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# Constituents of nutritional relevance in fermented milk products commercialised in Italy

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# Abstract

Fermented milk products represent an increasing share of the dairy products consumed in Italy. The nutritional value of these products is related to the milk utilised and to the eventual presence of other ingredients (milk powder, sugar, fruit puree and fruit extracts), whereas the microrganisms used can affect texture and organoleptic characteristics. In this paper constituents of nutritional relevance such as protein, fat, total carbohydrate, amino acids, minerals, vitamin A, vitamin E and cholesterol, have been evaluated in yoghurts, fermented milks (plain and with essences) and Quark cheeses (plain and with fruits). This study confirms the high nutritional quality of fermented milks and stresses the role of non-milk ingredients in modifying and, sometimes, improving the dietary contribution of these products.  $\odot$  1999 Elsevier Science Ltd. All rights reserved.

#### 1. Introduction

The fermentation of milk with lactic acid bacteria leads to peculiar organoleptic characteristics of the final product due to the reduction of the lactose content and, consequently, to the production of lactic acid and the coagulation of milk protein.

A variety of benefits have been associated with lactic acid bacteria. An improvement of calcium bioavailability and lactose digestibility in lactase-deficient individuals (Shermark, Saavedra, Jackson, Huang, Bayless & Perman, 1995), a balancing of the intestinal microflora, a hypothesised hypocolesterolemic effect (Eichholzer  $\&$ Stahelin, 1993) and a potential prevention of colon cancer (Kampman, Goldbolm, Van Den Brant, & Van't Veer, 1994) are reported in the literature on this subject.

Different lactic bacteria can be utilised in the manufacture of fermented milks but, according to the Codex Alimentarius definition (FAO, 1992) and to the Italian legislation (Ministero Della Sanitá, 1972), the fermented product can be called "yoghurt" only if the bacteria synergically growing in milk are Streptococcus thermophilus (new nomenclature: Streptococcus salivarius ssp. termophilus) and Lactobacillus bulgaricus (new nomenclature: Lactobacillus delbrueckii ssp. bulgaricus).

Traditional yoghurt bacteria do not belong to natural intestinal flora and cannot implant themselves in the intestines, but some selected intestinal bacteria can be isolated and utilised in the manufacture of fermented milks to increase their beneficial role to human health (Rosenthal, 1991).

Recently, fermented products utilising Bifidobacterium and *Lactobacillus*, that are reported to have healthpromoting properties (Gibson & Roberfroid, 1995), have been commercialised. These lactic bacteria are called "probiotics" i.e. "live microfloras which beneficially affect the host animal by improving its intestinal microbial balance'' (Fuller, 1989).

Other dairy products, containing living lactic bacteria, can be considered similar to the more traditional fermented milks. In particular, Quark cheese is a fermented product made from skimmed or partially skimmed cow milk previously heat-treated at  $70-90^{\circ}$ C for a short time and, afterwards, treated with liquid rennet and inoculated with Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremori and Lactococcus lactis ssp. diacetylactis. The thermal treatment leads to interactions between casein and whey proteins and a large part of the whey protein co-precipitates with casein on the acidification due to a prolonged incubation with lactic bacteria (Salvatori Del Prato, 1993). Finally the base-product can be treated with other ingredients of different origin such as fruit, vegetable or fish.

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In terms of overall composition, all fermented milk products have a nutritional value corresponding to the composition of the milk from which they are made, even though small differences in the concentration of a chemical constituent could be present, due to the manufacture and the fermentation process. The main modifications are: (i) a considerable formation of lactic acid and a consequent decrease of lactose; (ii) an increased content of free molecules, such as small peptides, amino acids and fatty acids. Furthermore, the composition of these products can be also modified and integrated by some ingredients of different origin, such as fruit (puree or pieces) and/or sugar.

In this work we studied, from a nutritional point of view, traditional and innovative fermented milk products such as yoghurt, fermented milks, and Quark cheeses, natural or treated with other ingredients. All these products are well represented in the Italian market and their consumption is increasing due to increased consumer attention. Finally, in homage to tradition, a fermented milk of very ancient origin in Italy (Sardinia) has also been studied. It was traditionally prepared from ewe or goat milk inoculated with a starter culture including bread yeast, a spoon of vinegar and a small quantity of rennet and is called "Gioddu". Nowadays, Gioddu is industrially produced with homogenized milk, heated at  $90^{\circ}$ C for 15 min, cooled to  $44^{\circ}$ C, inoculated with Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus salivarius ssp. termophilus for  $4-5$  h.

The curd is broken, cooled and packaged. The product is more consistent than yoghurt, has a white and shiny appearance and is rich in aromatic substances.

