Editorial


The scientific aspects are under review by Prof. Chr. Barth (Germany) and Dr. G.J. Schaafsma (Netherlands). IDF Secretariat has been asked to determine with FAO how to introduce a further round of thinking into the debate.

IDF's "Nutrition Newsletter" aims at periodically presenting and stimulating exchange of information in the field of human nutrition. The articles provided will be derived primarily from the IDF Groups of Experts concerned with nutrition, or constitute the account of special IDF events in the field, such as the Nutrition Week. The views expressed are those of the authors and not necessarily of IDF.

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IMPACT OF TECHNOLOGY ON NUTRITIONAL VALUE OF DAIRY PRODUCTS

EFFECTS OF LIGHT EXPOSURE, STORAGE AND PACKAGING ON THE NUTRIENT CONTENT OF MILK

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SUMMARY
Milk and milk products contribute substantially to the intake of different vitamins in western diets. A number of these vitamins are sensitive to oxidation upon exposure to light. This paper deals with the current knowledge about the effects of light on the vitamin content of milk and milk products, packed in glass bottles.

During storage in shops and at home milk is exposed to light – in shops mainly tube light, at home also sunlight. Penetration of light into the milk is dependent on the light intensity, the packaging material, the volume of the packaging and the fat content of the milk. Like polycarbonate, bright glass is transparent to light for about 90%. On the other hand, uncoloured cardboard is transparent up to only 4%. Penetration of light into skimmed milk is 40-50% more intense than into full fat milk.

Variables which appear to influence the sensitivity of vitamins to oxidation following light exposure are: kind of light, temperature, and oxygen concentration of the milk. No data are available concerning the possible effect of the homogenization process on the sensitivity of milk to oxidation upon light exposure.

The riboflavin content of full fat milk in glass bottles (600 ml) is decreased by 50% after 1 h of exposure to light at room temperature. Riboflavin losses are considerably lower after exposure of milk in glass bottles to tube light at lower temperatures (1-10°C). In milk in glass bottles (600 ml to about 4 l), losses vary from 10% after 24 h to 67% after 10 days of exposure to tube light having an intensity of 1000-4000 lux. Ascorbic acid in milk is converted into dehydroascorbic acid and consequently into diketogluconic acid. The latter compound has no vitamin C activity. The presence of riboflavin and copper in milk promotes oxidation following light exposure. In the presence of sunlight and oxygen, ascorbic acid in pasteurized milk, packed in glass bottles, is converted completely into dehydroascorbic acid within 30 min. In the absence of oxygen the decrease of ascorbic acid is not significant. The effect of tube light is less strong, but within 2 days almost all the ascorbic acid is converted, and after 5 days dehydroascorbic acid is hardly present any more.

The oxidation of folic acid in milk under the influence of light is highly dependent on the presence of oxygen. Vitamin C protects folic acid against oxidation. In pasteurized milk no losses of folic acid are detectable after exposure to sunlight or tube light, but in sterilized milk which is saturated with oxygen, more than 50% of the folic acid is lost after 8 h exposure to sunlight. The sensitivity of vitamin B12 in milk to light exposure is influenced by the kind of heat treatment of the milk. At higher treatment temperatures the losses following light exposure increase. Of the remaining water soluble vitamins, only vitamin B6 seems sensitive to light; however, the available information is limited.

Vitamin A (retinyl palmitate) is sensitive to oxidation by UV-light and by light with a wavelength up to 455 nm. In glass bottles vitamin A is more rapidly degraded by sunlight than by tube light. From several studies it appears that retinyl palmitate added to homogenized milk is more sensitive to light than the natural vitamin A in milk. Vitamin A in homogenized full fat milk is more stable than vitamin A is skimmed milk. This is related to the lower penetration of light into full fat milk. Losses of vitamin A in skimmed milk supplemented with retinyl palmitate and packed in glass bottles can increase up to 80%, depending on the intensity and duration of the light exposure.

From a nutritional point of view, losses of vitamins in milk following exposure to light have a negative effect on the intake of riboflavin and vitamin B12 for all age categories and on the intake of folic acid and vitamin A for young children. Losses of vitamin C in milk are not so relevant, considering the small contribution of milk to the intake of this nutrient.

