profiles are similar: 4 classes of caseins are present in both milks
- All caseins classes are more important in buffalo milk than that of cow milk

Proteins fractions	Surface area (µV*sec)	Buffalo milk (%)	Surface area (µV*sec)	Cow milk (%)	Buffalo/Cow
κ-CN + α_{s2}-CN	38077712	21	9134691	15	1.40
α_{s1}-CN	56047939	31	18835282	30	1.03
β-CN	71787660	39	22010568	35	1.11

Acidification

Size of Particles and Aggregates

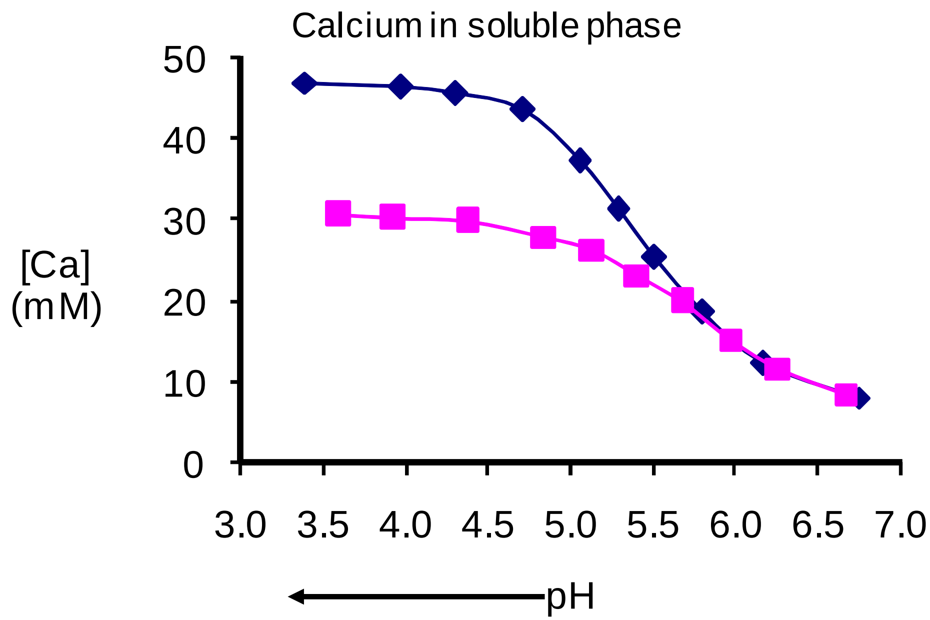


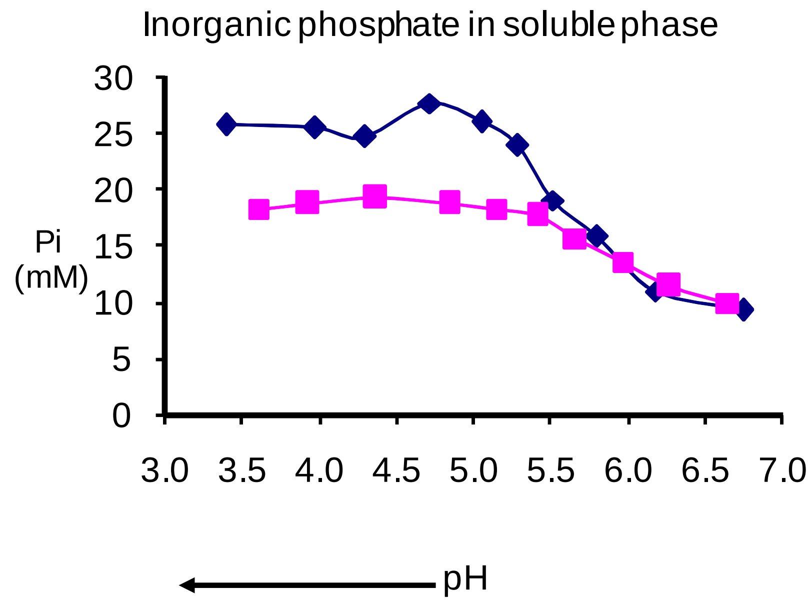


- Acidification induces aggregation of casein
- Identical process of protein aggregation during acidification in both milks