### 2. Materials and Methods

#### 2.1. Samples

In the following list some characteristics and principal ingredients of the studied products, as reported on the labels, are summarised: A yoghurt, natural. B milk fermented with Lactobacillus bulgaricus, Streptococcus thermophilus and Bifidobacterium bifidus. C yoghurt and milk fermented with Bifidobacterium and Lactobacillus acidophilus. D milk fermented with S. thermophilus and Lactobacillus acidophilus and integrated with skim-milk powder and milk proteins. D1: D product integrated with sugar, skim-milk powder, milk proteins and vanilla flavour. D2: D product integrated with sugar, skim-milk powder, milk proteins and strawberry preparation. E concentrated (1.5 times) goat fermented milk (Gioddu). E1 concentrated (1.5 times) goat milk treated with myrtle essence and fermented. E2 concentrated (1.5 times) goat milk treated with myrtle syrup and sucrose and fermented. E3 concentrated (1.5 times) goat milk treated with lemon syrup and sucrose and fermented. F Quark cheese, natural, with cream and salt. F1 Quark cheese with cream, sugar, syrup and puree of strawberry, blueberry and raspberry. F2 Quark cheese with cream, apple puree and sugar. F3 Quark cheese with cream, banana puree and sugar.

Gioddu samples were industrially produced and provided from the Sassari University, Italy. The other products were purchased from local supermarkets. The analytical sample was obtained by pooling at least three packages of each variety.

### 2.2. Chemical

All reagents (Carlo Erba, Milan) were of analytical or HPLC grade, as required. Standards of 13 cis-retinol (85% pure) and all trans-retinol (70% pure) alphatocopherol and cholesterol were obtained from Sigma Chemical Co (St. Louis, MO). Standards of amino acid were obtained from Beckman Inc. (Palo Alto, CA), sugars from Fluka Chemie (Buchs, Switzerland) and minerals from Merck (Darmstadt, Germany).

#### 2.3. Equipment

A Dionex-Biolc Ion system equipped with a Dionex CS12 Ion Pac column with a Suppressed Conductivity Detector and a CarboPac PA1 column with a Pulsed Amperometric Detector (Camberley, UK) were for mineral and sugar determination, respectively.

Amino acids were analysed by a Beckman 120C amino acid analyser (Beckman Instruments Inc., Palo Alto, CA), a column  $32\times0.9$  cm packed with a resin of polysulphonic acid and an ISCO spectrophotometer detector (Hengoed, UK).

A HPLC analytical system comprising a Waters (Milford, MA) Mod. 510 solvent delivery system with a column 5µm Beckman Ultrasphere Si 250 $\times$ 4.6 mm, a Gilson (Middleton, WI) autosampling Model 231-401 injector, a programmable Perkin-Elmer LS 40 (Norwalk, CT) spectrofluorometer and a programmable multiwavelength detector (Waters Model 490) were utilised to analyse vitamins and cholesterol.

# 2.4. Method

#### 2.4.1. Proximate composition

Water, protein and fat contents were determined according to the FIL-IDF procedures (International Dairy Federation, 1964; 1982; 1986). Carbohydrate level is calculated as the sum of glucose/galactose, fructose, lactose and saccharose contents determined by the chromatographic method described below.

#### 2.4.2. Carbohydrates

The samples were weighed and extracted in water by sonication for 30 min. A suitable volume was filtered

and injected into an anion exchange column. Carbohydrates were isocratically separated by NaOH (150 mM) at  $1$  ml/min flow rate and detected by a pulsed amperometric detector. The method does not allow separation between galactose and glucose. The relevant chromatographic peaks are perfectly overlapped and, as the response factors for the two monosaccharides are very similar and the difference is below the analytical error, the concentration was calculated as galactose and expressed as the sum of glucose plus galactose.

#### 2.4.3. Minerals

The samples were analysed after ashing:  $200-500$  mg sample was weighed into crucibles and ashed in the furnace at  $500^{\circ}$ C for 24 h. The ashes were dissolved with a few drops of nitric acid (70%) and diluted to 50 ml with deionised water. Sodium, potassium, magnesium and calcium were separated by an isocratic elution with a solution of methane sulfonic acid (20 mM) at 1 ml/min flow rate (Gambelli, Ingrao, Pizzoferrato & Santaroni, 1996) and revealed with a suppressed conductivity detector.

## 2.4.4. Total amino acids

Total amino acids analysis was carried out according to the method of Spackman, Moore and Stein (1958) by ion-exchange chromatography after protein hydrolysis. Methionine and cysteine were first oxidised by performic acid to convert methionine into methionine sulphone and cysteine into cysteic acid (Schram, Moore & Bigwood, 1954). Tryptophan determination was carried out by isocratic reversed-phase high performance liquid chromatography and fluorescence detection after alkaline hydrolysis (Steven & Jorg, 1989).

