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Importância da fração mineral na ciência e tecnologia de produtos lácteos

Importance of the mineral fraction in dairy science and technology

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Milk and dairy products are composed of proteins, lipids and sugar contributing to their nutritive and biological values. They also contain minerals in the form of macroelements (Ca, Mg, Na, K, P and Cl) and trace elements (Fe, Cu, Zn, Se, *etc*). Today, the analytical methods of quantification of these minerals allow us to have a precise idea of their contents in milk and dairy products. This knowledge plays a key role in dairy science and technology especially in the understanding on the structural organisation of milk, the transformation of milk in dairy products (acidification, heat treatments, *etc*) and the properties of dairy products (gel formation and texture, stability or formula containing minerals as a function of temperature and time, *etc*).

The objective of this presentation is to describe the actual knowledge on these minerals in milk and dairy products with special reference to their concentrations, locations and chemical forms. Some data regarding the role of minerals in human nutrition will also be presented. Finally, the future perspectives and actual research questions on the roles (technological and nutritional) of these minerals will be discussed.

1 Macroelements present in milk and dairy products

1.1 Phosphorus (P) and calcium (Ca)

Concentrations, chemical forms and locations of P and Ca in milk – P is an important element in milk. Its concentration expressed in total P is about 950 mg/L. P exists as organic and inorganic phosphates (Po and Pi, respectively). Po is the phosphate bound to organic molecules like casein molecules (phosphoseryl residues), phospholipids, RNA, DNA, nucleosides, nucleotides and sugar phosphate. Pi

corresponds to phosphate ion which can be more or less ionised depending on the pH value of milk and milk products. There is an acido-basic equilibrium between the forms HPO_4^{2-} and H_2PO_4^- at normal pH of milk (about 6.7). At this pH 6.7, Pi is distributed as 50% in the aqueous phase and 50% in the micellar phase to form nanoclusters of calcium phosphate (Figure 1).

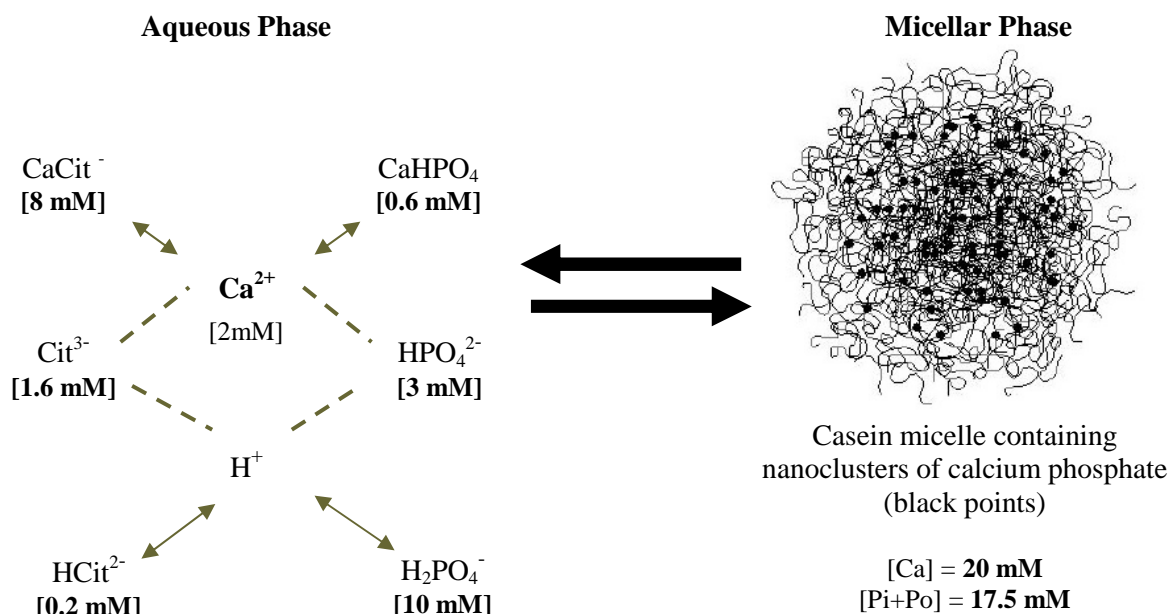


Figure 1. Calcium and phosphate distributions in milk at pH 6.7. In the aqueous phase, calcium is free and associated to inorganic phosphate (Pi) and citrate. In the micellar phase, calcium is associated to phosphoseryl residues of caseins (Po) and inorganic phosphate (Pi) to form nanoclusters. The concentrations of the different associations are indicated in mM.