Acidificati on

Micellar Ca & Pi Solubilization



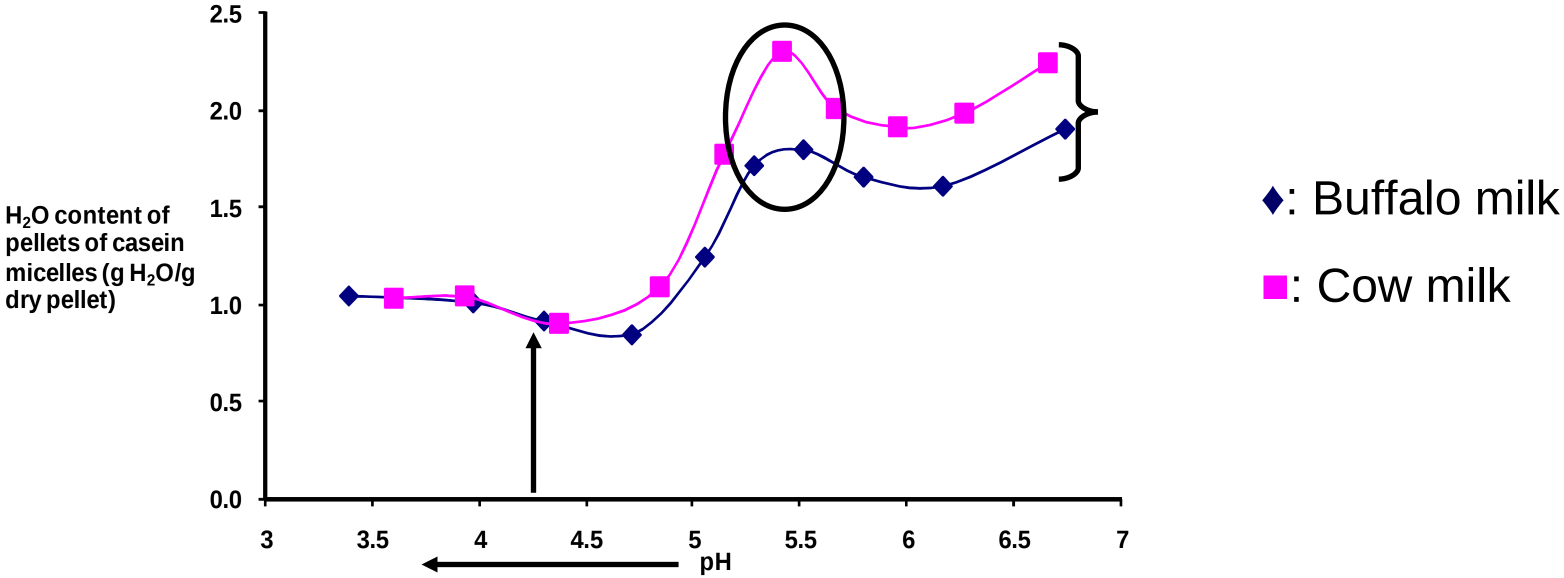


♦: Buffalo milk

■: Cow milk

- ➢ Diffusible Ca & Pi ⇗ when pH ⇘
- ➢ Solubilization of micellar calcium phosphate

Acidification on
Micellar hydrations



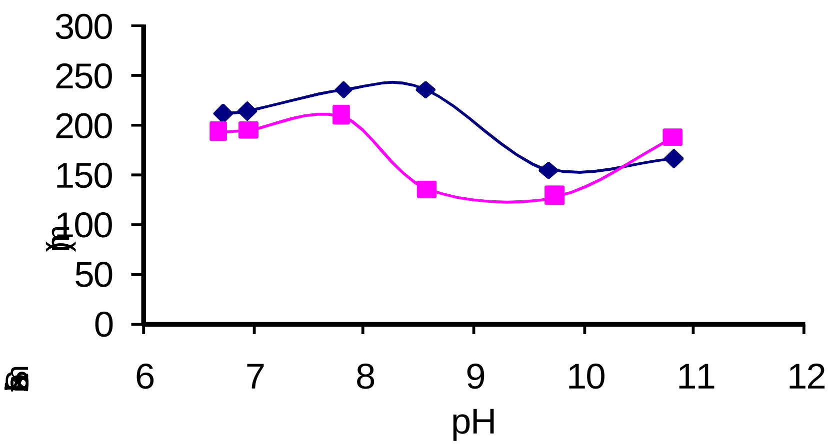
➢ ⇘ **micellar hydration with pH ⇘ (corresponds to collapse of outer hairy layer of casein micelles and dissociation of Ca and Pi)**

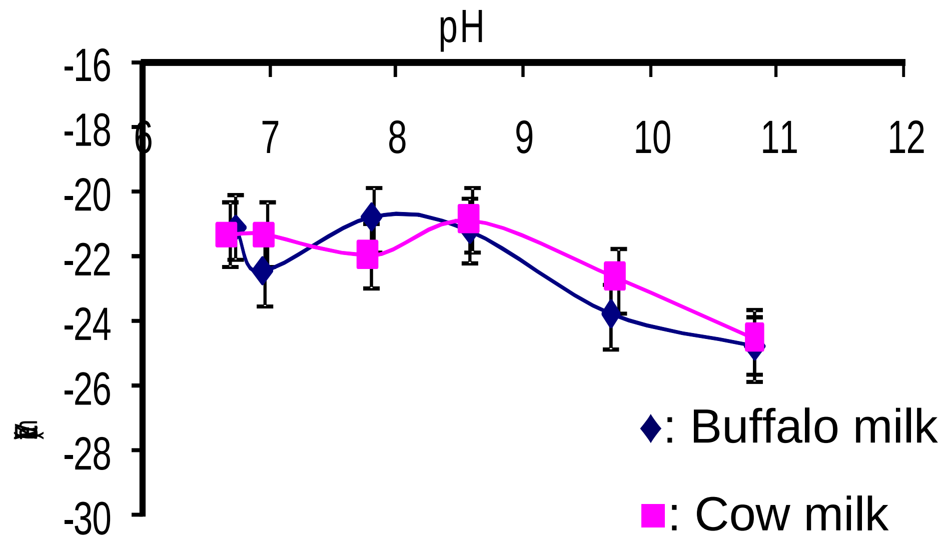
➢ ⇗ **micellar hydration with pH ⇘ (corresponds to ⇘ of electrostatic interactions between casein and increase of volume of casein micelles)**

➢ ⇘ **micellar hydration with pH ⇘ (corresponds to precipitation of casein micelles at pHi and general charge neutralization)**

Alkalinization

Micellar size and charge





- **Size of the casein micelles ↗ and ↘ which approves their disruption at pH > 8.6**

- **An ↗ in size is observed at pH > 10.8 may be due to reticulation of casein particles**

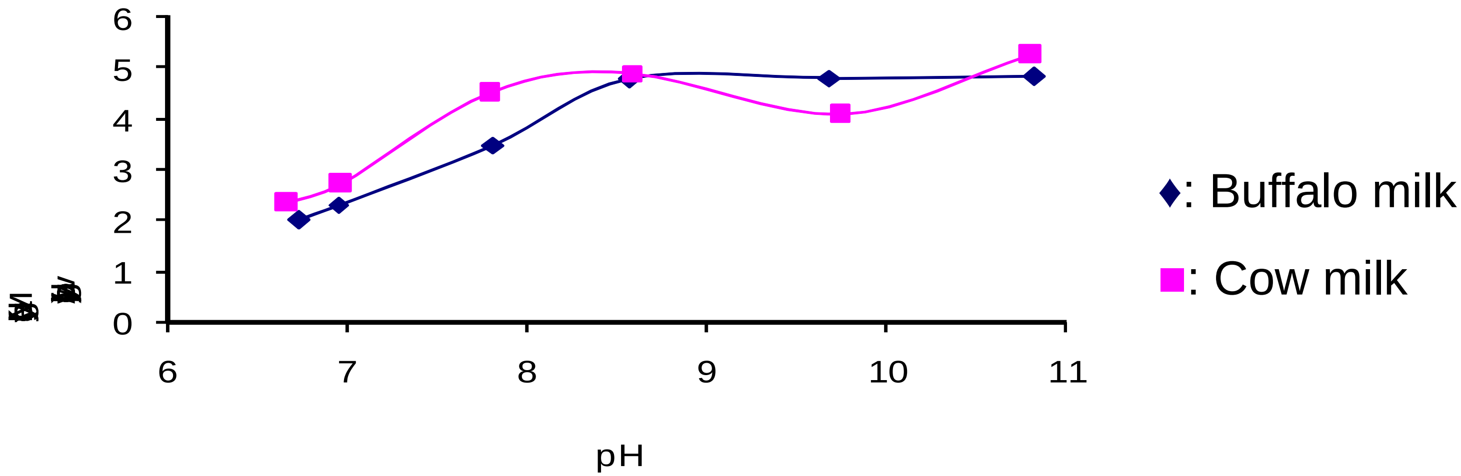
- **A significant ↗ in the net negative charge of the casein micelles may be due to:**

