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New functional milk-based products in the Italian market

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Abstract

The aim of this study is to obtain an overview, from a chemical and a nutritional point of view, of functional products such as lactosehydrolysed milk, milk with added probiotics, prebiotics and vitamins, probiotic fermented milk, and synbiotic fermented milk. For this purpose, six samples of lactose-hydrolysed UHT milk and two samples of milk with added functional ingredients, seven probiotic and four synbiotic fermented milk samples, have been studied. In lactose-hydrolysed milk samples, proximate composition, vitamins A, E and cholesterol contents have not shown significant differences, with the obvious exception of a skimmed milk sample containing small amounts of fat and related compounds. The other samples have shown a large compositional variability, and, in particular, cholesterol ranged from 3.6 to 12.7 mg/100 g, vitamin E ranged from 2.1 to 3300 μ g/100 g and vitamin A ranged from 3.5 to 182.0 Retinol Equivalents (μ g/100 g).

Lactose levels ranged from 0.26 to 0.77 g/100 g in lactose-hydrolysed milk samples and from 2.32 to 4.55 g/100 g in the other milk samples.

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1. Introduction

According to a widely accepted definition, a functional food is any modified food that may provide a health benefit beyond the nutrients it contains [\(FDA, 2004](#page-5-0)). These healthy foods include products with reduced fat, sugar or salt, fortified with vitamins, minerals, phytochemicals, bioactive peptides or ω 3 polyunsaturated fatty acids. In this work, different functional foods, widely consumed in Italy, such as semi-skimmed and skimmed lactose-hydrolysed milk, whole, semi-skimmed and skimmed functional milk fermented with probiotic bacteria and/or with added probiotic, prebiotic ingredients and vitamins, were studied from a chemical and a nutritional point of view.

Apart from ripened cheeses and, partially fermented milks, lactose-hydrolysed milk is the only kind of milk that lactose-intolerant subjects can consume. Lactose is digested by an enzyme which is present during infancy but its phy-

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siological synthesis decreases or even disappears with age, leading to lactose intolerance. The undigested lactose is fermented in the intestinal tract by colonic microflora to short chain fatty acids and gas and a relationship between the lactose consumed and the severity of symptoms is reported [\(King, 1996; Vernia, Di Camillo, & Marinaro, 2001\)](#page-5-0).

Lactose reduction in milk is industrially obtained by an enzymic hydrolysis to glucose and galactose, utilising a b-galactosidase isolated from several sources. The final product (low lactose milk or lactose-hydrolysed milk) is sweeter and easier to absorb in the intestinal tract than untreated milk. Moreover, lactose-reduced milk, yogurt and ripened cheeses are the best source for lactose-intolerant subjects of bioavailable calcium, a deficiency of which can lead to the development of osteoporosis ([Bannan & Levitt, 1996\)](#page-5-0).

Other functional foods selected in this work are fermented milks with probiotic bacteria, with or without prebiotics. Probiotics used in functional foods are of human origin, non-pathogenic, resistant to destruction by technical processing, and to gastric acid and bile physiological action. They should be able to adhere to intestinal epithe-

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lial tissues, colonise the gastrointestinal tract, produce antimicrobial substances, modulate the immune response, and influence metabolism ([Ziemer & Gibson, 1998; Saarela,](#page-5-0) [Mogensen, Fonden, Matto, & Mattila-Sandholm, 2000;](#page-5-0) [Teitelbaum & Walker, 2002](#page-5-0)).

Probiotic bacteria should be viable and at high concentrations ([Shah, 2001; Losada & Olleros, 2002\)](#page-5-0). Bifidobacterium (often referred as ''bifidus"), Lactobacilus casei and Lactobacillus acidophilus have been identified as probiotics and they are often added to milk to obtain fermented milk ([Saarela et al., 2000\)](#page-5-0).

[Gibson and Roberfroid \(1995\)](#page-5-0) defined prebiotics as non-digestible food ingredients which beneficially affect the host, by stimulating the growth of health-promoting bacteria in the intestinal tract. Hence, prebiotics are responsible for the growth of probiotics. Widely-used prebiotics are fructooligosaccharides (FOS) and complex oligosaccharides resistant to human digestive enzymes and therefore available for colonic fermentation by saccharolytic bacteria ([Shah, 2001; Losada & Olleros, 2002; FAO/](#page-5-0) [WHO, 2001\)](#page-5-0). Prebiotics and probiotics are often simultaneously present in functional dairy products, specifically called synbiotics, due to their synbiotic functional action.

