Iron Fortification in Dairy Industry

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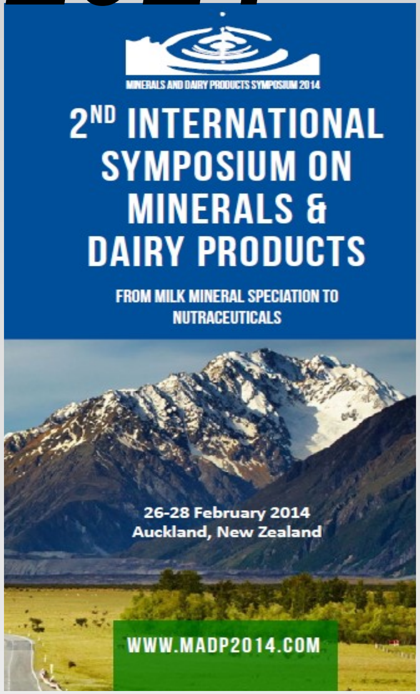

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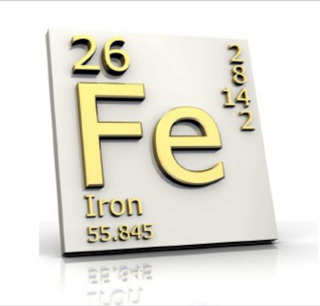


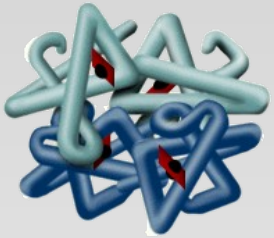
1. Importance of iron for the human organism
2. Iron in milk and dairy products
3. Iron sources for fortification
4. Interactions of iron with different individual dairy componen
5. Interactions of iron in milk and consequences
6. Some examples of dairy products enriched in iron

(No discussion on the nutritional effect)

Iron in human organism

- About 4 and 2.5 g of iron for man and woman, respectively.

- Exists under 2 forms:
  - ✓ **heminic iron** (70 %) Participate to the constitution of hemoglobin (structure of heme), myoglobin and different enzymes.
  - ✓ **non heminic iron** (30 %). Present in different enzymes and molecules of transport (transferrin) and storage (ferritin).


hemoglobin

Main physiological functions of iron

- Carries oxygen from the lungs to the rest of the body
- Maintains a healthy immune system
- Aids energy production (constituent of several enzymes including : iron catalase, peroxidase, and cytochrome enzymes)

One of most important consequence of iron deficiency

↳ anemia

Recommended dietary allowance (RDA) of iron (mg/day)

Age Group	Recommended Dietary Allowance (mg/day)	
	Male	Female
0-6 months	No RDA; Adequate Intake (AI) = 0.27	No RDA; Adequate Intake (AI) = 0.27
7-12 months	11	11
1-3 years	7	7
4-8 years	10	10
9-13 years	8	8
14-18 years	11	15
19-50 years	8	18
51+ years	8	8
Pregnancy	n/a	27
Lactation, equal to or less than 18 years	n/a	10
Lactation, 19-50 years	n/a	9

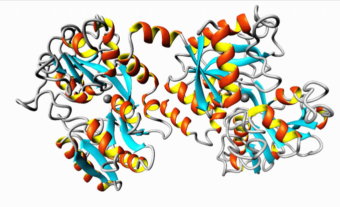
RDA between 7 and 27 mg/day

RDA depends age, sex, physiological state

Natural concentration and location of iron in milk

- **Iron concentration** : about 0.5 mg/L (low compared to 1200 mg/L of Ca)

- **Iron distribution in whole milk**
  - 14 % associated to fat (membrane of fat globule).
  - 24 % bound to caseins.
  - 29 % bound to whey proteins.
  - 32 % bound to compounds of low molecular masses



Lactoferrin

- **Iron distribution in skim milk**
  - 50 to 65 % of iron bound caseins.
  - 18 to 33 % bound to whey proteins (α-lactalbumin, β-lactoglobulin, lactoferrin with 2 atoms of Fe^{3+}, in the presence of 2 carbonate ions)
  - 15 to 33 % bound to non protein fraction (citrate and phosphate ions).

Milk and dairy products are considered as very poor sources of iron

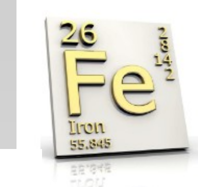
- Low contributions of dairy products to total iron intake (6 %)
- Fortification could be interesting

Iron fortification in dairy industry

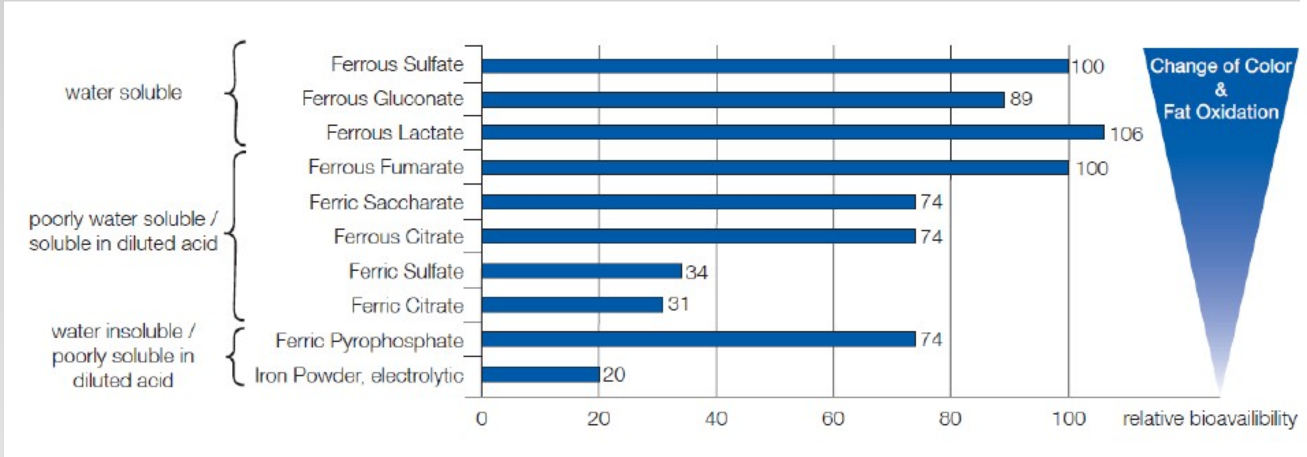
- Improvment of the nutritional quality of the dairy products
  - Milk
  - Yoghurts
  - Cheeses
  - Infant formula

- Improvment of the image of the product «good for health» (marketing)

Characteristics of ideal iron source for fortification



- Good solubility



- Good absorption and bioavailbility

- Don't decrease the nutritional value (↘ risk of interactions with protein and fat)
- Don't modify the sensorial quality (↘ risk of metallic taste and off-flavor)
- Resists to different technological treatments
- Low cost