- changes in the ionizations of proteins and κ-casein-associated sugars

- replacement of

Alkalinization

Micellar hydration



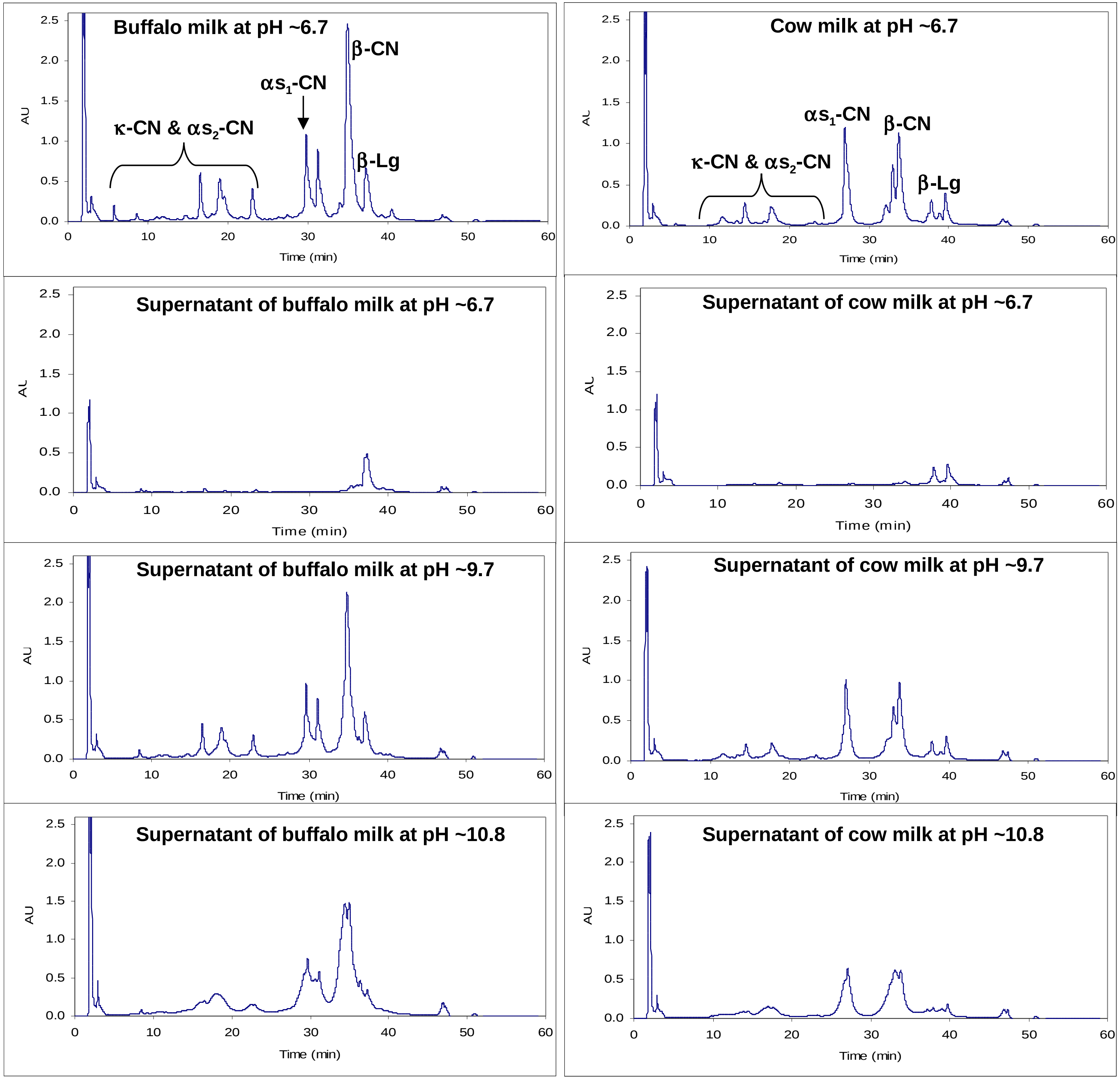
➢ **Ionic cosmotropes (Ca & Pi ions) promote association of hydrophobic areas of protein by binding water strongly and thus reducing the volume of water available to hydrate exposed protein surfaces**

Alkalinization

RP-HPLC

- All caseins are increasing in the supernatants as a function of pH which approves the increase of [TN]