Finally the last group of products studied are unfermented milks with added functional ingredients (e.g., probiotics, prebiotics, vitamins).

2. Materials and methods

2.1. Chemicals

All reagents (Carlo Erba, Milan) were of analytical or HPLC grade. Standards of lactose monohydrate, sucrose, glucose, galactose, 13-cis-retinol (85% pure), all trans-retinol (70% pure), α -tocopherol, β -carotene and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Equipment

An HPLC analytical system (Alliance Waters Model 2695; Milford, MA) and a refractive index detector (Waters Model 2414) were utilised for sugar analysis. An Ultra Amino Restek column (5 μ m; 150 \times 4.6 mm) was used to analyse lactose and sucrose and a Sugar Pak I column $(300 \times 6.5 \text{ mm})$; Waters) was used to analyse glucose and galactose.

A 5 µm Beckman Ultrasphere Si column (250 \times 4.6 mm), a programmable Perkin Elmer LS 40 (Norwalk, CT) spectrofluorometer and a programmable multiwavelength detector (Waters Model 490) were utilised to analyse vitamins and cholesterol.

2.3. Methods

Proximate composition (water, protein, carbohydrate, fat and ash contents) was determined according to the FIL-IDF procedures [\(International Dairy Federation,](#page-5-0) [1964, 1982, 1986\)](#page-5-0). Total energy was calculated according to the following equations ([CEE Directive, 1990](#page-5-0)):

Energy (kcal) =
$$
4 * (g
$$
 protein + g carbohydrate)
+ $9 * (g$ lipid)
Energy (kJ) = $17 * (g$ protein + g carbohydrate)

$$
+37*(g
$$
lipid)

Carbohydrates were extracted according to the method of [Indyk, Edwards, and Woollard \(1996\)](#page-5-0). The samples were weighted accurately and dissolved in ca. 15 ml warm water. Carrez reagents I and II were added sequentially. The extract, made to volume with water, was filtered $(0.45 \mu m$ membrane) and injected.

Glucose and galactose were isocratically separated with a Sugar Pak I column at 60° C with a mobile phase of water (0.5 ml/min). Quantitation of the separated compounds was carried out using a refractive index detector at 50 °C internal temperature.

Lactose and sucrose were isocratically separated with an Ultra Amino Restek column at 35° C with a mobile phase of acetonitrile/water (75/25 v/v) at 1 ml/min and quantitation of the separated compounds was carried out by the refractive index detector at 35° C internal temperature.

To determine vitamins A and E and cholesterol, all samples were saponified and extracted following the method of [Panfili, Manzi, and Pizzoferrato \(1994\).](#page-5-0) The extracted residue was dissolved in the mobile phase (2-propanol, 1% in n-hexane), injected and analysed by normal phase HPLC ([Panfili et al., 1994](#page-5-0)). The quantitation of the separated compounds was carried out using a spectrofluorimetric and a spectrophotometric detector connected in series.

2.4. Sample collection

All the samples were obtained from large Italian stores. Lactose-hydrolysed semi-skimmed or skimmed UHT milk (products A–F), probiotic fermented semi-skimmed or whole milk (products G–O), synbiotic fermented semiskimmed or whole milk (products P–S) and two milks with added functional ingredients (products T and U) were studied. A brief description of these products and their labeled ingredients are in [Table 1](#page-2-0).

3. Results

The chemical composition of the lactose-hydrolysed milk and fermented milk studied is shown in [Table 2.](#page-2-0) In the lactose-reduced milk group, there was no difference in the chemical composition (samples B–F). The only exception was the product A, which was a skimmed milk.

The probiotic and synbiotic fermented milk groups showed a large variability in chemical composition: fat content ranged from 0.2 to 3.6 g/100 g, protein from 2.7 to 5.8 $g/100 g$ and ash from 0.4 to 0.8 $g/100 g$. In Italy it is possible to purchase fermented milk, with or without added ingredients (sugar, oligosaccharides, starch, etc.).

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Proximate composition (g/100 g) and energy value of the studied functional milk products

Analytical data are means of triplicate analyses \pm standard deviation.

^a Sum of lactose, glucose, galactose and sucrose calculated according to the method section.

In particular, G, L, M, N, P, Q contained added sugar; total carbohydrates ranged from 3.4 g/100 g (product S) to $12.8 \frac{\mathrm{g}}{100 \mathrm{g}}$ (product N).