#### 2.4.5. Vitamins A and E and cholesterol

Samples were saponified and extracted following the method of Panfili, Manzi and Pizzoferrato (1994). The extracted residue was dissolved in the mobile phase (2 propanol 1% in n-hexane), injected and analysed by a normal phase HPLC methodology (Panfili et al., 1994). In this method, 2-propanol (1%) in *n*-hexane (A) and *n*-hexane (B) were utilised in a multi-linear gradient elution at a flow rate of 1.5 ml/min and the quantitation of the separated compounds was carried out by a spectrofluorimetric and a spectrophotometric detector connected in series.

# 3. Results

In Table 1 the proximate composition of fermented cow and goat milks and Quark samples is reported. Water and protein contents of fermented milk samples vary from 77.3 g/100 g to 88.3 and from 4.0 to 4.8 g/100 g respectively. Gioddu samples show water contents ranging from 81.8 to 85.8% and the protein content is variable as a function of the ingredients and decreases from 4.3% in the natural products and 4.1% in the product treated with a fruit essence, to  $3.6-3.7\%$  in the Gioddu treated with fruit syrups. Fat and carbohydrate levels present a large variability. Products made from goat milk show a greater content of fat than products made from cow milk because of a fat content usually higher in goat than in raw cow milk (USDA, 1997). Lower fat levels can be observed in fermented milk samples where milk-fat is diluted by the presence of other non-fat ingredients as such fruits. The presence of fruit puree and essence also explains the difference in carbohydrate contents, ranging from 3.5 to 13.1%.

Table 1

Chemical composition and energy value of fermented milks and Quark cheesesa

Samples	Water	Protein	Fat	Carbohydrate	Energyb	
					(kcal)	(kJ)
A (yoghurt natural)	87.8	4.7	3.8	4.3	70	294
B (fermented milk)	88.3	4.0	3.5	3.8	63	262
C (fermented milk)	86.8	4.8	3.8	3.5	67	282
D (fermented milk with milk proteins)	84.5	4.6	3.6	5.6	73	307
D1 (with vanilla essence)	77.3	4.6	3.2	13.1	100	419
D <sub>2</sub> (with strawberry essence)	78.3	4.7	3.2	12.0	96	402
E (Gioddu natural)	85.1	4.3	5.1	4.6	82	340
E1 (with myrtle essence)	85.2	4.1	4.8	4.7	78	327
E2 (with myirtle syrup)	85.8	3.7	4.6	5.0	76	318
E3 (with lemon syrup)	81.8	3.6	4.7	9.7	96	400
F (Quark natural)	67.0	9.0	18.6	3.0	215	892
F1 (with berry fruits)	62.5	7.2	9.6	18.9	191	799
F <sub>2</sub> (with apple)	62.5	7.2	9.6	17.8	186	780
F <sub>3</sub> (with banana)	62.5	7.2	9.6	16.6	182	760

<sup>a</sup> Data (mean value for duplicate determination) are expressed as  $g/100 g$ .

<sup>b</sup> Energy is expressed as kcal and kJ.

Quark cheeses show a similar trend. If fruit ingredients are present, protein and fat levels decrease and carbohydrate levels increase. This is obviously due to the difference in the product formulation leading to the substitution of milk and cream, good sources of protein and fat, with fruits, good sources of carbohydrates.

Finally, energy values are reported. Energy is calculated on the basis of the official conversion factor (CEE Directive, 1990): proteins 4 kcal/g and 17 kJ/g; carbohydrates 4 kcal/g and 17 kJ/g; fats 9 kcal/g and 37 kJ/g. Energy value ranges from 70 to 100 kcal/100 g  $(294-419)$ kJ/100g) in fermented milks to 182 to 215 kcal/100 g  $(760-892 \text{ kJ}/100 \text{ g})$  in Quark cheeses.

In Table 2 the amino acid contents of the studied products are reported. The differences in the amino acid levels of fermented cow and goat milks (A, B, C, D, E) and Quark cheeses  $(F)$  are essentially due to the different protein contents. The Chemical Score, i.e. the percentage of the recommended level (FAO/WHO, 1991) provided by the limiting amino acid, is always higher than one hundred, confirming the excellent nutritional quality of milk proteins. The limiting amino acid is generally tryptophan and, in some fermented cow milk products, threonine.