- At pH 10.8 protein molecules have been degraded



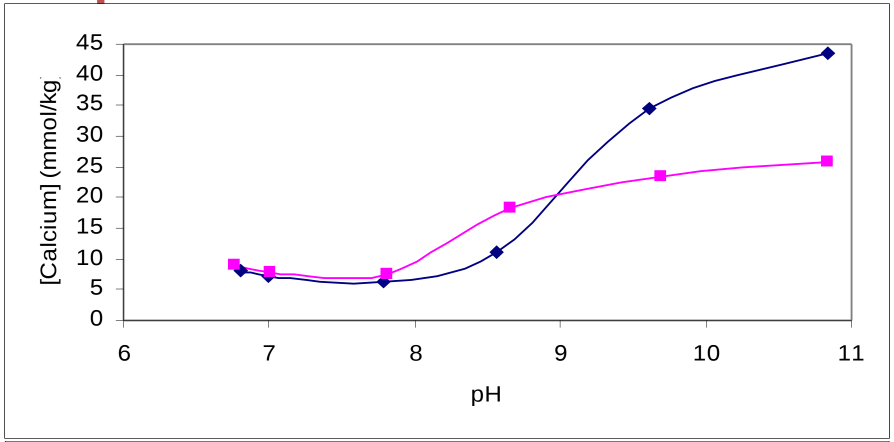
Alkalinization

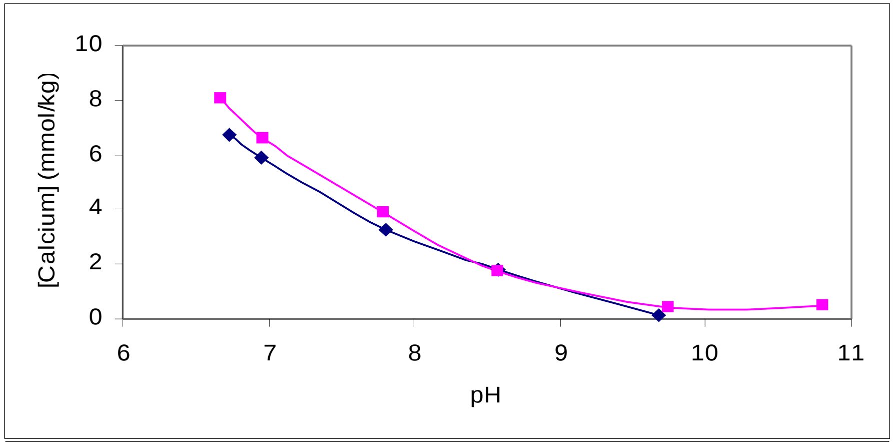
Micellar Ca and Pi

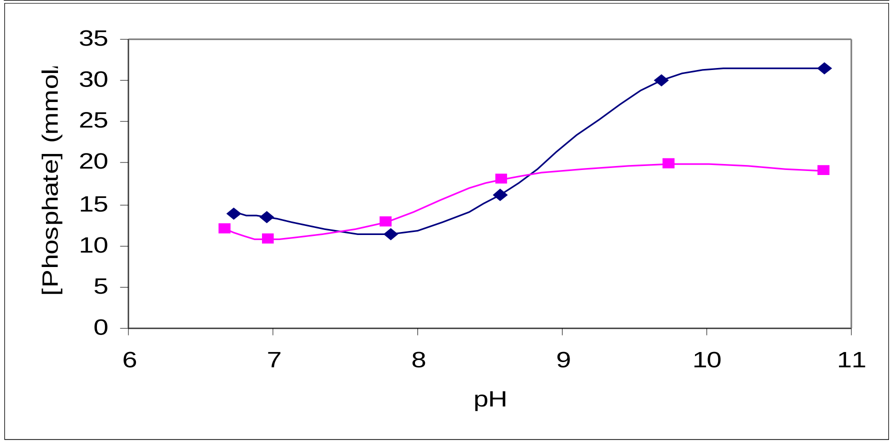
♦: Buffalo milk

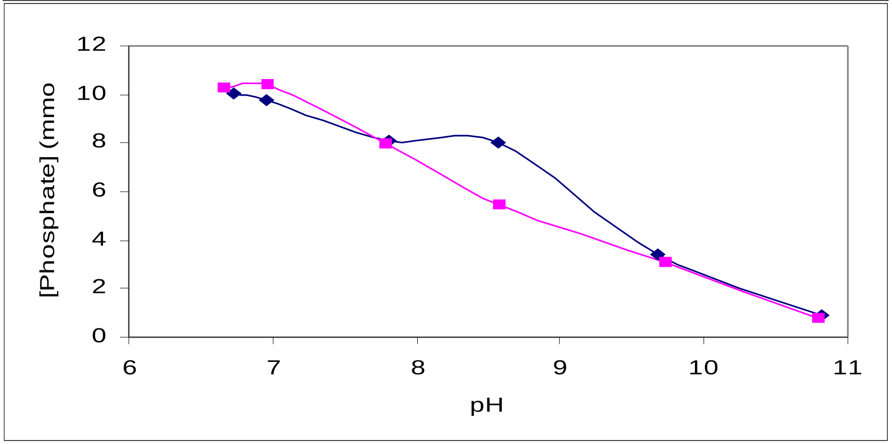
■: Cow milk

Supernatan | **Ultrafilterate**









- **[Ca] & [Pi] ↗ in supernatants and ↘ in ultrafiltrates of both milks**

- **Association of calcium phosphate onto disrupted casein**

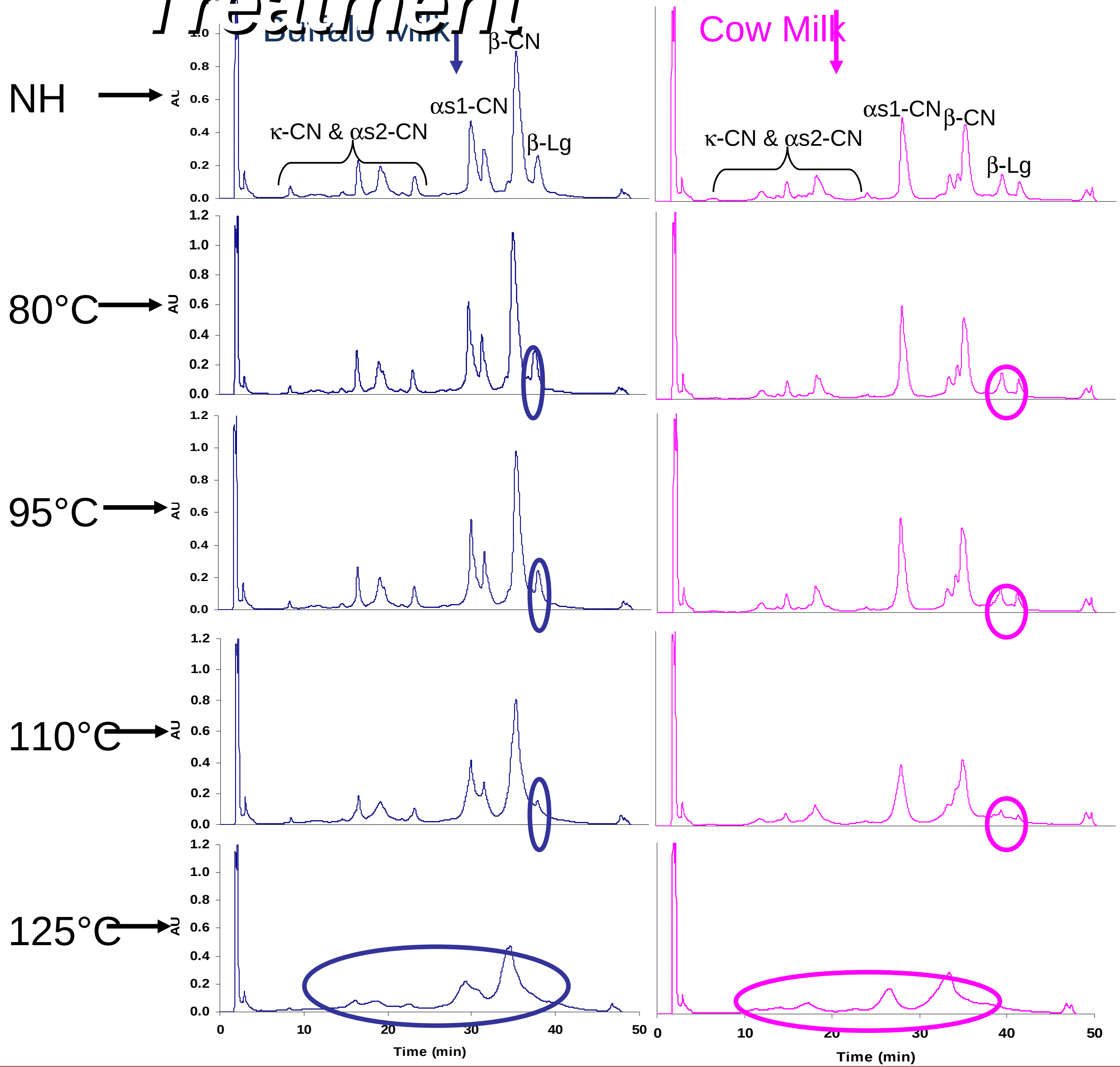