- This ideal iron source don't exist
- Different iron categories can be used (with advantages and disadvantages)

Some different iron sources for dairy fortification
↳ Classification as a function of their properties

Product	Product Properties	Solubility in Water
Ferric Pyrophosphate, micronized	Almost inert High iron content Whitish powder Slightly metal taste Good bioavailability	Insoluble
Ferrous Gluconate	Slightly sweet and metallic taste Low Iron content	High
Ferrous Lactate	Low hygroscopicity Neutral to metallic taste Good bioavailability	Low
Ferric Ammonium Citrate	High iron content Slightly metallic taste	Good
Ferric Citrate	Neutral taste Off-white powder	Low
Ferrous Sulfate 7-hydrate or dried	Relative high iron content Greenish-blue crystals (7-hydr.); greyish-white powder (dried) Metallic taste	High
Ferrous Fumarate, micronized	High iron content Strong metal taste, brown powder	Low

Food	Iron form	Doses	References	Special mention of determined aspects
Infant formulas: whole milk powder	Ferrous sulphate microencapsulated by spy drying using glycerol monostearate as the wall material	6% ferrous sulphate +15% GMS	7	Improve oxidative stability of lipids
Milk and dairy products	Ferrous sulphate microencapsulated with lecithin		3	
Cheese	Iron casein and ferric chloride		19	Sensory evaluation showed the best flavour for these two forms of Fe fortification
Ras cheese	Iron citrate Iron chloride Iron gluconate	40, 80 and 120 mg Fe/kg	17	Physicochemical analysis
Mozzarella cheese	Casein-chelated Fe Whey prot-chelated Fe Ferric chloride	25 and 50 mg/kg	20	Physicochemical analysis
White soft cheese	Electrolitic iron Ferric chloride Ferrous sulphate	40, 60, 80 mg Fe kg 1	16	Physicochemical and sensorial analysis
Havarty style cheese	Ferrous sulphate Ferrous sulphite Ferrous sulphate + ascorbic acid	4 mg/oz	24	Free Fe not suitable for fortification of its high reactivity Malonaldehyde (Undiserable oxidiza flavours)
Cheddar cheese	Ferric chloride Iron-casein Iron-whey protein complexes	40 mg/kg	43, 44, 45	Fat oxidation for Fe
Yogurt	$FeCl_3$	10 mg Fe/100 ml	21	TEM analysis of samples to locate added Fe, Don't affect the sensorial properties
	Fe complexed with casein Fe complexed with whey proteins			
Edam cheese	Ferrous chloride, Ferrous citrate, Ferrous glyconate	150 mg/kg	15	Physicochemical and sensory properties
Flavoured yogurt		10, 20, 40 mg/kg yoghurt	36	No significant increases in chemical oxidation. Consumer panel not detect Fe presence
Milk	Ferrous sulphate	15 mg/l	38	Sensory properties or shelf life

Some results on

Interactions of free iron with individual dairy compounds (studies on model systems)

- In different physico-chemical conditions
- With different iron sources and especially with reactive iron

Dairy proteins are able to bind iron

Native protein	Charge at neutral pH	Mineral binding
Caseins		
α_{s1}-casein	-42.6	**Fe**, Zn, Ca
α_{s2}-casein	-31.2	Zn, Ca
β-casein	-30.5	**Fe**, Zn, Ca, Mg, Mn, Cu
κ-casein	-6.9	Ca
Whey proteins		
α-Lactalbumin	-2.6	Ca, Zn, Mg, Cu, Mn, **Fe**
β-Lactoglobulin	-19	Mg, Cu, Zn, **Fe**, Mn, Ca
BSA		**Fe**, Cu, Al
Lactoferrin		**Fe**, Zn
Immunoglobulins		Ca

Main binding sites : phosphoseryl residues and negative charge (COO^-) or other groups

Addition of iron to sodium caseinate ↳ total binding

Main binding of iron to phosphoseryl residues /Coordinative bound

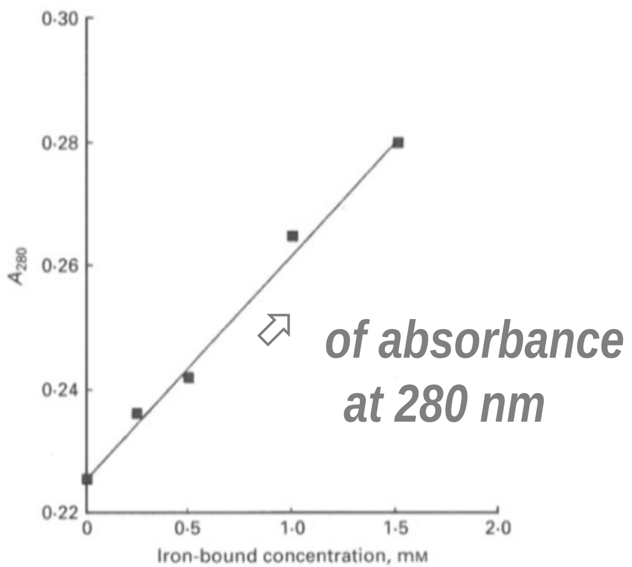


Fig. 2. Absorbance of iron-supplemented casein solution at 280 nm as a function of iron-bound concentration. Iron-supplemented casein solutions were diluted in water to give a final concentration of 0·25 g/l. The accuracy of the absorbance value was ~5%.

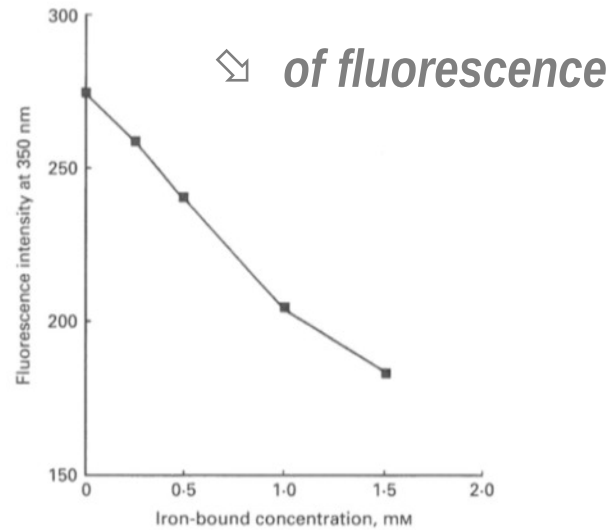


Fig. 3. Intrinsic fluorescence of iron-supplemented casein solution as a function of iron-bound concentration. Iron-supplemented casein solutions were diluted in 50 mM-Tris-HCl, pH 8·0 to give a final casein concentration of 0·50 g/l. The relative fluorescence intensities of tryptophan were measured at 20 °C with excitation and emission wavelengths of 295 and 350 nm. Slit widths were 2·5 nm for excitation and emission. The accuracy of the fluorescence value was ~5%.