Mineral contents of the studied products are reported in Table 3. In all the fermented milks potassium was the

Table 2

	Amino acid content in fermented milks and Quark products <sup>a</sup>				
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<sup>a</sup> Data (means of duplicate determination) are expressed as  $g/100$  g.

b See Materials and Methods for samples identification.

#### Table 3





<sup>a</sup> Data (means of triplicate analysis  $\pm$  standard deviation) are expressed as mg/100 g.

mineral present in the largest amount  $(107-256 \text{ mg}/100$ g). The difference between fermented cow milks and goat milk natural products are significant ( $p < 0.01$ ), as already observed in cow and goat raw milk (Cerutti, 1997), but, in this case, it is probably also due to the concentration of goat raw milk before processing of Gioddu. It is also notable that the potassium content of the Quark containing banana puree is higher if compared with the other Quark products and this is due to the high level of this mineral in banana fruit  $(0.396 \text{ g})$ 100 g) (USDA, 1997).

In the Quark samples sodium level is higher in the natural product containing added salt than in the ``sweet'' products treated with fruits. The contents of the other minerals are significantly lower in these products, than in the fermented milk samples. This is probably due to the industrial procedure utilised in the production of Quark cheese and, in particular, to the acid-coagulation step inducing a demineralisation of the product collected. On the other hand, in the fermented milks mineral contents are quite similar to the original raw milk because no whey separation occurs.

Table 4, reporting the carbohydrate levels in some fermented products both natural and treated with other ingredients, shows a large variability due to the difference in the recipe of each product. As an example, the presence of fructose and saccharose can be observed only in the samples with the addition of some other ingredient (puree fruits, syrup, sugar).

The content of lactose can be affected by the addition of ingredients different from milk. A dilution effect can be observed in Quark cheese samples: 2.92 g/100 g lactose in the natural product  $(F)$  and 1.80 g/100 g, on average, in the treated samples (F1, F2, F3). The variation of the lactose content  $(4.42-4.74 \text{ g}/100 \text{ g})$  in D fermented milks is not statistically significant ( $p > 0.05$ ). In Gioddu samples the lactose level decreases from the natural (E) sample  $(3.67 \text{ g}/100 \text{ g})$  to the sample (E2)



treated with myrtle syrup  $(2.49 \text{ g}/100 \text{ g})$ , but increases when lemon syrup  $(E3)$  is added  $(4.71g/100g)$ . To explain this apparent inconsistency, it should be noted that, according to the processing, the fruit addition is performed after the inoculation but before the incubation, so that the greater acidity of the sample treated with lemon syrup can interferes and slows down the fermentation, causing a higher residual lactose level in this product  $(4.71 \text{ g}/100 \text{ g})$  than in the myrtle syrup product  $(2.49 \text{ g}/100 \text{ g})$ . As in the fermented milk samples, the addition of myrtle essence (E1) is without any significant consequences  $(3.84 \text{ g}/100 \text{ g})$ .

It is also noteworthy that Gioddu with myrtle essence (E1) does not contain saccharose but only lactose (from milk), fructose and glucose (from fruit) while, in the sample with myrtle syrup (E2), saccharose is present as an ingredient in the recipe of the product.

Finally, in Table 5, fat-soluble vitamin and cholesterol contents are reported. Significant differences  $(p<0.01)$ , observed between fermented cow milks (A, B, C, D) and Gioddu (E), are due to levels of fat and alpha-tocopherol greater in goat milk than in cow milk. It is also important to highlight, in the Gioddu samples, as in goat milk, the absence of carotenes (Panfili et al., 1994).

The content of fat-soluble vitamins and cholesterol is higher in natural (F) than in Quark products with fruit (F1, F2, F3). This is due to the greatest content of fat in natural Quark cheese, and a diluting effect of fat and fat-soluble vitamins caused by the addition of the fruit, a non-fat ingredient. Also relevant are the level of total carotenes in the sample with banana, a particularly rich source of carotenes, and the level of tocopherol in the sample treated with berry fruits containing high concentrations of this compound (USDA, 1997).

In conclusion, this study confirms the high nutritional quality of the fermented milk products quite separately from the different lactic acid bacteria utilised during the



Data (means of triplicate analysis  $\pm$  standard deviation) are expressed as g/100 g.

 $b$  ND, not detectable (minimum detectable level 0.04 g/100 g).





<sup>a</sup> Data are means of triplicate determinations  $\pm$  standard deviation.

<sup>b</sup> ND not detectable (minimum detectable level 0.4  $\mu$ g/100 g).

fermentation. In fact, any nutritionally interesting differences in composition of fermented milk products, should be ascribed to the presence of non-milk ingredients rather than to the fermentative activities of the working microrganisms and such modifications should be carefully considered in particular dietary restrictions.

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