Heat Treatment

RP-HPLC

- ⮚ **β-Lg disappeared with ⇗ of temperatures**
- ⮚ **Modification of casein molecules particularly at 125°C**

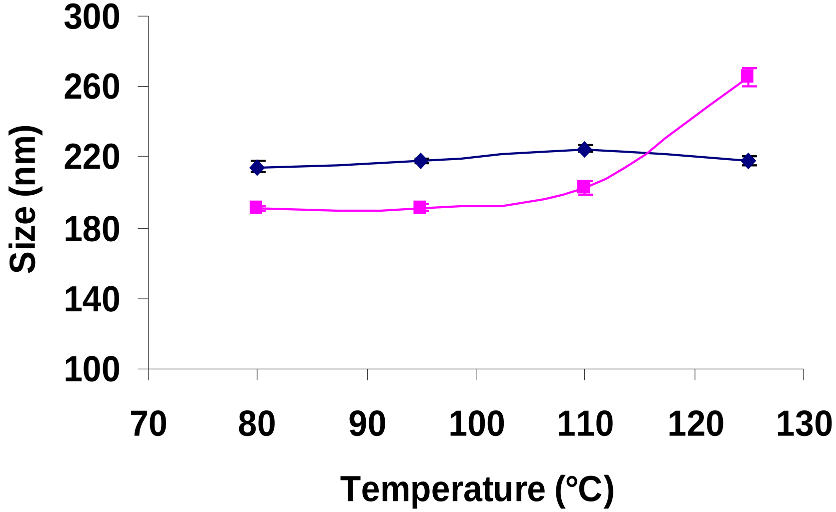
- ⮚ **Disappearance of β-Lg was due to its denaruration and interaction with κ-CN**

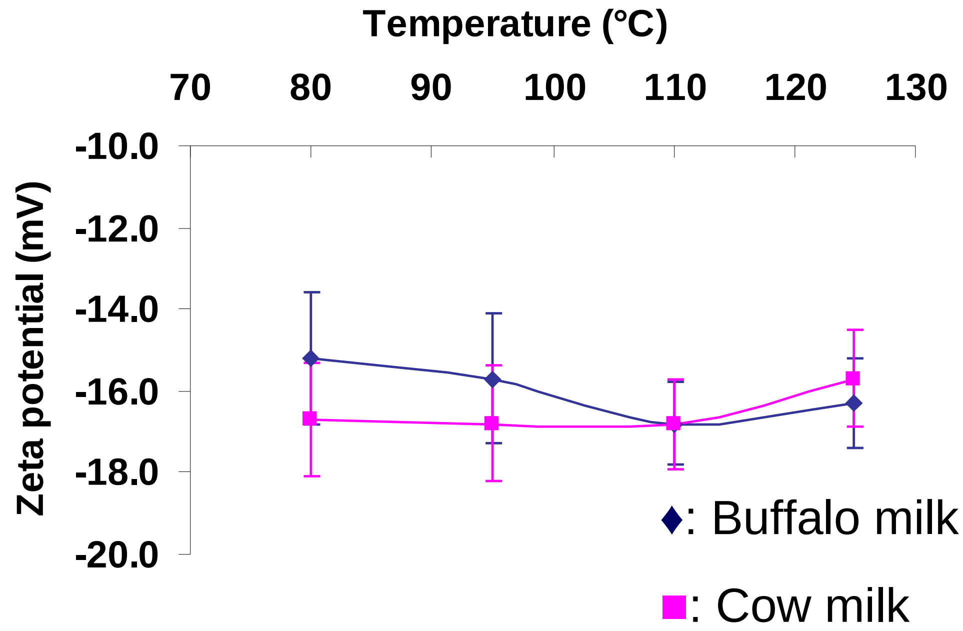
- ⮚ **Modification of casein molecules may be due to:**
  -proteolysis
  -deamidation
  -dephosphorylation
  -lactosylation
  -formation and breakdown of s-s bridge