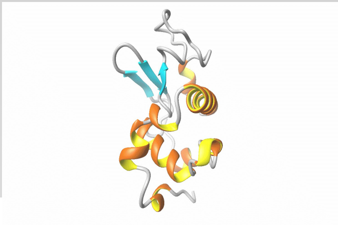
- Formation of iron-casein complexes → change in the oxydation state of iron

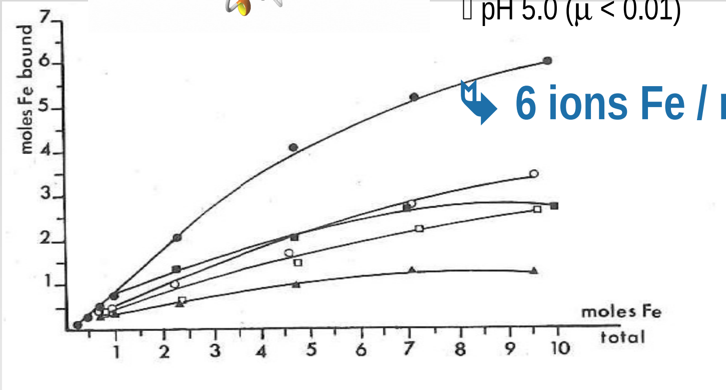
$$4\,Fe^{2+} + O_2 + 4\,H^+ \rightarrow 2\,H_2O + 4\,Fe^{3+}$$

Iron interacts also with whey proteins

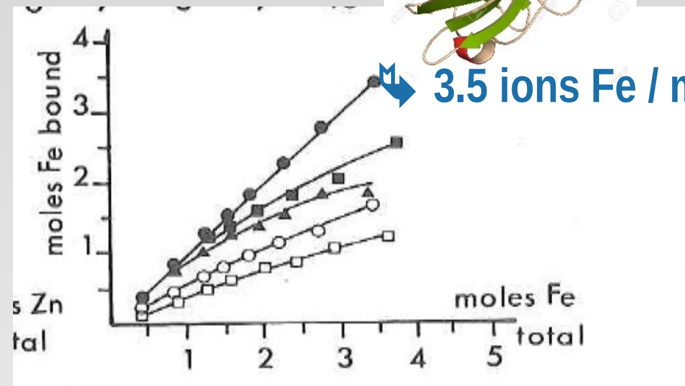
BINDING CURVES IN DIFFERENT PHYSICO-CHEMICAL CONDITIONs

● pH 6.6 (μ < 0.01) / ○ pH 6.6 (μ = 0.05) / □ pH 6.6 (μ = 0.1)
■ pH 5.5 (μ < 0.01)
▲ pH 5.0 (μ < 0.01)

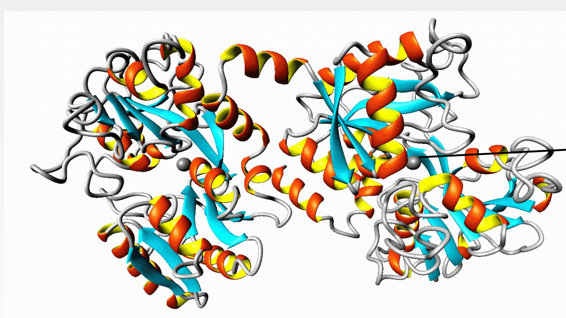


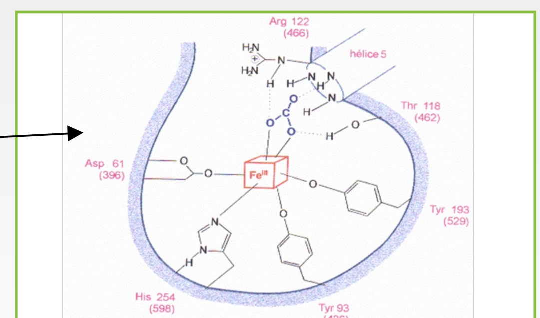


Interactions added iron / α-lactalbumine



Interactions added iron / β-lactoglobuline





Interactions natural iron / lactoferrine (2 atoms of iron)

Iron can also interact with anions like citrate and inorganic phosphate

	Fe^{3+}	Cu^{2+}	Zn^{2+}	Ca^{2+}	Mg^{2+}
Cation-Cit^{3-}	11.20	5.90	4.86	3.50	2.80
Cation-$H_2PO_4^-$	3.61	1.70-1.20	1.20	0.60	0.60
Cation-HPO_4^{2-}	8.13	3.30	2.40	1.30	1.80

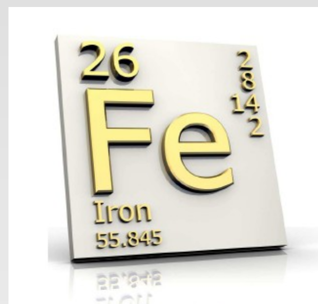
Log (stability constant) of some cation-citrate and cation-inorganic phosphate salts
(25°C, ionic strength = 0.1 M)

and the solubility of iron-phosphate is very low

Smith and Martell, 1976.

Summary on the interactions between reactive iron (ferrous or ferric suphate, chloride…) and individual dairy compounds

Casein molecules

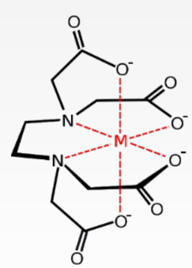


Catalyser of fat oxidation

Whey proteins

Anions like Pi, citrate

These interactions don't exist or are lesser in intensity with unreactive iron (iron EDTA) or iron encapsulated



Some results on

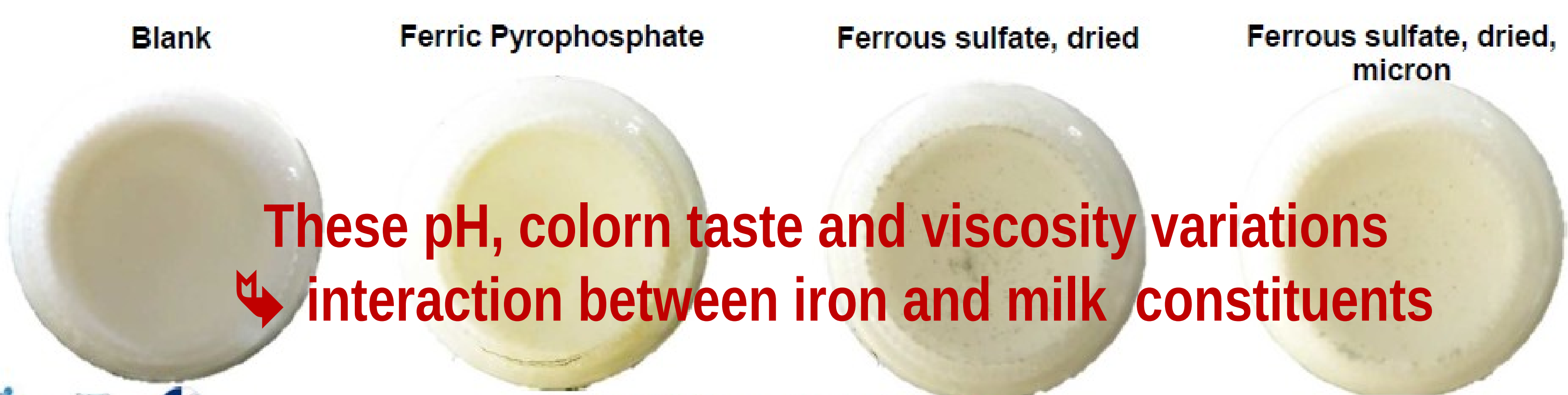
Interactions of soluble iron with skimmed milk