Ca is one of the most important mineral in milk. Its concentration in cow milk is about 1200 mg/L (30 mM). 99 % of the Ca is in the skim milk where it is distributed between micellar (about 800 mg/L – 20 mM) and aqueous phase (about 400 mg/L – 10 mM) (Figure 1). In the aqueous phase, Ca exists under different forms *i.e.* ionic (Ca^{2+}) and associated to citrate and inorganic phosphate (Pi) to form the corresponding salts (calcium citrate and calcium phosphate). The aqueous phase is saturated with calcium phosphate (with a concentration < 1 mM). In this aqueous phase, α -lactalbumin and osteopontin contain Ca in their structure. In the micellar phase, Ca is bound to phosphoseryl residues of casein molecules (Po) and Pi. In casein micelles, these associations between Ca and phosphates form granules named nanoclusters. Today, the exact chemical composition of these granules is not well known. However, the micellar calcium phosphate has been determined as amorphous (McGann, 1983; Holt, 1993,

1997, 2004; Holt & Hukins, 1991) with a diameter of about 2.5 nm (Marchin *et al.*, 2007). It is considered as a cross-linking and neutralizing agent of phosphoseryl residues. For these reasons, the micellar calcium phosphate contributes to the structure and stability of casein micelles (Tuinier & de Kruif, 2002; Horne, 2006; Dalgleish & Corredig, 2012).

Salt equilibrium - Concerning Ca and Pi, it exists in dynamic equilibrium relatively well described between the aqueous and micellar phases (Figure 1). This dynamic equilibrium depends on the physico-chemical conditions (pH, addition of mono or divalent cations, additions of chelatants, temperature, *etc*) of milk and milk products (De la Fuente, 1998; Gaucheron, 2004, Gaucheron *et al.*, 2004). Figure 2 shows schematically the different changes in the salt equilibrium. Ca and Pi present in one phase can be transferred in the other phase depending on the physico-chemical conditions of milk. The intensity of these changes depends on the intensity of the applied treatments. On the other hand, these changes of Pi and Ca distribution have effects on the organisation, structure and stability of casein micelles.

