ELSEVIER

Food Chemistry 80 (2003) 573–578

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Total and soluble contents of calcium, magnesium, phosphorus and zinc in yoghurts

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Received 23 May 2002; received in revised form 20 October 2002; accepted 20 October 2002

Abstract

This paper examines the content of major minerals (calcium, magnesium, phosphorus and zinc) and its distribution between soluble (non-sedimentable) and micellar fractions of 16 commercial yoghurts from five different branches. These elements were determined by atomic absorption spectrometry after mineralisation by dry ashing. Total mineral contents showed wide intervals of variation (calcium 1090–2050 mg l^{-1}, magnesium 101–177 mg l^{-1}, phosphorus 878–1560 mg l^{-1} and zinc 4.0–7.3 mg l^{-1}) due to the addition during manufacturing of different dairy products or fractions. Most of the minerals were found in the non-sedimentable fraction. Practically all calcium was measured in the supernatants of ultracentrifugation. Lower proportions of magnesium and zinc were determined in the non-sedimentable fraction (between 87 and 96%) whereas the lowest ones (63–77%) were found for phosphorus.

Keywords: Yoghurt; Calcium; Magnesium; Phosphorus; Zinc

1. Introduction

Yoghurts are gaining in popularity due its acceptability for the consumers as well as their nutritional properties and potentially beneficial effects in human health. There is currently considerable growth in industrialised countries in the economical importance of yoghurts. In the last few years, this tendency has driven the manufacturers to develop and produce a wide variety of these products with different characteristics. Yoghurt can be a good source of essential nutrients as minerals in the human diet. It could contribute significantly to the recommended daily requirements for calcium and magnesium to maintain the physiological processes. Yoghurts are also a good dietary source of phosphorus (besides calcium, considered the most important nutrient for bone health) and its contribution to total phosphorus intake has been reported as 30–45% in western countries (Flynn & Cashman, 1997). Other key nutrients supplied would include zinc.

Analysis of total content of mineral in yoghurts have been carried out (Buttriss, 1997; Kim & Choi, 1993) and studies of mineral composition of milk fruit-added yoghurts (Moreno-Rojas, Sánchez-Segarra, García-Martínez, Gordillo-Otero, & Amaro-López, 2000; Sánchez-Segarra, García-Martínez, Gordillo-Otero, Díaz-Valverde, Amaro-López, & Moreno-Rojas 2000) and fermented milks fortified with minerals (Hekmat & McMahon, 1998; Pirkul, Temiz, & Erdem, 1997) can also be found in the literature.

However, it is now generally recognised that mineral absorption does not depend solely on the amount of the element present in the dairy product but also on other factors such as solubility (Delisle, Amiot, & Doré, 1995). Mineral availability in dairy products is affected by the nature of the complex. The chemical form of the nutrient may influence bioavailability: free or soluble form are well absorbed, whereas those that are bound could be poorly. Technological treatments used in the processing of dairy products are known to modify the proportions of its various chemical forms (De la Fuente, 1998). Bacterial production of lactic acid from lactose of milk is an essential step in yoghurt manufacture. Lowering pH causes important alterations in the composition, structure and reactivity of casein micelles and

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0308-8146/03/$ - see front matter
PII: S0308-8146(02)00505-8

modifying the mineral equilibrium. The acid nature of yoghurt would also have positive effects on the gastrointestinal absorption of calcium from milk.

Yoghurt manufacture may result in the redistribution of nutrients as mineral, mainly those associated with caseins. Changes in this distribution may have effects on nutritional properties. Therefore, their occurrence in yoghurts is of particular concern in view of the demanding quality standard that this product must meet with respect to nutrition. However, no studies have been carried out to evaluate the extent and nature of mineral redistribution arising from yoghurt processing. This paper was undertaken to determine the partition of calcium, magnesium, phosphorus and zinc between micellar and non-sedimentable phases in a wide spectrum of yoghurts commercialised in the market as a contribution to improve the knowledge and the nutritional characteristics of these of dairy products.

2. Materials and methods

2.1. Samples and reagents

Sixteen yoghurts made of milk with different fat content (0.1–5.5%), belong to five different brands were purchased in local markets. One of them (sample 16) was manufactured from goat's milk. In all cases sodium azide was added as preservative (0.1 g l^{-1}). High purity water with a resistance of 18Ω cm was used to prepare all samples and standards. All reagents used were of the highest purity available and at least of analytical grade.