HEAT TREATMENT OF INFANT FORMULA: PHYSIOLOGICAL AND CLINICAL EFFECTS ON PROTEIN NUTRITION IN INFANCY

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ABSTRACT
Several different types of heat treatment are used in the production of commercial infant formulas. Previous in vitro studies have indicated that the extent of heat treatment has different physicochemical effects on the milk proteins and will affect their solubility and digestibility. In the present study 40 very low birth weight infants were randomized at the age of 2 weeks to be given one test feed of one of the following: fresh human milk, protein fortified human milk, a conventionally in-can sterilized liquid formula, a spray-dried powder formula or a UHT-treated liquid formula. The mean volume of the nasogastric test feed was adjusted so that the protein intake was equal in each study group of infants (0.45 g/kg). Venous blood samples were taken preprandially and at 30, 60, and 120 min after the test meal. α-Amino-nitrogen (total amino acid concentration) was assayed by the ninhydrin reaction. The concentration of α-amino-nitrogen rose rapidly after the protein fortified human milk feed and reached a...
peak value at 30 min. After the peak was reached, the serum values progressively declined, reaching baseline values at 120 min after the start of the test meal. The areas under the curve for all formulas were significantly different from that found for the human milk. At 30 min all formulas had significantly lower α-amino-nitrogen values than fortified human milk; at 60 min, all formulas had significantly higher mean concentrations than human milk, and at 120 min, none of the formula-fed infants had reached baseline values. In particular, the in-can sterilized formula-fed infants showed values significantly above baseline. By extrapolation, it was estimated that baseline was not reached before about 180 min after the test meal in the infants fed this formula. The UHT-sterilized formula had an area under the curve most similar to the human-milk-fed infants. These results demonstrate that heat treatment of formula has significant effects on in vivo amino acid absorption in the very low birth weight infant and possibly also in normal newborn infants. Further studies are needed to test whether these differences in formula protein absorption may have any significant effects on growth and metabolism of the infants. The results also indicate that differences in the time when postprandial serum amino acids reach baseline values in infants fed breast milk or different formulas may confuse the interpretation of plasma amino acid data.

Among the different purposes of food irradiation, two are of interest to dairy products:
- elimination of pathogen strains to prevent foodborne infections,
- lowering of total microbial load to increase the shelf life of products.

Such as for other preservation processes, the degree of protection depends on the absorbed dose by a specific product; a cold (as opposed to heat treatment) pasteurization by irradiation is called radurization (doses range 1–10 kGy). Higher doses are seldom used because of off-flavour development. Complete sterility is achieved at about 50 kGy.

The energy of radiations used for food purposes is unable to induce radioactivity in the product.

The term ionization refers to the ability of radiations to split off electrons from atoms creating unstable ions. Irradiation of water has two effects: it induces highly chemically reactive products and, in its liquid state, provides a good medium for free movement of reactants; the reactive free radicals initiate autocatalytic peroxidation which involves mainly fat; it produces breakdown radioisotopes products and random crosslinking between the chains of larger molecules.

The breakdown products differ little from those induced by conventional heat treatment: their yield parallels the absorbed dose; breakdown products are the basis of detection methods and induce alterations of the sensory properties of foods even at low concentrations which are far below the threshold of nutritional alterations; absorbed doses up to 35–40 kGy do not produce significant changes in amino acid or fatty acid profiles.

Although only modest destruction of amino acid occurs, the formation of free radicals is greater in proteins having a higher sulfur content, producing small radiolytic products (fatty acids, mercaptan and sulfur compounds).

Changes in secondary and tertiary structures depend on the dose: casein splits into small peptides at low doses but undergoes aggregation at higher doses, leading to changes in viscosity; globular proteins having a tight structure favour recombination reactions and are more resistant to changes. Structure alterations could modify functional properties of dairy proteins such as described for ovalbumin.

Oxygen plays an important role in lipid alteration by promoting their oxidation which is accelerated by irradiation. Changes in triglycerides are similar to fatty acids. Cleavages occur preferentially at bonds in the vicinity of carbonyl groups. No specific lipid breakdown products are induced by irradiation; however, the detection and quantification of food irradiation can be performed by determining the ratio between n-1 and n-2 hydrocarbons of their major lipids; this will be the subject of a European Standard. This method has recently been validated on cheeses by Hasselman and colleagues.

The depletion of vitamin activities parallels depletion through cooking or similar processes, that is, mainly loss of vitamins B1, B6; antioxidant vitamins (A, E, C) are more vulnerable to ionizing (peroxidative) radiations: in fats and milk vitamin A losses are high; vitamin E is easily oxidized by the products of the oxidation of unsaturated fats, mainly in the presence of O2 and during storage in air. The irradiation of foods generally produces little loss of vitamin D.

Animal populations have been fed sterilized diets (absorbed dose: 50 kGy) for years without side effects. Human trials have involved several hundred humans. Initial studies showed children had developed polyploidy; later works did not confirm these results.