Situation is more complex due to the presence of different dairy constituents able to react with free iron

Modifications of pH, color, taste and viscosity

Iron for Milk 3.5% Fortification – Test Results

Name	pH	color ΔE:	taste	dosage 100 ml	viscosity
Blank	6.6	-	-	-	4.5 mPa*s
Ferrous sulfate, dried	6.1	0.3	slightly metallic and dots on the ground	6.56 mg to 15% RDA	4.7 mPa*s
Ferrous sulfate, dried, micron	6.1	0.4	a bit stronger metallic taste, but less dots	6.65 mg to 15% RDA	4.8 mPa*s
Ferric Pyrophosphate	6.2	0.2	similar to blank, but brown sediment	8.61 mg to 15% RDA	4.9 mPa*s
Ferrous lactate 3-hydrate	6.2	0.5	similar to blank (slightly metallic)	10.24 mg to 15% RDA	4.7 mPa*s
Ferrous Gluconate	6.1	0.5	slightly metallic, musty, unacceptable	18.26 mg to 15% RDA	5.2 mPas*s



These pH, colorn taste and viscosity variations
➥ interaction between iron and milk constituents

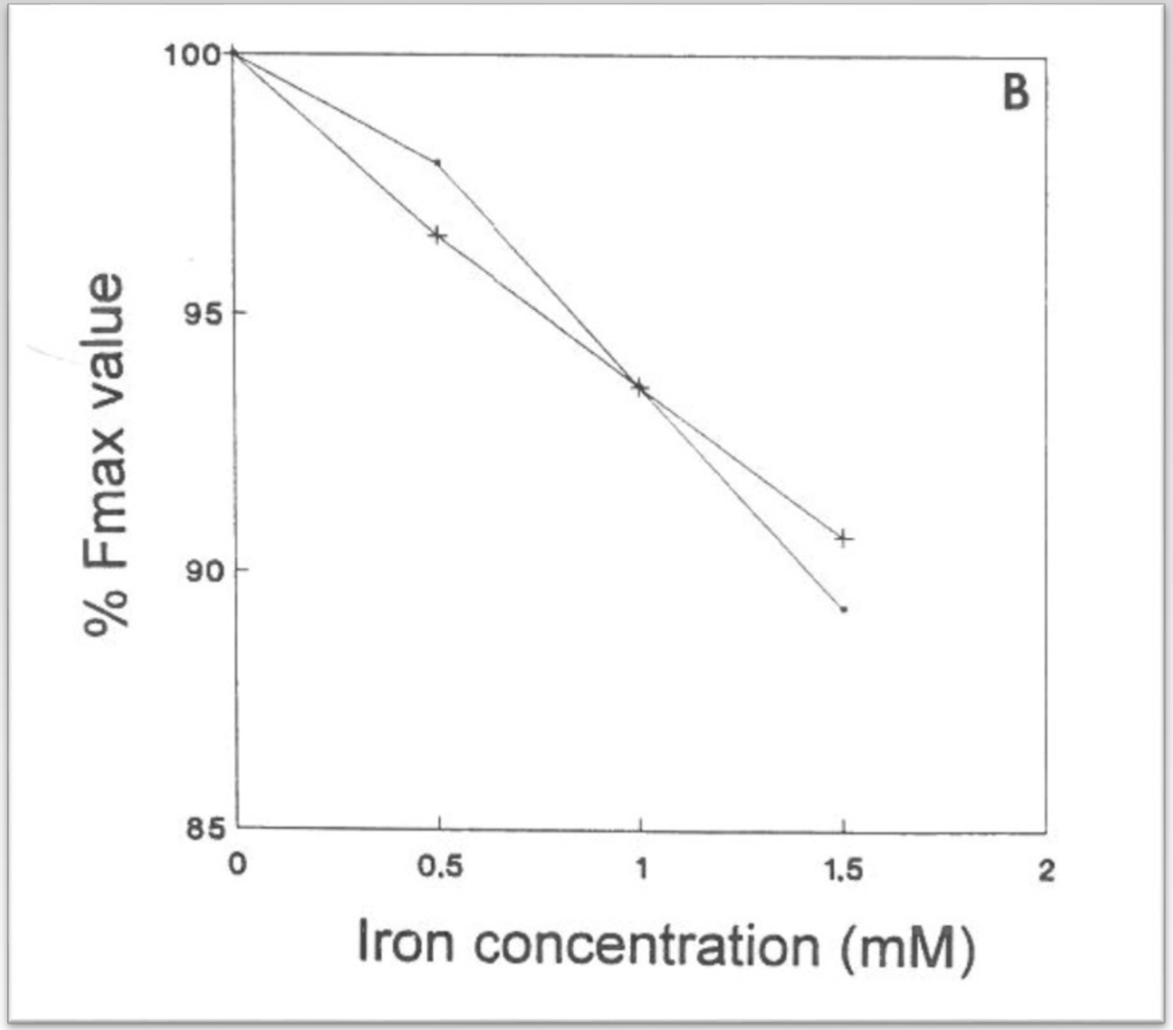
[iron] in the aqueous phase after addition of $FeCl_2$ and $FeCl_3$ to skim milk

(0 - 0.50 – 1.00 and 1.50 mM)

	Control	$FeCl_2$			$FeCl_3$		
Total iron (mM)	0	0.50	1.0	1.50	0.50	1.00	1.50
ultracentrifugable iron (µM)	0	55	96	140	49	87	117
(%)	-	(11%)	(9.6%)	(9.3%)	(9.8%)	(8.7%)	(7.8%)
utrafiltrable iron (µM)	0	28	49	67	18	35	47
(%)	-	(5.6%)	(4.9%)	(4.5%)	(3.6%)	(3.5%)	(3.1%)

- After iron addition to milk ➷ Low [iron] in the aqueous phases ➷ Association of more than 90 % of added iron to micellar phase
- No important difference between $FeCl_2$ and $FeCl_3$
- Results could be different with other iron source like iron-EDTA for example (iron ➷ less exchangeable than with other anions like chloride, sulfate, ...)