In the last group, milk with added functional ingredients, but not fermented, there were two products: a semiskimmed milk (T) with a fat content of $1.5 \frac{g}{100}$ g and a whole milk (U) with a fat content of $3.7 \frac{g}{100 \text{ g}}$. Their proximate compositions, apart from the fat, showed levels of nutrients and energy in accordance with the relevant recipe ([Table 1](#page-2-0)).

In Table 3, lactose, glucose, galactose and sucrose contents of the studied products are shown. The carbohydrate has been studied with two different chromatographic methods to allow the separation of glucose from galactose and lactose from sucrose.

In particular, lactose ranged from 0.26 (product D) and 0.77 g/100 g (product A) in lactose-hydrolysed milk and these data suggest that this molecule was present, even though in slight amounts, in these products. In lactosehydrolysed milk, sucrose was absent. Glucose and galactose, products of lactose hydrolysis, were the main sugars: they ranged from 1.84 (product B) to 2.27 $g/100 g$ (product D) and from 1.61 (product B) to 2.02 g/100 g (product D), respectively.

In fermented milk samples the amount of lactose was greater than in lactose-hydrolysed milk and ranged from 2.32 (product N) to 2.82 $g/100 g$ (product G). The highest value of this group $(4.50 \text{ g}/100 \text{ g}$ in sample H) is due to the product recipe comprising the addition of an extra milk

Table 3

Lactose, glucose, galactose and sucrose content $(g/100 g)$ in functional milk products

Product	Lactose	Glucose	Galactose	Sucrose
	Lactose-reduced milk			
A	0.77 ± 0.01	1.96 ± 0.05	1.69 ± 0.02	
B	0.76 ± 0.01	1.84 ± 0.01	1.61 ± 0.00	
C	0.66 ± 0.04	1.99 ± 0.02	1.75 ± 0.02	
D	0.26 ± 0.02	2.27 ± 0.03	2.02 ± 0.04	
E	0.30 ± 0.02	2.14 ± 0.03	1.86 ± 0.02	
F	0.70 ± 0.04	1.86 ± 0.03	1.59 ± 0.02	
	Probiotic fermented milk			
G	$2.82 + 0.02$	0.03 ± 0.00	0.39 ± 0.01	6.02 ± 0.03
H	4.50 ± 0.26	0.02 ± 0.00	0.87 ± 0.00	
I	2.69 ± 0.16	0.16 ± 0.01	0.28 ± 0.00	7.92 ± 0.03
L	2.53 ± 0.02	0.02 ± 0.00	0.66 ± 0.00	5.51 ± 0.01
M	2.74 ± 0.02	0.05 ± 0.00	0.68 ± 0.00	5.96 ± 0.01
N	2.32 ± 0.14	0.20 ± 0.00	0.40 ± 0.00	9.83 ± 0.07
O	2.56 ± 0.15	0.25 ± 0.01	1.01 ± 0.01	
	Synbiotic fermented milk			
P	2.33 ± 0.14	0.18 ± 0.00	0.60 ± 0.01	6.69 ± 0.05
Q	2.58 ± 0.02	0.46 ± 0.01	0.49 ± 0.00	3.90 ± 0.04
R	3.42 ± 0.03	0.03 ± 0.00	0.94 ± 0.01	
S	2.81 ± 0.16	0.01 ± 0.00	0.56 ± 0.00	
	Milk with added functional ingredients			
T	4.55 ± 0.05	nd	0.01 ± 0.00	
U	4.36 ± 0.03	nd	0.01 ± 0.00	

 $nd = not detectable$

Data are mean of triplicate determinations \pm standard deviation.

component. Glucose level ranged from 0.01 to 0.46 g/100 g, while galactose ranged from 0.28 to 1.01 g/100 g. The concentration of glucose was always lower than that of galactose. This trend is in agreement with the fermentation mechanism of producing energy, 1 mole of galactose, which is not fermented by lactic bacteria, and 2 moles of lactic acid, which is derived from glucose fermentation. Lactose in milk with added functional ingredients is neither hydrolysed nor fermented, and the amount of glucose and galactose (the hydrolysis products) detected was zero or negligible. In some products sucrose was added in large amounts, from 3.90 (product O) to 9.83 $g/100 g$ (product N).