The most efficient method to detect ionization of dairy products remains taste: low molecular weight radiolytic products are volatile and responsible for undesirable off odours; dairy products, rich in water and fat, display a poor tolerance to ionization: organoleptic alteration of milk occurs at 0.25 kGy, of milk powder at 0.3–0.5 kGy; the tolerance limit is below 3 kGy for most cheeses: Gammelort, 2.75–3.0 kGy; Saint-Paulin, 2–4 kGy, Cheddar, Munster #.1 kGy.

This dose does not significantly destroy surface moulds or enzyme activity, thus ripening of cheeses is not significantly altered. However, the tolerance can be improved by adding antioxidants, by modifying atmosphere packaging or by ionizing frozen products, these values are far below the safety limit of 10 kGy below which the WHO does not require toxicological studies.

**CONCLUSIONS**

Irradiation doses consistent with unaltered organoleptic properties are not susceptible to induce significant chemical or nutritional alterations in dairy products.
IMPACT OF UHT-TREATMENT ON NUTRITIONAL VALUE OF MILK PROTEINS

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ABSTRACT
Studies were conducted on model milks prepared in pilot plants for direct and indirect heating. In the heated milk samples furosine, hydroxymethylfurfural (HMF) and partially lysinoalanine (LAL), lactulose and N-carboxymethyllysine (CML) were determined. The results showed a clear relationship between the intensity of heat treatment in terms of heating time and temperature and the concentrations in the different markers. Indirect heating by plate heat-exchangers produced a greater degree of lysine damage than direct heating by steam injection. The possible explanation for this phenomenon is that during direct heating saturated steam is injected into the preheated milk, diluting the milk and thus reducing the concentrations of reactants susceptible to heat damage. Steam injection and steam condensation increase the temperature of the directly heated milk almost immediately, whereas this is not the case in the indirect system with heat-exchangers. Results of experiments with a miniature pilot plant characterized by extremely short heating and cooling phases supported this theory.

In addition to the model experiments, 200 commercial UHT milks were collected during milk control in 7 German states and analysed for furosine, HMF and LAL. Most of the commercial milks analysed so far contained much higher levels of the markers than were predicted from the model experiments described above. Also here the lowest values were found in samples prepared by the direct heating technique.

From the furosine values the losses in available lysine were calculated. They were between 0.7 and 6.1% of the initial lysine value (72% of the samples were between 1.7 and 3.5%). Considering the high absolute values of lysine in milk the damage to lysine is of only small nutritional significance. However, measurements of the used markers will be an aid to good manufacturing practice.

The impact of these results on the nutritional value of the milk proteins is discussed.

INTRODUCTION
Heating of milk results in chemical alterations of the proteins with some degradation of essential amino acids or formation of undesired flavour compounds. To evaluate the intensity of the heating process it is possible either to measure losses of nutrients or to analyse the concentrations of new substances whose formation depends on different heating conditions. Milk is a very sensitive material and soon after the introduction of the UHT-treatment to increase its keeping quality the question arose as to whether also the protein quality would be damaged even by this very short heating process. The first amino acid determinations and animal experiments showed no differences between UHT-treated and untreated milk (Lang et al., 1965; Hostettler, 1965). However, later several more sensitive indicators of distinct reactions were found, suited to measuring the technical impact on protein quality. An important review on this question was given by Burton (1984).

Milk proteins are very rich in the essential amino acid lysine, which has a free ε-amino group able to react with, for example lactose in the Maillard condensation. The first intermediate formed in this way, lactuloselysine, is degraded during acid hydrolysis of the protein, but can be estimated by analysing for furosine which is formed during hydrolysis with strong HCl.

MARKERS FOR HEATING DAMAGE & PROTEIN QUALITY
Since the detection of furosine as a stable indicator of the Amadori products like lactuloselysine in milk (Erbersdöbler & Zucher, 1966; Brüggemann & Erbersdöbler, 1968; Heyns et al., 1968; Finot et al., 1966) it has been used as an indicator of thermal damage in food science and medical biochemistry (see Erbersdöbler, 1986a). Recently its importance was enhanced by analytical improvements, starting with the proposal of Resmini et al. (1990). Additionally, the fact that now a pure and stable standard is commercially available has led to further analytical activities (for example Hartkopf & Erbersdöbler, 1993; Henle et al., 1995). Furosine has the disadvantage that it is formed from the Amadori products only at a rate of 30-40%. However, this recovery is reproducible if constant analytical conditions are applied.