Heat Treatment

Micellar Size and Charge



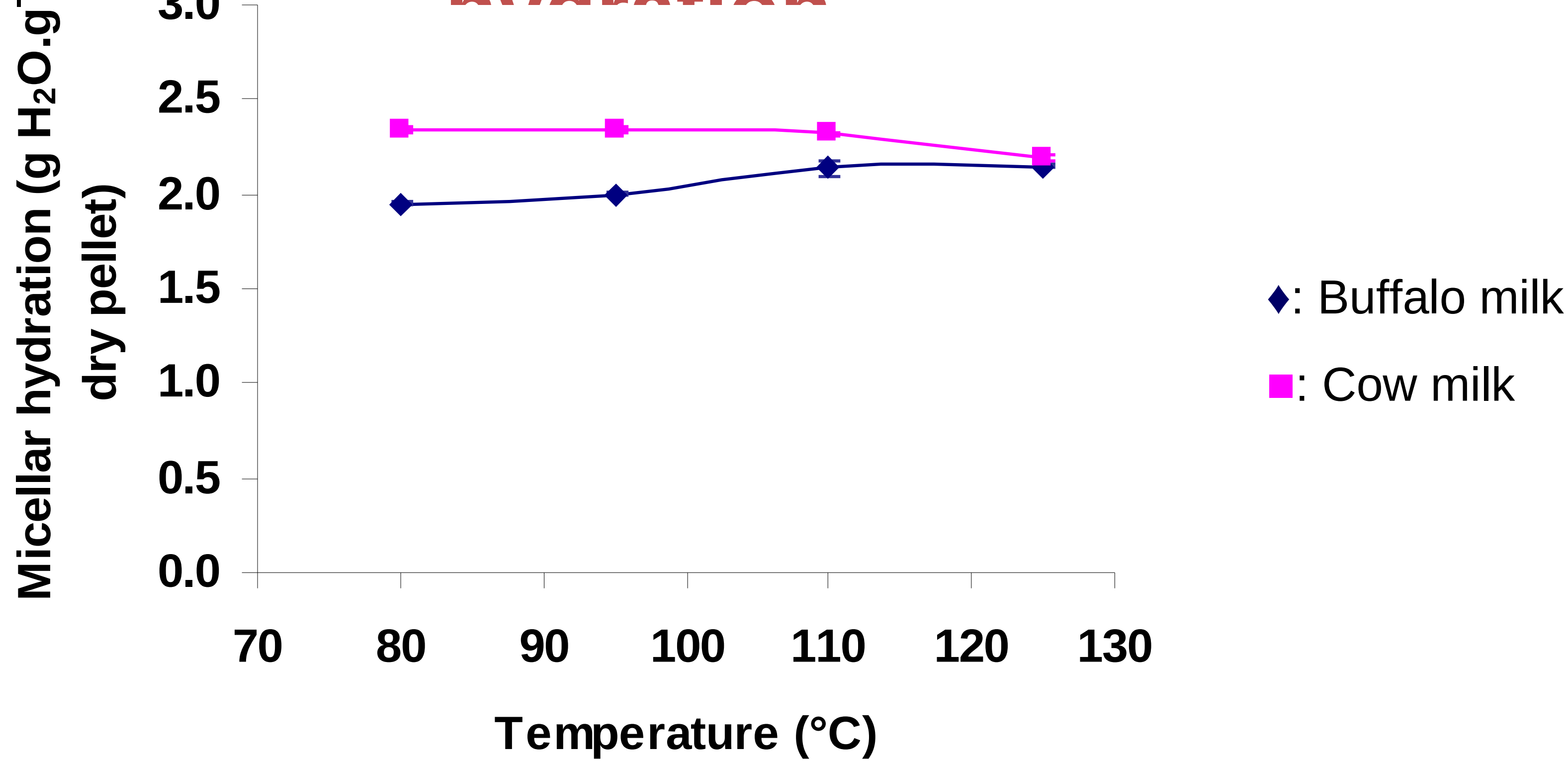


➢ **Overall size ↗ with ↗ temperature in both milks**

➢ **No effects of heat treatment on the zeta potential around casein micelles of milks of both species**