Vitamins A, E and cholesterol contents are reported in [Table 4.](#page-4-0) In lactose-reduced milk the vitamin content and cholesterol content were similar with the exception of sample A (totally skimmed milk). In fermented milk, there was great variability: cholesterol ranged from 3.6 (product G) to 11.7 mg/100 g (product S), and α -tocopherol from 2.0 (P) to $3299.5 \mu g/100 g$ (S, supplemented product). Beta-carotene is present in cow milk and varied from 1.6 (product R) to 11.8 μ g/100 g (product O), and *trans*-retinol ranged from 3.2 (product P) to $44.0 \mu g/100 \text{ g}$ (product S), depending on the fat content of each sample. Vitamins and cholesterol contents of milk with added functional ingredients (products T and U) were compatible with product recipes. The 13-cis-isomer of retinol is absent in a well stored raw milk and its presence and concentration depends on other external variables, such as process temperature, storage temperature and time, and microorganism activity. In particular, the ratio 13-cis/all trans-retinol, an index of process and storage severity ([Panfili, Manzi, & Pizzoferrato,](#page-5-0) [1998](#page-5-0)) ranged from not detectable (products A and R) to 23.7% (product G).

[Fig. 1](#page-4-0) shows cholesterol level, expressed in mg per 100 g of product and in mg per g of fat, versus fat percentage. Linear rise in cholesterol (expressed as mg /100 g of product) as a function of increasing fat concentration can be observed. It is interesting to note the trend of cholesterol expressed as mg/g of fat; an exponential function means that the highest levels of cholesterol are present in the fat of low-fat products. Milk skimming procedures allow the separation of the largest fat globules, which contain less cholesterol than the smallest ones. Hence, the fat globules remaining in low-fat products are the richest in cholesterol ([Pizzoferrato & Manzi, 1999\)](#page-5-0).

Another particular aspect of light products can be observed in [Fig. 2](#page-4-0), showing the DAP value versus cholesterol, expressed as mg/g of fat. DAP, meaning degree of antioxidant protection, is a parameter recently proposed to estimate the potential oxidative stability of fat in foods ([Pizzoferrato & Manzi, 1999; Pizzoferrato, Manzi, Rubino,](#page-5-0) [Fedele, & Pizzillo, 2000; Esti et al., 2004](#page-5-0)). In dairy products, DAP is calculated as the molar ratio between a-tocopherol and b-carotene (antioxidant compounds), and cholesterol (an oxidation target). The higher the DAP value, the higher is the stability of the food to oxidation.

 $nd = not detectable$

Data are means of triplicate determinations \pm standard deviation. The percentage of retinol isomerisation (13-cis/all trans-retinol) is also shown.

Fig. 1. Cholesterol level, expressed as mg/100 g of product (\square) and as mg/ g of fat (\blacksquare) , versus fat concentration $(\%).$

Fig. 2 shows that the protection from oxidation decreases from a low-level of cholesterol expressed as mg/g of fat (fat products) to a high level (skimmed products). In other words, cholesterol, an important constituent of milk fat, often implicated in the aetiology of atherosclerosis and coronary heart disease, is more vulnerable to oxidation in light than in fat products. This consideration is particularly important both from a chemical and a physiological point of view. Actually cholesterol oxides, rather than cholesterol itself, are reported to be implicated in the initiation of atherosclerotic plaque formation ([Kumar & Singhall, 1991;](#page-5-0) [Caboni et al., 1994\)](#page-5-0).

Fig. 2. Degree of antioxidant protection (DAP) values versus cholesterol expressed as mg/g of fat.

In [Table 5](#page-5-0) the percent contribution of 125 g of each studied product to the daily allowances for vitamin A and E is shown. The percent contribution of lactose-hydrolysed milk to vitamin A ranges from 0.1 (product A) to 5.0% (products B and C) in male and from 0.1 (product A) to 5.9% (product B) in female, while the percent contribution to vitamin E ranges from 0.0 (product A) to 0.6% (products B, C, D, E and F) in adults. For fermented milk samples the percent contribution to vitamin A ranged from 0.6 (products P and R) to 8.3% (product S) in male and

Table 5 Contribution (%) of a portion of the studied functional products (125 g) to the daily allowances of vitamins A and E, and cholesterol (LARN, 1996)

from 0.7 (products P and R) to 9.7% (product S) in female, while vitamin E ranged from 0.0 (product P) to 51.6% (product S) for the adult. Product T, in the group of milks with added functional ingredients, contained the highest amounts for both vitamins A and E, in agreement with its recipe.

Finally, in Table 5 the percent contribution of these products to the daily intake of cholesterol is illustrated. To calculate these data, the maximum limit of 300 mg/ day (LARN, 1996) has been applied. A portion of 125 g of lactose-hydrolysed milk or fermented milk contributes only 2.1% or 2.5%, respectively, of the cholesterol daily intake and for this reason these products can be safely consumed in a nutritionally balanced daily diet by healthy individuals.

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