Interactions of $FeCl_2$ and $FeCl_3$ with milk proteins determined by intrinsic fluorescence



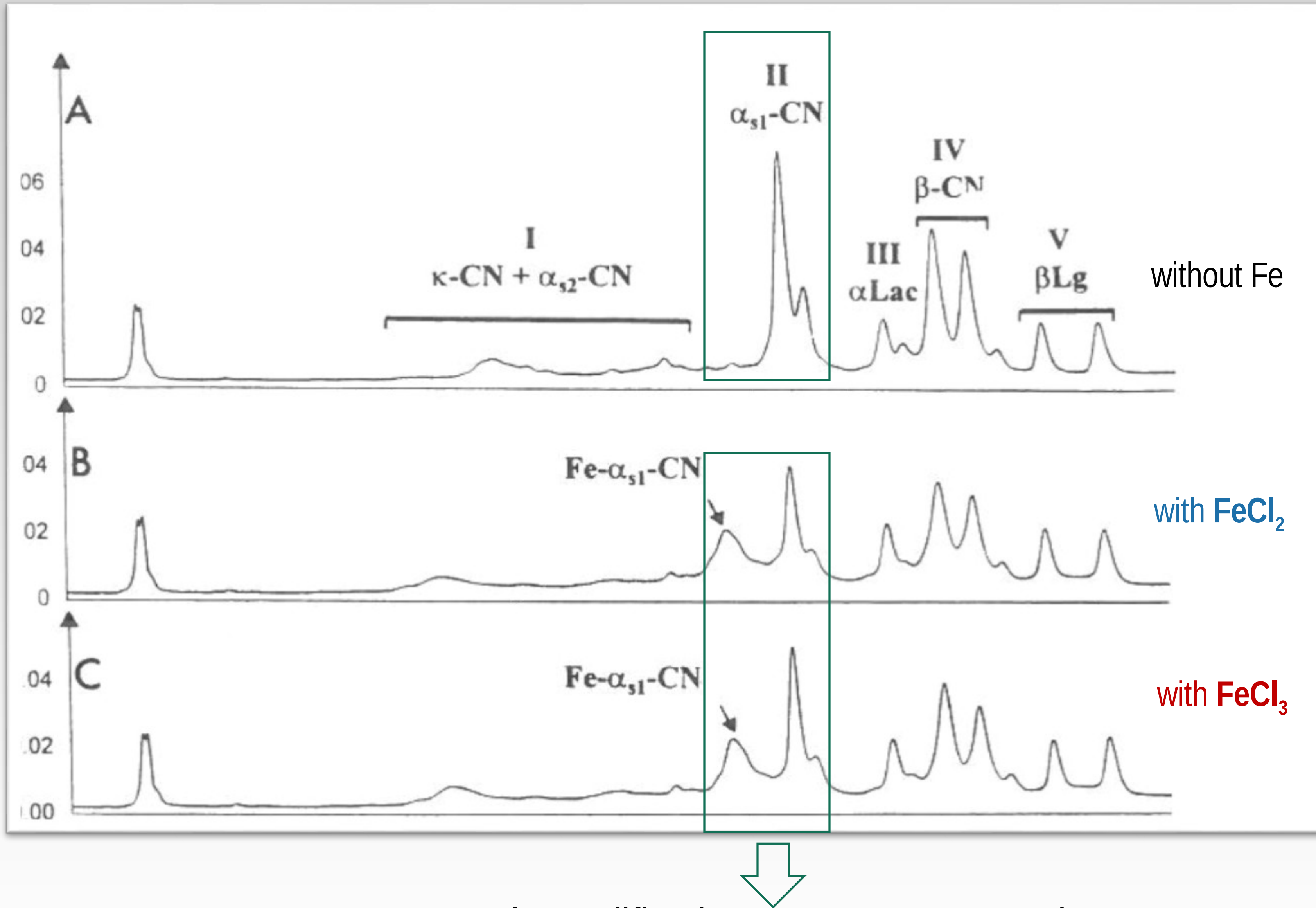
⇘ in intrinsic fluorescence

⇩

-Interactions of iron with milk proteins

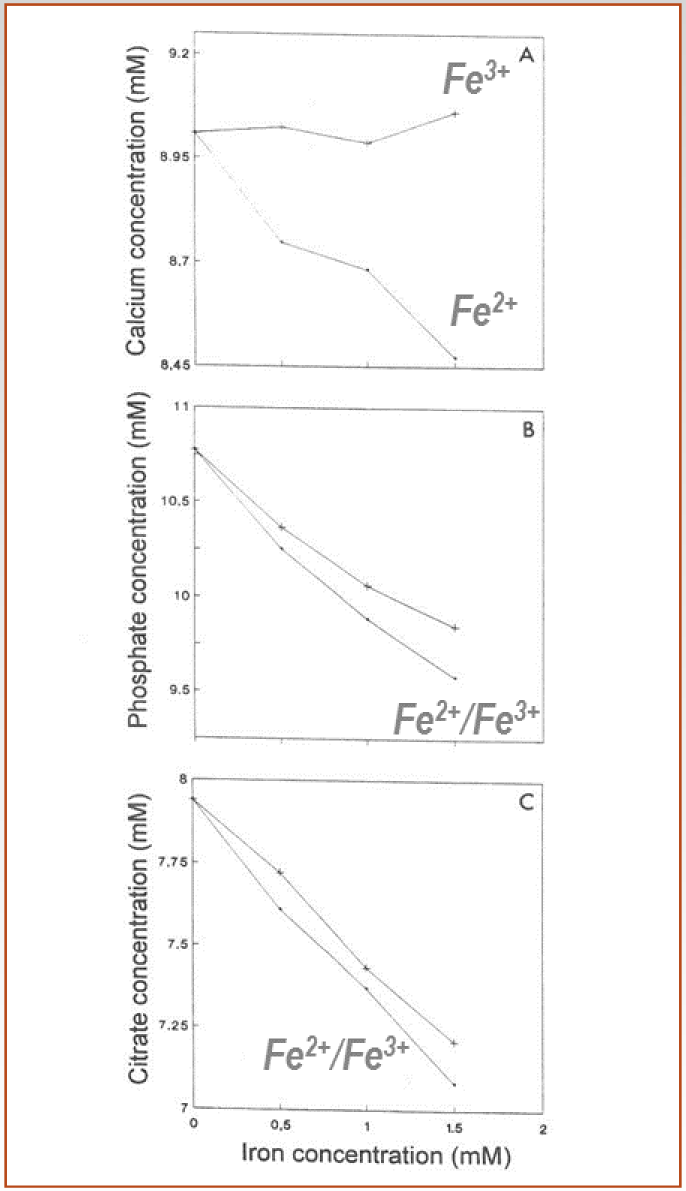
-No difference between $FeCl_2$ and $FeCl_3$

RP HPLC profiles of milk proteins after addition of $FeCl_2$ and $FeCl_3$ to skim milk