2.2. Apparatus

The determination of potassium and sodium was carried out by atomic emission spectrometry using a model 5100PC atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA) in an air–acetylene flame. Calcium, magnesium and zinc were determined by atomic absorption spectrometry with the same instrument using a multi-element (Ca–Mg–Zn) hollow-cathode lamp. Phosphorus was measured using an UV–visible spectrophotometer, model Lambda 15 (Perkin-Elmer, Norwalk, CT, USA).

2.3. Methods of analysis

Total solids were determined according to FIL-IDF (1991). The non-sedimentable or soluble fraction was separated by high speed centrifugation. Thirty grams of yoghurt was centrifuged at 100,000 *g* using a 50-RT-1250 rotor at 20 °C in an ultracentrifuge (Sorvall Combi Plus, Wilmington, DE, USA). The supernatant fluid was carefully removed and filtered through Whatman 40 paper. It was then stored for analysis at 5 °C before digestion.

Samples (yoghurts and its supernatants) were mineralised by dry ashing in a muffle furnace. Five gram samples were weighed into a porcelain crucible and dried on a hot plate. After charring, samples were incinerated in a muffle furnace at 450 °C for 24 h. If necessary, the ash was bleached by treatment with HNO_3 and heating in the muffle furnace for 1 h. Finally, the ashes were diluted with concentrated nitric acid and distilled water and measured by atomic absorption.

Calcium, magnesium, sodium, potassium and zinc were measured in the digested samples by flame atomic absorption in the conditions specified by De la Fuente and Juárez (1995a). Phosphorus was determined by visible spectrophotometry at 750 nm by the molybdenum blue method according to De la Fuente and Juárez (1995b). All analysis were performed in triplicate.

3. Results and discussion

3.1. Total content of minerals

Total and non-sedimentable calcium, magnesium, phosphorus and zinc contents in yoghurts can be seen in Table 1. Total mineral content showed wide intervals of variation (calcium 1090–2050 mg l^{-1}, magnesium 101–177 mg l^{-1}, phosphorus 878–1560 mg l^{-1} and zinc 4.0–7.3 mg l^{-1}). There are numerous factors which affect yoghurts chemical composition, mainly the methods of fortification used to increase the solid content. This is a common practice during yoghurt manufacture. A wide range of total solids and other minerals (sodium and potassium) was also found in the yoghurts studied (Table 2) indicating the possible addition of different dairy fractions or products. Although, whey protein concentrates, milk protein concentrate or caseinates have been successfully assayed (Guzmán-González, Morais, & Amigo, 2000; Guzmán-González, Morais, Ramos, & Amigo, 1999) the most common approach in the industry is the utilisation of milk modified by the addition of skim milk powder. Yoghurt 4 had the highest content in calcium, magnesium, phosphorus and zinc. At the same time it showed very high levels in sodium and potassium either. This would indicate the enrichment of the raw milk with dairy additives abundant in these minerals as milk powders.

Although other samples (1, 5, 11 and 15) have mineral concentrations similar to the generally reported for raw milk, most of the yoghurts showed higher contents in these nutrients, suggesting the possible addition of dairy ingredients. This supply can represent an advantage from a nutritional point of view as a source of essential nutrients in diet in comparison with other dairy products. Moreover yoghurt could act as an alternative source of mineral for sufferers of lactose intolerance.

Table 1
Total and non-sedimentable contents (mean±standard deviation) of calcium, magnesium, phosphorus and zinc in commercial yoghurts[a]

Sample	mg l^{-1}							
	Ca		Mg		P		Zn	
	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
1	1170±5	1147±10	115±3	101±3	899±10	697±7	4.7±0.1	4.4±0.1
2	1522±38	1472±7	144±7	128±1	1178±15	825±8	5.3±0.1	5.0±0.1
3	1474±49	1454±48	131±1	127±1	1140±3	801±2	4.6±0.1	4.3±0.1
4	2050±37	2003±7	177±1	167±1	1560±14	1089±10	7.3±0.3	6.9±0.1
5	1088±8	1068±18	101±1	95±1	878±15	613±9	4.8±0.4	4.5±0.1
6	1314±34	1274±17	129±2	124±2	1157±26	822±12	5.4±0.2	5.1±0.2
7	1807±63	1780±8	136±3	126±2	1391±16	987±1	5.9±0.1	5.1±0.2
8	1560±6	1530±5	141±2	134±1	1283±61	952±8	5.9±0.1	5.5±0.4
9	1540±40	1520±10	137±3	133±1	1328±17	911±11	5.2±0.1	4.9±0.3
10	1534±7	1513±11	132±2	127±2	1326±20	927±10	5.2±0.1	5.0±0.1
11	1090±8	1080±2	112±3	101±1	1006±1	675±10	4.5±0.1	4.2±0.1
12	1400±4	1380±5	118±2	114±1	1058±10	792±11	4.8±0.1	4.6±0.1
13	1660±20	1640±40	124±1	109±1	1222±2	793±8	5.9±0.1	5.5±0.3
14	1480±28	1459±12	114±1	105±1	1181±32	746±40	4.6±0.1	4.4±0.2
15	1130±20	1110±10	123±2	108±1	901±23	603±8	4.3±0.5	4.0±0.3
16	1310±30	1290±3	123±2	108±1	952±6	665±17	4.0±0.1	3.7±0.1