Heat Treatment

Micellar hydration

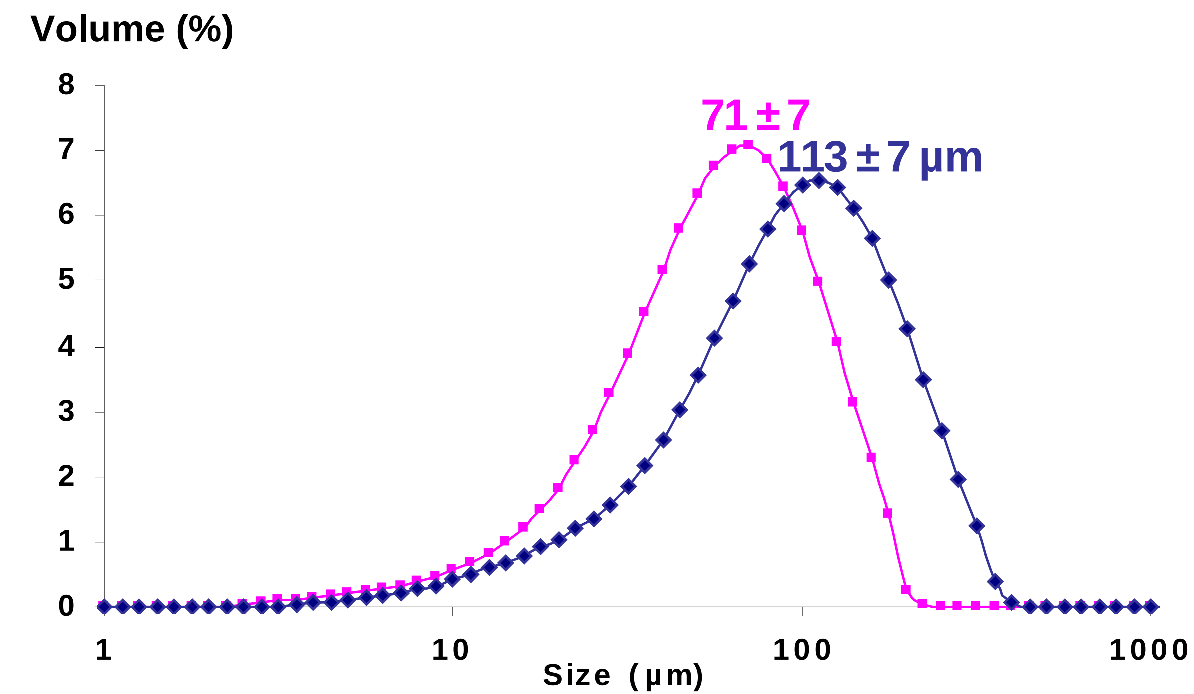


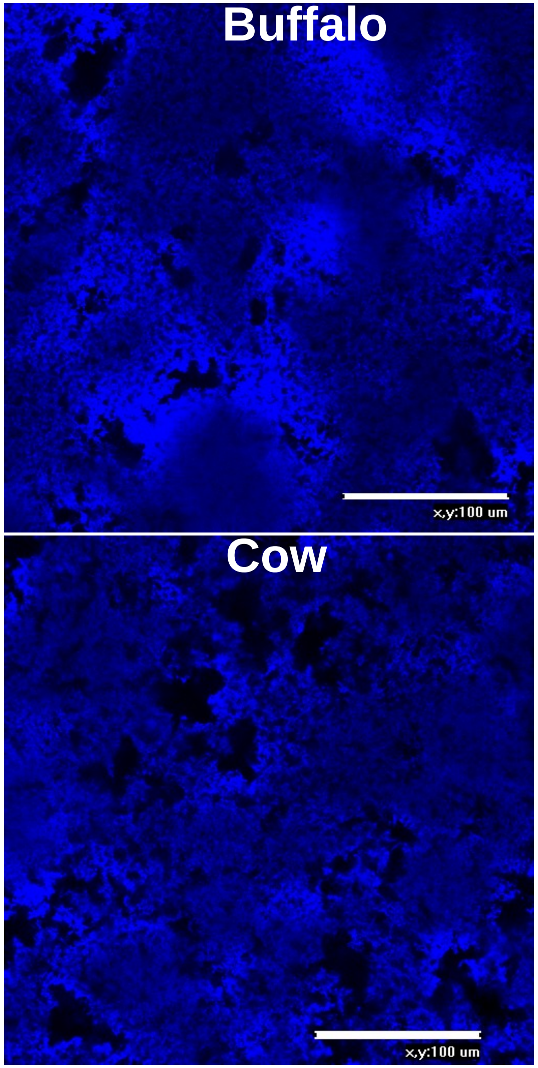
- ➢ Micellar hydration remained constant