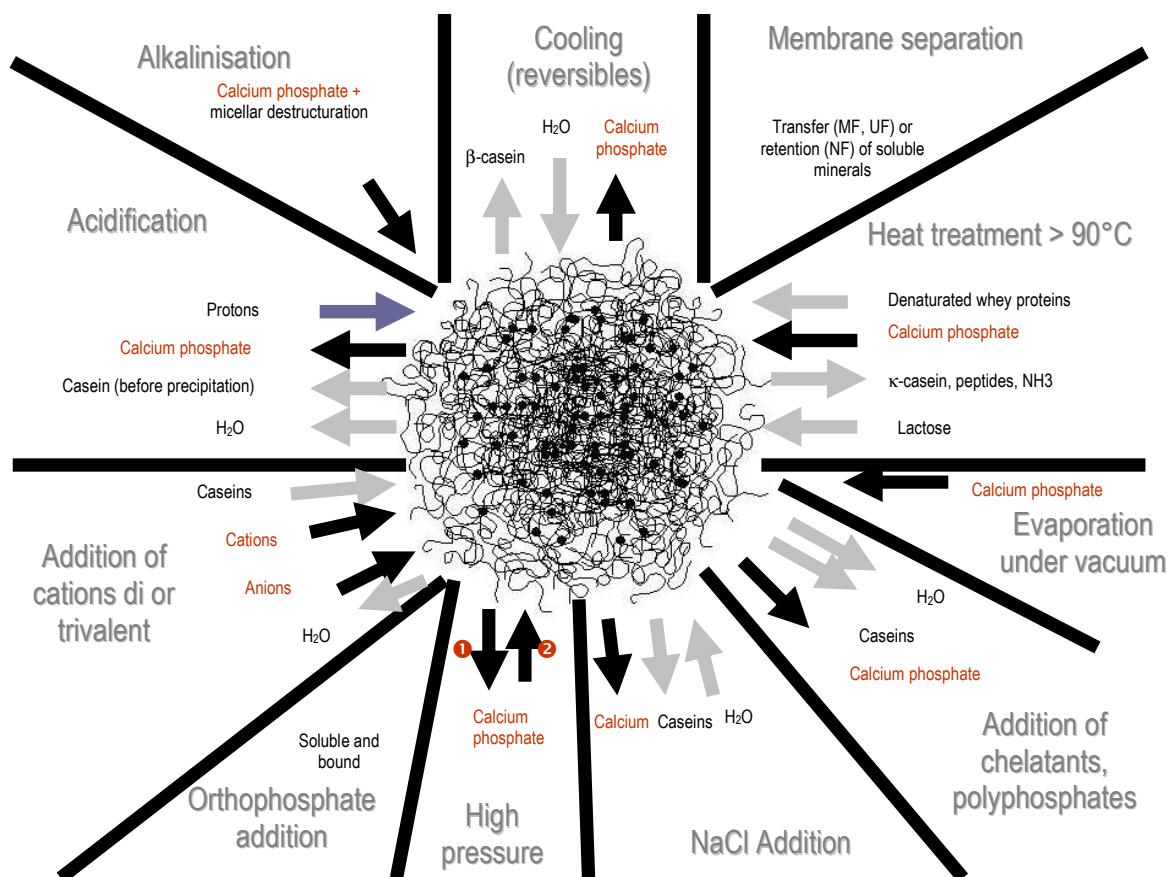


Figure 2 : Salt equilibrium as a function of physico-chemical conditions or technological treatments of milk

Today we are able to calculate theoretically the distribution of minerals between aqueous and micellar phase as a function of different physico-chemical conditions. The calculations are made by software developed in collaboration with the French dairy association. The calculations are based on the combination of anions and cations according to their reciprocal affinities (association constants) and considering pH, ionic strength, and solubility of calcium phosphate. Good correlations between experimental and calculated values were obtained (Mekmene *et al.*, 2009 and 2010). With this software, several hundred combinations of dairy formula taking into account concentrations in proteins, minerals and pH can be calculated. It facilitates the understanding of salt equilibrium and could explain relationships between mineral environment and functionalities of milk proteins. On the other hand, it can help the the development of dairy products (enriched milks, cheeses, yoghurts, *etc*) by modifying the ionic environment.

Ca and P Contents in different dairy products - Acidification is an important step in the manufacture of different dairy products like cheeses and fermented milks. Micellar calcium phosphate is dissolved with transfers of Ca and Pi (not Po which is covalently bound to casein molecules) from the micellar to the aqueous phase during the decrease in pH (Le Graët and Brulé, 1993) (Figure 3). The intensity of this phase transfers depends on the pH value.

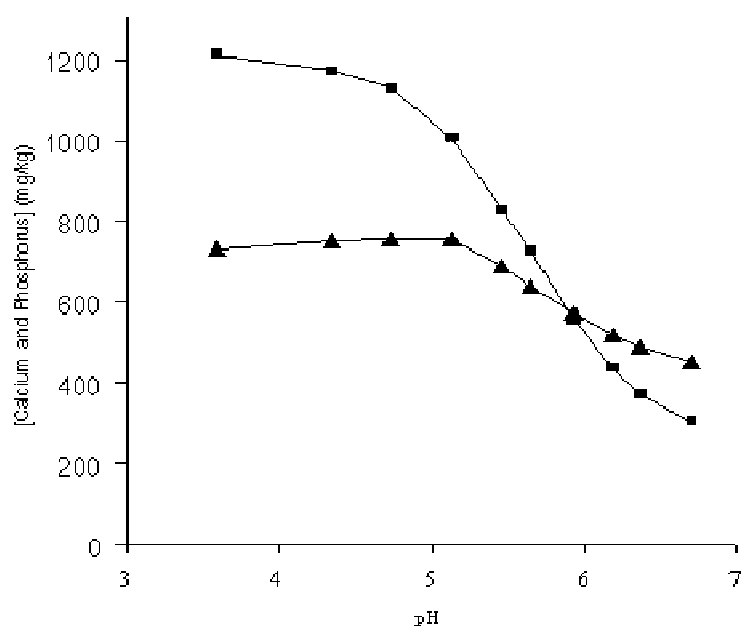


Figure 3: Concentrations of calcium (■) and phosphorus (▲) in the aqueous phase of milk between pH 6.7 and pH 3.5 (Le Graët and Brulé, 1993).