Main modification concerns α_{s1}-casein

[minerals] in the aqueous phase after iron addition



Differences of modifications between Fe^{2+} and Fe^{3+} :

With Fe^{2+} : linear ⇘ in [calcium], [inorganic phosphate] and [citrate]

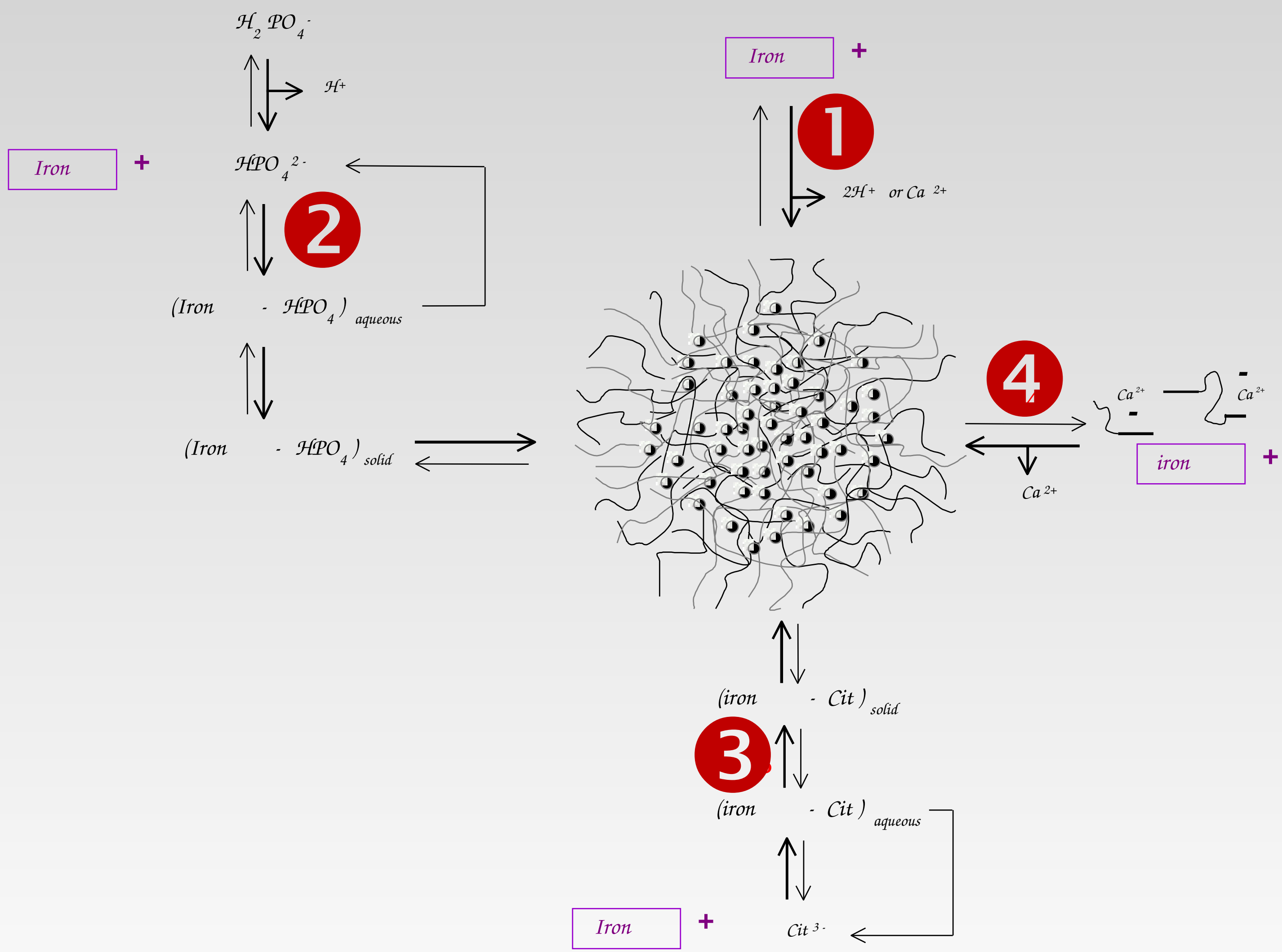
With Fe^{3+} : ⇘ in [inorganic phosphate] and [citrate]

⇩

Modification of salt equilibrium

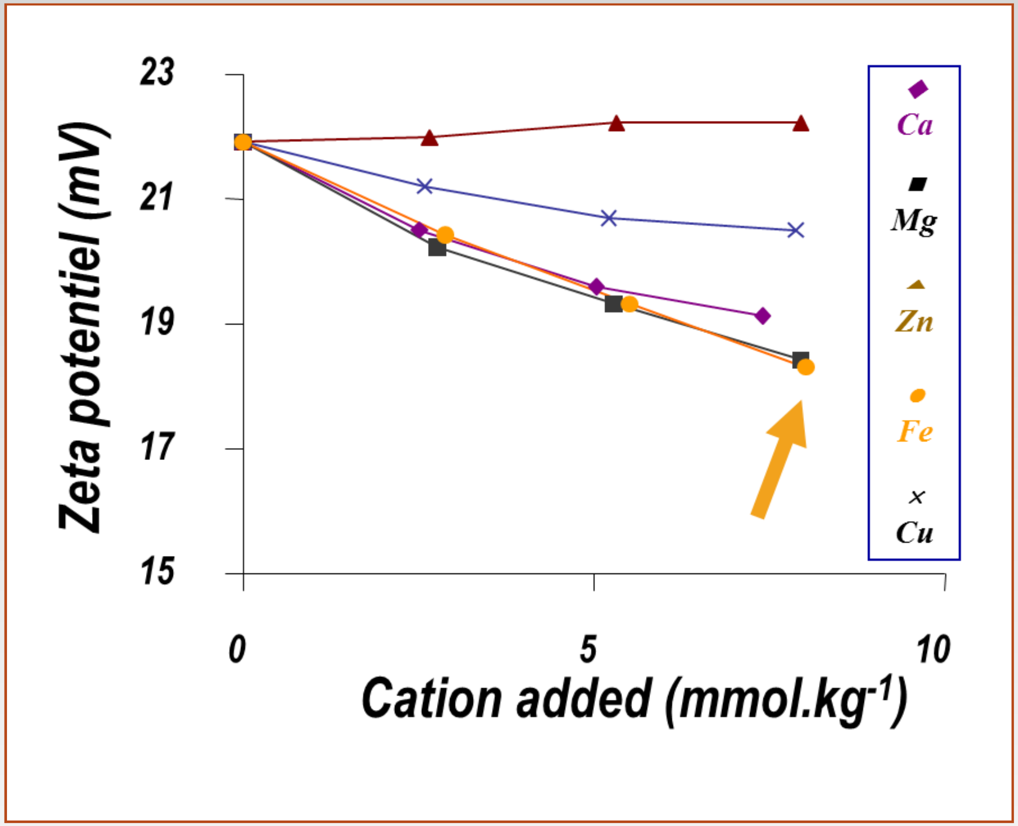
Modification of salt equilibrium after iron addition