♦: Buffalo milk

■: Cow milk

Casein Gel Microstructure

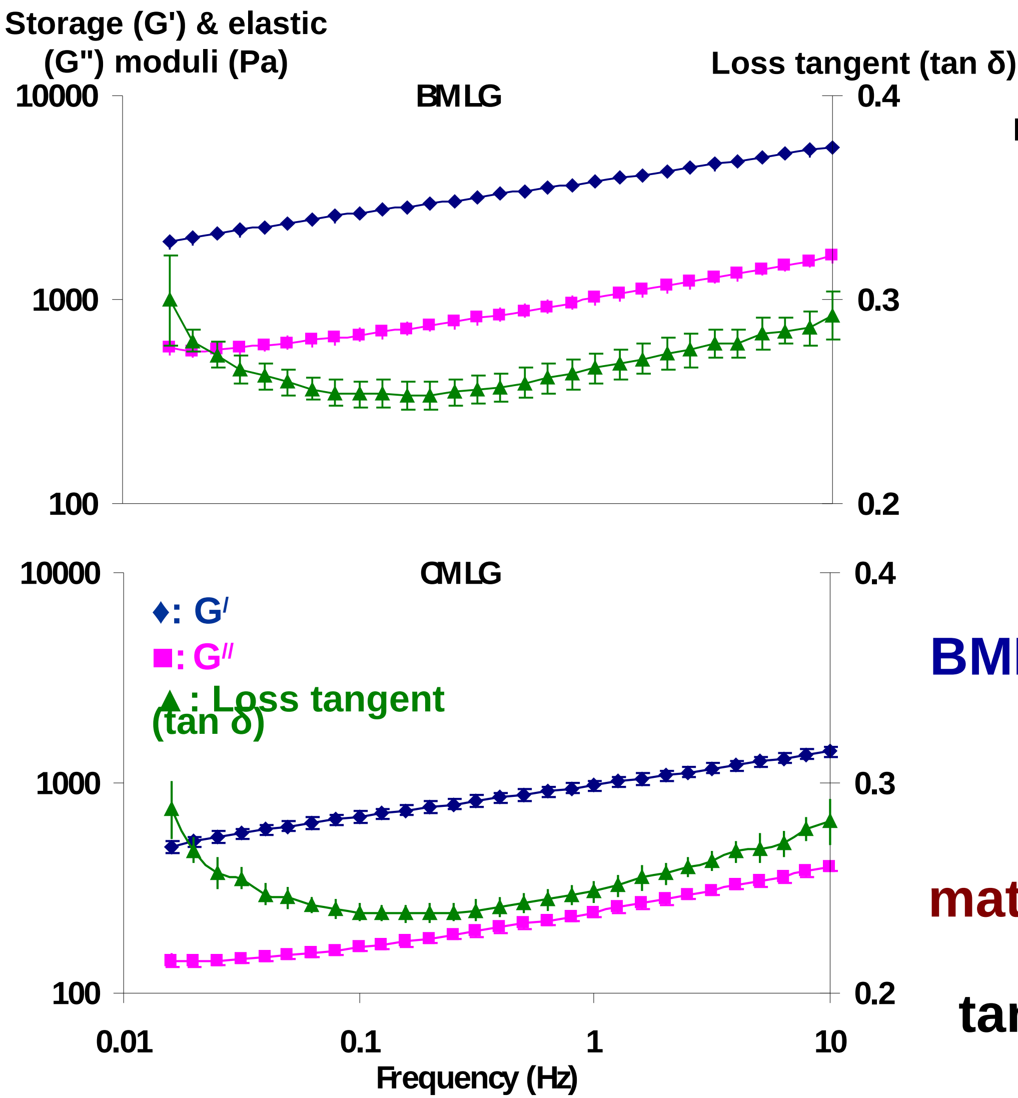


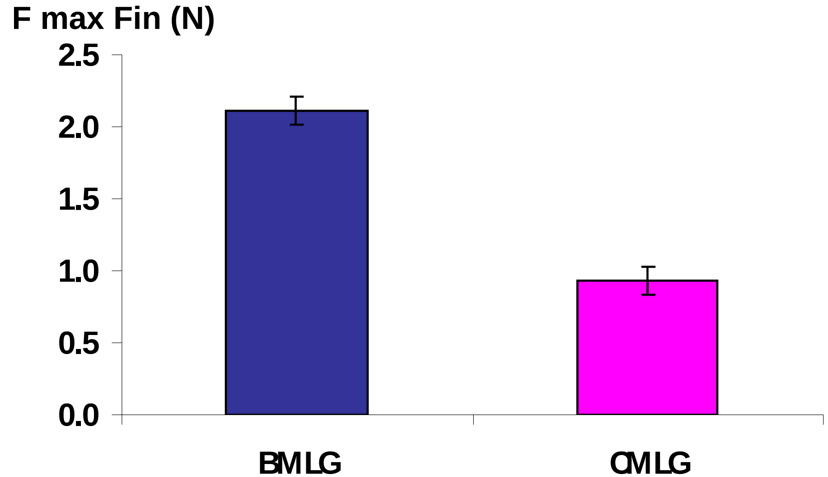


Buffalo casein gel presents

- bigger particles than that of Cow casein gel with less

Casein Gel Rheology



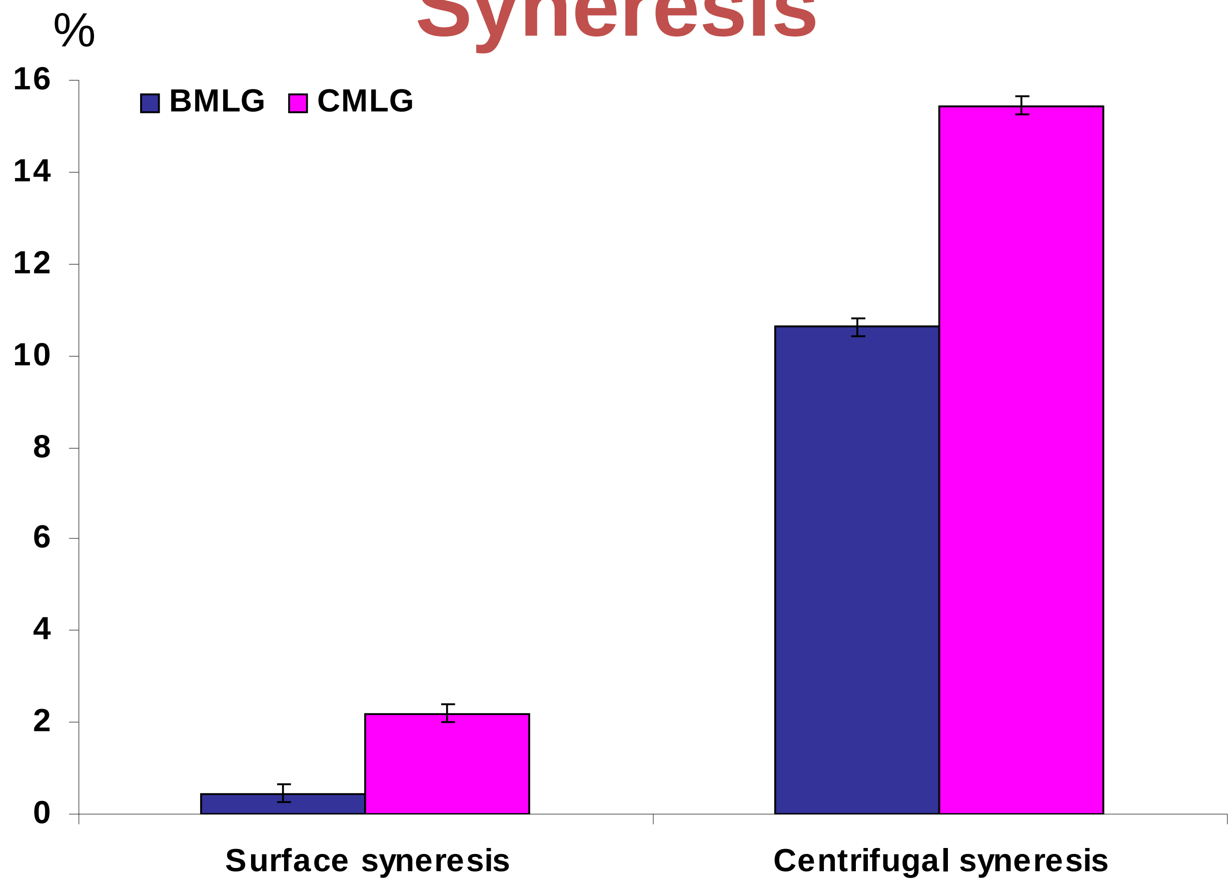


BMLG has

- higher visco-elastic moduli (G´ & G˝)
- more interactions or more building material?
- Both have similar loss tangent (δ) = same structure, same solid/liquid like properties

BMLG is firmer

Casein Gel Syneresis



BMLG shows

- less syneresis with both methods
- more water holding capacity

Conclusio
ns

Microfiltration-Native Casein

- **Native casein micelles from buffalo milk were bigger in size, less hydrated, more mineralized with similar charge than that of cow milk**
- **Similar classes of caseins in both milk but higher concentration in buffalo milk**

Acidification

- **The molecular changes were similar for both milks qualitatively**
- **Precipitation/aggregation of casein suggested similar isoelectric pH**
- **Minerals solubilized in the same manner (pH for total solubilization of Ca ~ 3.5 and of Pi ~ 4.7)**
- **Micellar hydration (decrease and increase showing the similar behavior and also complete neutralization at pH 4.6)**
- **Acidification process (already well established for cow**

Conclusions

Alkalinization

- Ca & Pi ⇘ in soluble phase due to possible precipitation onto casein
- disruption & aggregation of casein molecules ⇗ and size of micelles ⇘ upto 120 nm
- net - ive charge ⇗ due to loss of net + ive charge ; micellar hydration ⇗ and solubility of casein micelles ⇗

Heat treatments

- Effects of heat treat treatments were qualitatively similar for both milks but quantitatively different related to the compositional differences
- The biochemical modifications were progressively occurred with ⇗ of intensity of heat treatments with major effects observed at 125°C in both milks

Casein gel

- Bigger size, undissolved Ca and lower hydration of casein micelles of buffalo milk resulted into a firmer gel

Acknowledgement

THANKS FOR YOUR ATTENTIO N