In cheese manufacture, the aqueous phase is removed during the draining and consequently Ca and P concentrations are different and depend on the parameters used for cheese-making (Table 2). The main parameters influencing the contents of calcium and P in cheese are the pH and removal water during the draining and pressing. Thus hard cheeses are more mineralised than soft or lactic cheeses. Butters are poor in minerals except when it is salted.

Locations and forms of Ca and P in different dairy products - In fermented products (pH close to 4.6), Ca and Pi are mainly free under ionic form (Ca^{2+} and H_2PO_4^-). The concentration of Ca and Pi varies in whey liquids and depend on its pH value. Thus, mineral content is higher in acid whey than in sweet whey. In ripened cheeses (Camembert, Cheddar and Emmental), Ca can exist under different chemical and physical forms (precipitates of calcium phosphate, calcium lactate and calcium carbonate) (Bottazzi *et al.*, 1982; Brooker *et al.*, 1975; Brooker, 1987; Karahadian & Lindsay, 1987; Metche & Fanni 1978; Morris *et al.*, 1988). Ca can also exist in interaction with phosphopeptides and free fatty acids to form Ca-phosphopeptide complexes and soap, respectively.

Manufacture of ingredients with different contents in Ca and P - It is also possible to combine physico-chemical modifications of milk (heat treatment, modifications in pH, Ca-chelators or mineral additions,...) and membrane separation techniques (microfiltration 0.1 μm , ultrafiltration or nanofiltration associated or not to diafiltration with different solutions) to prepare casein with different mineral contents. As it is admitted that Ca and P play important role in the structure and stability of dairy products, these types of technological pathways could be a way to create new dairy ingredients having interesting technological and functional properties.

Table 2. Concentrations of minerals in different dairy products. Concentrations are expressed in mg /100 g of product except for Se expressed in µg / 100g). (<http://www.composition-des-aliments.fr/>)

	Ca	Mg	Na	K	P	Zn	Fe	Cu	Se
POWDER									
Whole milk powder	912	85	371	1330	776	3.34	0.47	0.08	16.3
Skim milk powder	1254	115	420	1468	990	3.96	0.433	0.2	10.5
LIQUID MILK									
Skim milk	125	11	42	156	101	0.42	0.03	0.013	3.1
UHT whole milk	117	10	43.9	150	84.2	0.38	0.05	0.007	2.2
UHT skimmed milk	113	10.6	41.8	173	88.8	0.41	0.05	0.003	0.8
UHT semi skimmed milk	115	11.6	49.6	165	85.7	0.51	0.16	0.003	0.9
Concentrated whole milk	264	25	114	287	213	0.89	0.15	0.05	1.9
BUTTER									
Salted butter	24	2	576	24	24	0.09	0.02		1
Unsalted butter	24	2	11	24	24	0.09	0.02	0.016	1
YOGHURTS									
Yoghurt with skimmed milk	127	11	48	172	94	0.44	0.1	0.008	2.2
Yoghurt with whole milk	126	12.6	45.4	186	100	0.42	0.08	0.009	1.4
CHEESES									
Edam	731	30	965	188	536	3.75	0.44	0.036	14.5
Feta	493	19	1116	62	337	2.977	0.67	0.032	15
Brie	184	20	629	152	188	2.38	0.5	0.019	14.5
Cheddar	721	28	621	98	512	3.11	0.68	0.031	13.9
Gruyere	1011	36	336	81	605	3.9	0.17	0.032	14.5
Camembert	388	20	842	187	347	2.38	0.33	0.021	14.5
Provolone	756	28	876	138	496	3.23	0.52	0.026	14.5
Port Salut	650	24	534	136	360	2.6	0.43	0.022	14.5
Switzerland Emmental	791	38	192	77	567	4.36	0.2	0.004	18.2
Cottage cheese 1%	61	5	418.5	86	134	0.39	0.144	0.028	9
Parmesan	1184	44	1602	92	694	2.75	0.82	0.032	22.5
Mozarella	575	21	415	75	412	2.46	0.2	0.022	16.1
Hard cooked cheeses	1050	43.8	405	103	690	5.2	0.4	0.13	8.2
Bleu de Bresse	450	17	602	118	320	2.5	1	0.1	16.1
Bleu d'Auvergne	563	18.1	113.6	84.8	301	2.68	0.3	0.07	3.71
Fresh cheese 0 %	118	11.7	42.1	124	102	0.49	0.16	0.01	1.9
Processed Cheese (25 % fat)	346	24	282	152	990	8	0.3	0.5	7.4
Fresh cheese (type Petit suisse 20 %)	117	10	34	98	125	0.48	0.2	0.01	2
Pont L'Évêque	485	20	690	128	431	5.1	0.37	0.05	4.8
Livarot	620	21.3	729	112	427	5.8	0.37	0.11	8.7
Beaufort	995	39.5	628	117	728	5.18	0.24	0.09	7.22
Maroilles	350	40	937	130	320	9	0.4	0.07	7.36
Morbier	760	30	990	100	520	7	0.3	0.06	8
Reblochon	514	25.1	555	152	339	8.5	0.32	0.11	5.1
Saint Marcellin	138	20.2	1009	187	442	0.85	2.74	0.07	0.51
Saint Nectaire	539	30.4	477	98	304	5.3	0.22	0.07	5.1
Saint Paulin	741	31.6	750	87.9	401	4.81	0.24	0.09	8.9
Comté	909	48.9	412	118	664	5.1	0.49	0.1	7
Roquefort	662	30	1809	91	392	2.08	0.56	0.034	14.5