[a] Each value is the average of three analysis and triplicate determinations.

The mean concentrations of the minerals studied in this work were not affected by the different fat content (Table 2). Although a very important fraction of other minerals (copper and iron) is linked to fat globule, any of the elements studied in the present work has been reported in high percentage bound to dairy lipids (Flynn & Cashman, 1997).

Table 2
Total solids, fat, sodium and potassium (mean±standard deviation) in commercial yoghurts

Sample	Total solids (%)	Fat labelled (%)	K[a] (mg l^{-1})	Na[a] (mg l^{-1})
1	10.1	3.1	1641±6	476±14
2	9.9	0.1	1990±12	637±14
3	13.0	3.9	1990±29	652±36
4	12.8	0.1	2630±19	766±7
5	11.0	3.9	1547±10	591±9
6	7.8	0.1	2325±8	766±2
7	11.4	0.1	2028±10	658±2
8	12.5	2.0	2275±37	716±19
9	12.6	2.1	2338±54	777±3
10	10.6	0.1	2230±25	768±5
11	11.3	2.2	1812±20	752±12
12	10.3	0.1	2074±39	746±9
13	16.9	3.8	1696±3	605±11
14	12.6	3.4	2071±25	654±14
15	11.6	3.1	1582±37	604±14
16	12.2	5.5	1628±38	558±2

[a] Each value is the average of three analysis and triplicate determinations.

3.2. Percentage of non-sedimentable minerals

The physico-chemical changes of the casein micelles brought about by acidification due to biotransformation of lactose to lactic acid are reflected in changes in the salt balance.

3.2.1. Calcium and phosphorus

The percentage of non-sedimentable calcium ranged from 96.7 to 99.1% whereas the levels of phosphorus detected in the supernatants were within the interval of 63.2–77.5% (Fig. 1).

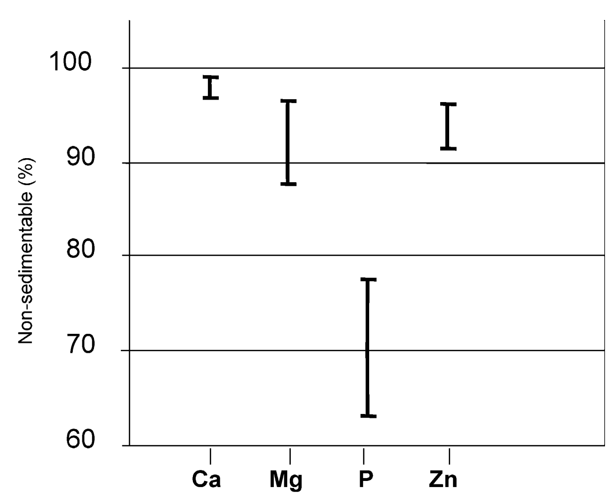

Fig. 1. Intervals of variation of non-sedimentable calcium, magnesium, phosphorus and zinc in commercial yoghurts.

At pH 6.7, in raw milk, approximately two thirds of the calcium are associated with casein micelles and being sedimentable after centrifugation. However, during bacterial acidification changes in micellar calcium and phosphorus take place. As the milk pH decreased from pH 6.7 to 4.0 the amount of calcium and phosphorus associated with the micellar proteins decreases while an increase of calcium and phosphorus in the serum milk can be observed (Dalgleish & Law, 1989; Gastaldi, Lagaude, & Tarodo de la Fuente, 1996; Le Graet & Brulé, 1993; Van Hooydonk, Hagedoorn, & Boerrigter, 1986). Previous studies (Dalgleish & Law, 1989; Le Graet & Brulé, 1993) have shown that the dissociation of calcium and phosphate from the casein micelles is sigmoidal in shape as the milk is acidified. This decrease is relatively slow until about pH 6.0–5.8 and then became faster. At pH 5.1 most of the micellar inorganic phosphorus was solubilised, whereas about 17% of the calcium was still present in the casein micelles (Gastaldi et al., 1996). Dalgleish and Law (1989) even at pH 4.9 estimated that 1 mM calcium was sedimentable while Hekmat and McMahon (1998) found at pH 4.5 small amounts of calcium remaining in the pellet after ultracentrifugation. Le Graet and Brulé (1993) also observed solubilisation of calcium remaining amounts (245 mg l^{-1}) within the region at pH 5.2–3.5.