4 possible interactions



Formation of iron salts having low solubilities and consequences on structures-functions of casein micelles

Zeta potential of casein micelles in presence of different cations (iron)



Casein micelles are less charged due to interaction with negative charge (phosphoseryl residues and carboxylic groups)

Summary of bio-physico-chemical modifications of dairy constituents after iron addition to milk

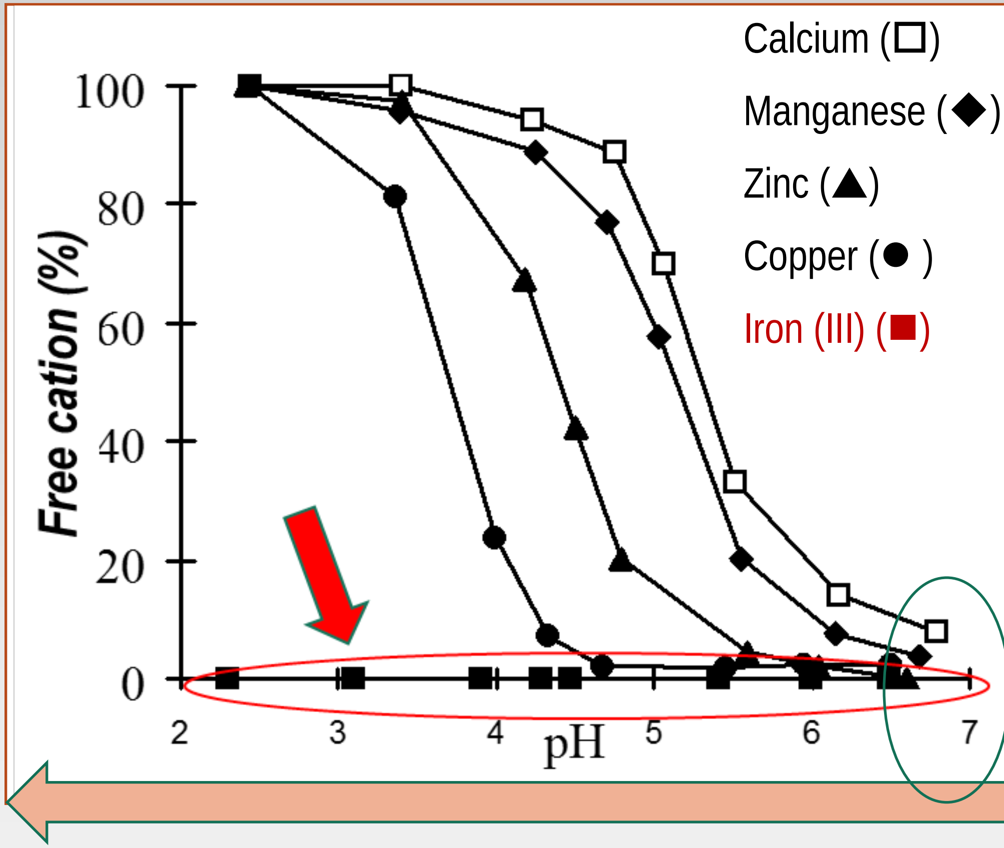
Iron = very reactive ➷ several effects

- pH decrease
- Modifications of caseins.
- Modifications of whey proteins
- Modifications of salt equilibrium
- Modifications of flavour and lipid oxydation (not showed).
- Change of iron oxidation state of with Fe^{2+} ⇨ Fe^{3+}

Intensity of these modifications depends on the:

- Added amounts
- Types of dairy products
- Iron source (oxidation state, contre-ion).

Stability ➥ effect of acidification on caseins-cations interactions



Each cations is totally bound to casein as there is no free cation at neutral pH

↘ *of pH* ➥ ↘ *of the cation-casein interaction with their progressive releases in the aqueous phase (except for iron)*

Binding types are different : electrostatic or coordinative (iron)

Stability ↳ other physico-chemical conditions on the interactions of iron with milk

Irreversible binding of iron to caseins

-Acidification (previous slide)

-Addition of NaCl

-Heat treatment (95°C 30 min)

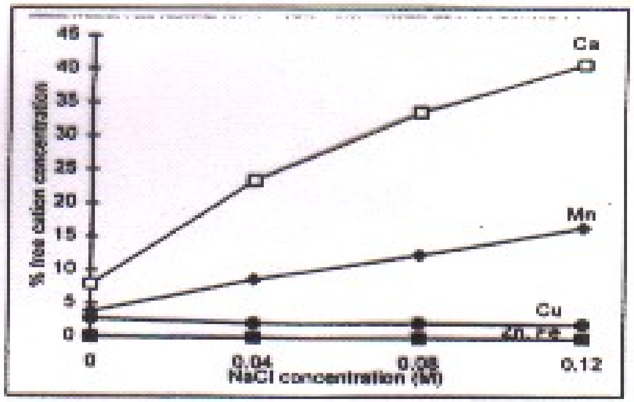
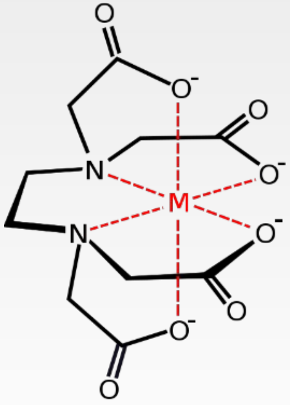


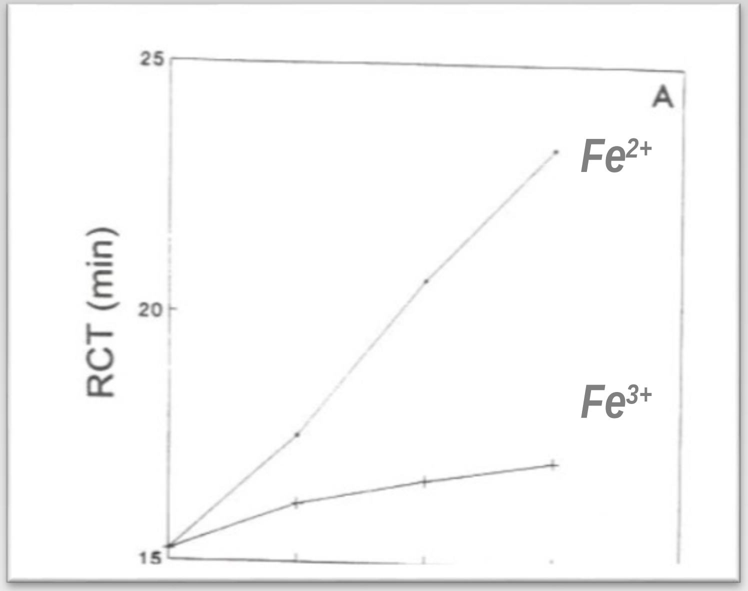
Fig. 6: Effect of NaCl concentration of cation-supplemented caseinates on the % of free cation concentration. 100% correspond to 1.5 mM-cation