Nutritional aspect of P and Ca - Human needs for P are inferior to those of Ca. There is no deficiency for this element which is found in different foods and especially in milk and dairy products (Pointillart & Guéguen, 2004). The recommended dietary allowance for Ca is about 800 mg for adults and children aged 1-10 years and 1200 mg for adolescents, young adults, and pregnant and lactating women. From these recommendations, milk and dairy products are important sources of Ca. So, 600 ml of milk correspond to 720 mg of Ca. 80 % of the recommended dietary allowance can be met by consuming milk and dairy products. Thus, the dairy products contribute to 70% of the Ca nutrition for human.

1.2 Magnesium (Mg)

Mg is not abundant in milk and dairy products (Table 2) and the research concerning this element in milk and dairy products is limited. Its concentration in milk is about 120 mg/L (against 1200 mg/L for Ca). 99 % of this ion is found in the skim milk with as distribution 50/70 ratio between aqueous and micellar phases, respectively. This ion is probably associated to Pi and citrate in the aqueous phase and in the nanoclusters of casein micelles. As Ca and Pi, Mg distribution between aqueous and micellar phases is sensitive to the physico-chemical conditions (Le Graët & Brulé, 1993). Thus, during milk acidification, Mg is solubilised from the micellar to the aqueous phase. In dairy products, the concentration of Mg is variable and depends on the type of the dairy products (Table 2).

In spite of the low concentration of Mg in milk and dairy products, the dairy products are considered as interesting sources of Mg for human needs as 600 ml of milk gives 65 mg (*i.e.* 16 % of the human recommended daily allowance).

1.3 Sodium (Na), potassium (K) and chloride (Cl)

In milk, the concentrations of Na, K and Cl are 450, 1500 and 1100 mg/L, respectively. These monovalent ions are mainly in the aqueous phase where they are free or weakly associated to ions of opposite charge. They contribute to the ionic strength of milk. In dairy products especially cheeses, the concentration of NaCl is increased by salting or brining and contributes to the draining of the curd, organoleptic properties of the cheeses and selection of micro-organisms and enzyme activities during ripening. The concentrations of NaCl depend on the types of cheese (Table 2). Some cheeses contain important amounts of NaCl like Roquefort blue-veined cheese (Table

2). Other cheeses do not contain supplemented NaCl like lactic cheeses. Today, in our diet, the NaCl concentration is very important with negative impacts on Human health like increase of the blood pressure. For this reason, different researches are in progress to reduce the quantity of NaCl in the products or replace partially NaCl by KCl.