The remaining sedimentable calcium levels at pH below 5.0 have been attributed to the adsorption of calcium directly to the casein molecules (Dalgleish & Law, 1989; Le Graet & Brulé, 1993). Decreasing of pH below 5.0 causes the calcium phosphate to dissolve but also removing the calcium directly bind to casein which is not bound to phosphate. Most of the remaining sedimentable calcium levels calculated in the present study for yoghurts therefore they could be ascribed to calcium bound to casein without being involved in calcium phosphate formation.

Phosphorus had the lowest percentages of non-sedimentable minerals in yoghurts (Fig. 1). Micellar phosphorus calculated represents the covalently bound phosphate of the phosphoserine groups of caseins. In cow's milk, about 25% of phosphorus in milk is associated with caseins in the micelles as calcium phosphate, whereas 25% occurs as organic phosphate esterified to casein. The another half is soluble mainly as free phosphate ions. The organic phosphate esterified to casein would not removal when pH is decreased during yoghurt manufacture and would stay in the pellet after ultracentrifugation.

Although milk calcium absorption in vivo is controlled by complex homeostatic mechanisms and this matter is still subjected to controversy, different studies have suggested that calcium in a soluble form would be a prerequisite for its better absorption in the gastrointestinal tract. Delisle, St-Amand, Gosselin, Pichette, and Amiot (1993) postulated that the high content in ionic calcium generated in yoghurts during simulated gastro-intestinal digestion could be an index of high bioavailability of this dairy product. In that case, the high content of non-sedimentable calcium in yoghurts may represent an advantage from a nutritional point of view.

On the other hand, calcium phosphate can form insoluble precipitates from which calcium can not be absorbed. Casein phosphopeptides, formed during the digestion of caseins, have a positive effect on calcium absorption because of their ability to bind calcium and maintain it in a soluble form in the intestinal lumen, and therefore available for absorption. Although information available from human is still scarce, numerous animal studies have shown that phosphopetides enhance the absorption of calcium in the intestine (FitzGerald, 1998; Jovaní, Barberá, & Farré, 2001). The use of milk powders as enrichment vehicles in yoghurt manufacture could increase such phosphopeptides to enhance calcium absorption.

The real significance that any of the above would have on calcium absorption and human nutrition is still a matter of study. However, it would seem evident that the choice of fortifier agent could have consequences on calcium bioavailability and, therefore, on the yoghurt nutritional quality.

3.2.2. *Magnesium*

Between 88 and 97% of total magnesium in yoghurt was non-sedimentable (Fig. 1). In cow's milk 65% of magnesium is in a soluble form while the remainder is colloidal associated with the casein micelles (about half associated with colloidal calcium phoshate and half bound directly to phosphoserine residues in caseins) (Flynn & Cashman, 1997).

This micellar magnesium is progressively released as the pH was decreased from 6.7 to 4.9 (Dalgleish & Law, 1989). At pH 4.9, the lowest pH which was used by those authors, there was still some magnesium remaining on the casein. It appeared from Dalgleish and Law (1989) results that the behaviour of the small amount of micellar magnesium paralleled that of the calcium. Le Graet and Brulé (1993) also remarked the similarity of the magnesium solubilisation with the calcium and determined that the remaining micellar magnesium (around 1/3) was only solubilised at pH between 5.2 and 3.5.

As for calcium, magnesium may bind to the non-phosphorylated binding sites in the caseins. However it has been suggested that magnesium occur in combination with casein rather that with micellar inorganic phosphate (Van Hooydonk et al., 1986). This could help to explain why lower percentages of magnesium than calcium were found in the non-sedimentable yoghurt fraction (Fig. 1). These percentages would correspond to magnesium linked to non-phosphorylated binding sites in the caseins.