Reversible binding of iron to caseins

-In the presence of EDTA (chelatant agent)

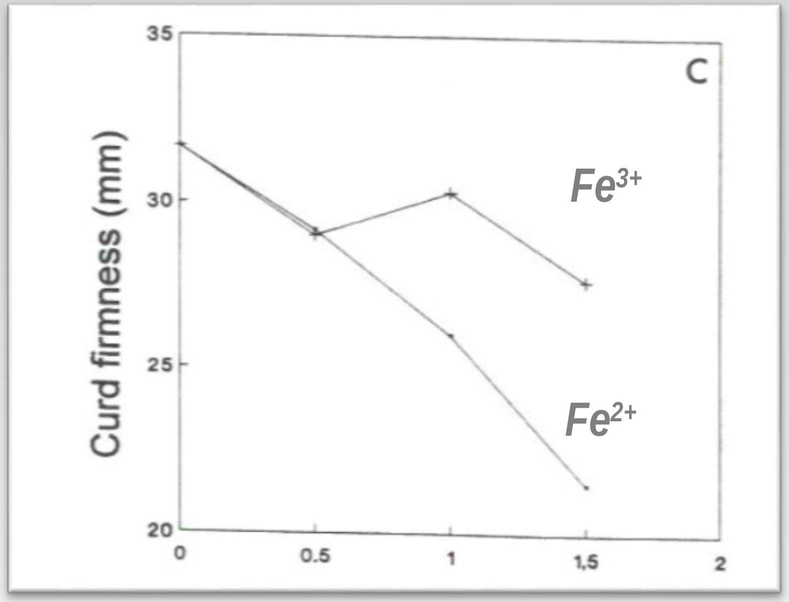


Modifications of rennet coagulation of skim milk enriched with $FeCl_2$ and $FeCl_3$



↗ of rennet coagulation time

$FeCl_2 > FeCl_3$



↘ of gel firmness

$FeCl_2 > FeCl_3$

These technological modifications are related to the previous described modifications of physico-chemical properties of casein micelles (↘ zeta potential, mineralisation change, ↘ hydration, conformation change,…)

Some examples on

Iron enrichment of dairy products

Characteristics of Cheddar manufactured with milks enriched with different iron sources

- Recoveries of iron in cheese
  - 71 to 81% for $FeCl_3$,
  - 52 to 53% for ferric-citrate
  - 55 to 75% for complexes iron-caseins
  - 70 to 75% for complexes between whey proteins and ferric-polyphosphate

- Slight ⇗ in lipid oxidation but acceptable

- After 3 months of ripening :
  - Absence of bad flavor
  - Good quality of cheeses especially those containing 40-50 mg/kg fortified with ferric polyphosphate-whey proteins, iron-casein and ferric chloride

Characteristics of Mozzarella manufactured with milks enriched with different iron sources

Preparation of cheese containing 25 and 50 mg of iron fer/kg of cheese with milks enriched with iron-caseins, iron-whey proteins or $FeCl_3$

- No effect of 25 mg of iron/ kg of cheese on the physical properties of cheese.
- Slight increase in the viscosity of cheese containing 50 mg of iron/kg of cheese
- No chemical oxidation
- Slight increase of oxidized and metallic flavors
- Sensorial analyses ➥ no difference between control cheese and cheese containing iron

Characteristics of Baker and Cottage cheeses manufactured with milks enriched with iron

Preparation of cheeses with ferric ammonium citrate

- About 14 and 58 % of iron retained in cheeses, respectively
- No effect on microbiological development
- Absence of bad flavor

Dairy products enriched with different iron sources

Fortification iron in dairy products (for references, see Table 4)

Food	Iron form	Doses	References	Special mention of determined aspects
Infant formulas: whole milk powder	Ferrous sulphate microencapsulated by spy drying using glycerol monostearate as the wall material	6% ferrous sulphate +15% GMS	7	Improve oxidative stability of lipids
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Flavoured yogurt		10, 20, 40 mg/kg yoghurt	36	No significant increases in chemical oxidation. Consumer panel not detect Fe presence
Milk	Ferrous sulphate	15 mg/l	38	Sensory properties or shelf life

Conclusion on iron dairy fortification

- The **knowledge** (physicochemical, technological and nutritional) on iron enrichment **is relatively low** (compared to calcium enrichment).

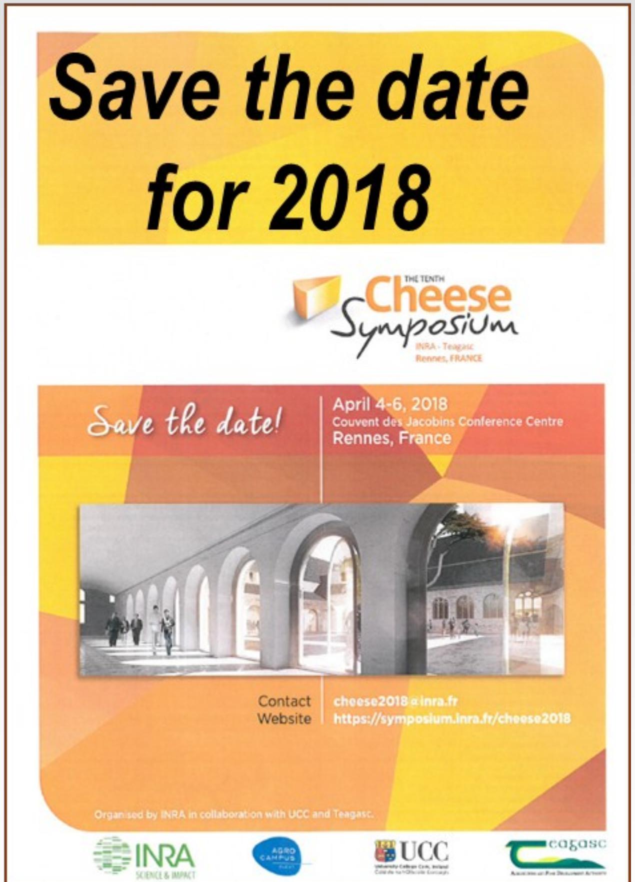
- **Results depends on the iron source** (solubility, chemical forms, ….)

- **Some limits of iron fortification of the dairy products due to**
  - Strong reactivity of dissociated iron towards the dairy compounds (lipids, caseins, proteins and certain minerals)
  - Deteriorations of the physicochemical, organoleptical and technological characteristics
  - These risks could be limited by use of iron having a low reactivity / solubility

- **Added iron is considered as not natural element.**

Needs to increase the knowledge on the effects on health


3rd International Symposium on Minerals & Dairy Products
Sep 20-22, 2017, Wuxi, China
Thanks for all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