2 Trace elements present in milk and dairy products

2.1 Iron (Fe)

Fe is not an abundant element in milk (Table 2). Its concentration is about 0.5 mg/L. It is noteworthy that the concentrations of Fe reported in the literature are sometimes variable. This variability is related to the different methods of sample preparation and analytical methods used for the determination. It can exist in two different states of ionisation (Fe^{2+} or Fe^{3+}). In skim milk, Fe is mainly associated to casein molecules, whey proteins, Pi, citrate (Brulé & Fauquant, 1982) and lactoferrin. When Fe is bind to casein molecules, it is not by a electrostatic bond but a coordinate bond.

Milk and dairy products are considered as very poor sources of Fe (Table 2) and their contributions to the total Fe intake are very low. Thus, 600 ml of milk gives 0.1 mg *i.e.* about 2.5 % of the recommended daily allowance. In this context, different researches are performed to enrich milk and dairy products with different forms of Fe. In these studies on Fe supplementation, the technological, organoleptic and nutritional aspects are evaluated (Demott, 1971; Demott & Dincer, 1976; Gaucheron *et al.* 1997; Hekmat & Mahon 1998; Gaucheron, 2000).

2.2 Copper (Cu)

Like Fe, Cu is not an abundant element in milk and dairy products (Table 2). Its concentration in cow milk is about 0.1 mg/L distributed as follows: 2% in the lipid phase, 8% bound to whey proteins, 44 % to casein and 47% in a low molecular weight fraction (Pi and citrate ions). It is noteworthy that the concentrations of this element reported in the literature are sometimes variable. This variability is also related to the different methods of sample preparation and analytical methods used for the determination. Table 2 reports some Cu concentrations present in different dairy products.

Milk and dairy products are considered as poor sources of Cu and their contributions to the intake of copper are very low (600 ml of milk gives 0.3 mg *i.e.* 5 % of the recommended daily allowance) (Lönnerdarl *et al.*, 1981).

2.3 Zinc (Zn)

Zn is a divalent cation present in milk at a concentration ranged between 3 and 4 mg/L. Most of the Zn (99 %) is present in skim milk associated with the casein micelles (95%). Zn is usually associated with Po and/or Pi of casein micelles. Secondly, in the aqueous phase, Zn is found in association with citrate molecules (Brulé & Fauquant, 1982; Blakeborough *et al.*, 1983; Singh *et al.*, 1989). Table 2 reports some Zn concentrations in dairy products.

Zn present in milk and dairy products contributes significantly to the health state of human (Lönnerdarl *et al.*, 1981 ; Cashman, 2011). 600 ml of milk gives 2.4 mg of Zn corresponding to 20 % of the recommended daily allowance.

2.4 Selenium (Se)

It is a metalloid showing great similarity with sulphur. It is often described in association to glutathione peroxydase with antioxidant role. Its concentration in milk is 30 µg/L. It is mainly present in skim milk where it is associated to casein molecules and whey proteins (Van Dael *et al.*, 1991; Sanz Alaejos & Diaz Romero, 1995; Foster *et al.*, 1998; Navarro-Alarcon & Cabrera-Vique, 2008). Values relating the concentrations of Se in different dairy products are not well described (Table 2).

Concerning its nutritional implication, milk is an important source of Se (600 ml of milk gives 20 µg of Se). Depending on the country, the contribution of dairy products to daily intake of Se is ranged between 8 to 39 % of the human recommended daily allowance.

3 Conclusion and perspectives

Milk and dairy products are unique sources of minerals subjected to extensive investigations since over 60 years. It is concluded from these researches that minerals may vary in concentrations, types, forms, locations and nature of their interactions with the other compounds in milk and dairy products.

The knowledge is relatively complete and precise and the key roles of minerals to the Human health are evident from many researches. All these micronutrients influence significantly our state of health. Despite of this knowledge, several technological and nutritional questions must be resolved in the future to understand milk and dairy products and to improve their general qualities. These questions are as follows:

- What are the physico-chemical structures of calcium phosphate in different dairy products?
- What are their contributions to the functional properties of dairy products (heat stability, rheological properties, etc)?
- What are the concentrations of each minerals especially trace elements in different dairy products?
- What are the effects of external compounds present in the food on the absorption of these minerals?
- Is it nutritionally interesting to enrich milk and dairy products in minerals?

All these questions should be addressed in future research projects to understand the importance of minerals in dairy science and technology but also in nutrition.

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