There is little information on the bioavailability of magnesium from dairy products in humans. Apparent absorption of magnesium in rats fed with different dairy products varied widely (Deslisle et al., 1995). In skim milk, milk powders and yoghurt magnesium absorption was high (40–45%) whereas the lowest levels were found in cheese (15%). A previous work (Pantako, Passos, Desrosiers, & Amiot, 1992) showed that the onset of magnesium absorption was faster with diets based on caseins, mainly β-casein, compared to those based on lactoserum proteins demonstrating the importance of dairy additives in the magnesium bioavailability. According to this, commercial yoghurts manufactured with dairy ingredients enriched in caseins as fortifiers, could potentially enhance magnesium absorption. However more studies should be carried out to confirm it.

3.2.3. Zinc

About 95% of the zinc in yoghurts was found in the supernatants after ultracentrifugation (Fig. 1). This is in contrast with the low level of this mineral in the soluble phase in raw milk. Zinc in cow's milk is primarily bound to casein (≈90–95%), and only small amounts are linked to whey proteins and low molecular weight ligands in the serum (De la Fuente, Fontecha, & Juárez, 1996; Shen, Robberecht, Van Dael, & Deelstra, 1995; Singh, Flynn, & Fox, 1989).

Singh et al., (1989) documented that non-micellar zinc levels increased when the pH of milk was reduced before ultracentrifugation so that ~95% of the zinc was non-sedimentable at pH 4.6. Within the casein micelles, one-third of the zinc is loosely bound to casein phosphoserine residues, whereas two-thirds are more tightly bound to colloidal calcium phosphate. Most of the zinc directly bound to casein is readily removed when pH decrease to low values. It was inferred that the more tightly bound zinc fraction in skim milk was largely that associated with colloidal calcium phosphate. Nevertheless at pH 4.5, as we mentioned above, virtually all calcium phosphate has been solubilised. Consequently the remaining sedimentable zinc calculated in yoghurts should be attributed to the fraction bound directly to casein phosphoserine residues.

It is well known that binding of a significant fraction of the zinc in human milk to a low molecular weight zinc-binding ligand may enhance zinc absorption either by facilitating zinc transport across the gastrointestinal tract wall or by preventing the sequestration of zinc by other substances in the gastrointestinal tract that reduces its availability for absorption. On the contrary, the binding of a large fraction of zinc in cow's milk to casein may result in the entrapment in casein curds formed in the stomach which may be incompletely digested in the small intestine, thus rendering a significant proportion of the zinc unavailable for absorption.

Nowadays, it is not well clarified if the chemical form of zinc in dairy products can represent an advantage from a nutritional point of view. Few studies have been specifically devoted to the estimation of the bioavailability of zinc in different dairy products. However, it does not seem likely that zinc absorption from yoghurt were reduced in comparison to raw milk. Fermentation of milk with *Staphylococcus thermophilus* or *Lactobacillus bulgaricus* to yield yoghurt significantly increased zinc availability (12.5%) compared with raw milk (Van Dael, Shen, & Deelstra, 1993). Bobilya, Ellersieck, Gordon, and Veum (1991) showed that zinc was absorbed and retained as well from nonfat dry milk as lowfat plain yoghurts in diets fed to neonatal pigs. The low pH of yoghurt would facilitate the high levels of soluble zinc bound to low-molecular weight ligands. Such ligands may enhance zinc absorption either by facilitating zinc transport across the gastrointestinal tract wall or by preventing the chelation of zinc by other substances in the gastrointestinal tract, which would lower its availability for absorption (Shen et al., 1995). Moreover, milk acidified to pH 4.6 and then readjusted to pH 6.7, simulating conditional of gastrointestinal tract, contained significantly more non-sedimentable zinc than untreated skim milk (≈50%) (Singh et al., 1989). Although a positive correlation between high content in soluble form and absorption is far to be completely demonstrated and more research is required, the consumption of yoghurt as a potential source of zinc more easily available in the diet should not be left aside.

4. Conclusions

High concentrations of minerals detected in the present study in most of the samples confirmed the role of yoghurts as source of essential nutrients in comparison with raw milk. Moreover, this food could represent an excellent alternative to milk for lactose intolerant population. The distribution of minerals (calcium, magnesium, phosphorus and zinc) in yoghurts differed dramatically from those of milk showing that elements associated with casein were redistributed during manufacturing. The noticeable increase in the levels of non-sedimentable minerals could favour the absorption of these nutrients in the gastrointestinal tract. However the implications of these findings for nutritional properties should be investigated further.

Acknowledgements

The authors acknowledge the financial support for this research project (AGL2000-0727-C03-02) furnished by the *Comisión Interministerial de Ciencia y Tecnología* (CICyT).

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