For the determination of 5-hydroxymethylfurfural (HMF), which also results from the Maillard condensation (Keeney & Bassette, 1959), precursors of browning products in milk are transformed to HMF after addition of oxalic acid and following heating (Konietzko, 1981). Principally, the HMF value of a milk can be used as an indicator for the heating process, but data from literature offer a wide range for this value which leads to the conjecture that the HMF determination is insufficiently reproducible between laboratories. In particular, the level of HMF in untreated milk, measured during the determination of "total HMF" and subtracted from the levels in treated milks, is a source of variation (Burton, 1984). A comparison between the furosine and the HMF method demonstrated the usefulness of the HMF method as a rapid and simple measure of heat damage caused by the UHT process (Dehn-Müller, 1989).

Furosine does not increase linearly with increasing heat damage since the Amadori products are only intermediates which react to further compounds in the advanced and late Maillard reaction. Also the correlation of HMF values to heat damage is not linear while the third main parameter of heat damage - lactulose - is formed linearily from lactose during heating or storage (Geiler & Klostermayr, 1983; Andrews, 1989). However, the formation of lactulose changes, depending also on the secondary conditions of heat treatment, like dry matter content or pH.

A comparison between the three parameters from the results of a model experiment (Hewedy et al., 1994) is given in Figure 1. The comparison shows that lactulose exhibits the most linear response to heating while furosine has a curvilinear characteristic.

On the other hand, furosine has the big advantage that it is a direct marker for a real existing reaction product of lysine, which has nutritional relevance. It represents the main reaction product of the initial stage of Maillard condensation and in this way a sector of heat damage, which is most interesting for milk products and especially for the UHT process.

An additional indicator for more severely heated products may be N-carboxymethyllysine (CML) because it seems to be more stable than lactuloselysine (Büser & Erbersdöbler, 1986; Lüdemann & Erbersdöbler, 1990). However, in UHT milk CML was detectable in small amounts only in the more severely heated samples (Hartkopf et al., 1994).

In addition to the above mentioned and widely used markers, lysinoalanine (LAL) was determined in model experiments and in commercial UHT milks. LAL is formed throughout heat and/or alkali treatment of proteins by nucle-
Furosine, HMF, lactulose, CML and LAL exist, if at all, only in very small amounts in raw milk. They represent, therefore, a good means to characterize the reactions during the heating process. A direct measure of the losses of total or chemical reactive ("available") lysine is complicated by the uncertain original lysine content of milk which changes to a certain degree and is mostly unknown. On the other hand, losses of nutritional significance will be detected in any case. Renner (1983) found losses in available lysine (fluoro-dinitrobenzene method) of 2.5% and 4.6% in direct and indirect UHT-treated milk, respectively.

**DIRECT VERSUS INDIRECT HEATING TECHNIQUES**

Because of better recovery rates of energy, indirect working plants in UHT heating are meanwhile predominant. Of 190 commercial samples collected in 1985/86 from 45 different dairies in the western part of Germany only five came from plants working with direct heating. However, recently some dairies offered "premium quality UHT milks" which are produced by direct heating plants and which are of real high quality.

A comparison of the furosine values from model experiments on a pilot plant working either by direct or indirect heating is given in Figure 2. The data for this Figure (Dehn-Müller, 1989), of which until now only the part with direct heating has been published, are corrected according to the results with the meanwhile available pure furosine standard (Hartkopf & Eberhard, 1993).

The results of the determinations in the model milk samples show that there is a clear relationship between the

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**Figure 1:** Relative values of furosine, HMF and lactulose after UHT-heating in a pilot plant (calculated from: Hovestey et al., 1994).

ophophilic reaction of the lysyl--e-amino group with the activated double bond of dehydroalanine, which is formed by $\beta$-elimination of cystine and phosphoserine in the peptide chain. Unlike furosine, LAL crosslinking creates not only a decrease in lysine availability but also a reduction in protein digestibility.

Moreover, LAL is rather controversial as, in rats, it produces cytomegaly in certain regions of the kidneys. Although there is no direct evidence that this is also possible in man, there is common agreement in food technology that the production of greater amounts of LAL should be avoided.

**Figure 2:** Comparison of furosine values in milk heated directly or indirectly in a UHT pilot plant (recalculated from Dehn-Müller, 1989 and corrected according to the new furosine standard).
severity of heat treatment in terms of heating time and temperature, and the furosine values of the milks. Similar results were obtained with HMF in the directly heated samples. Generally the directly heated milk samples show lower furosine than the indirectly heated milks, as is also demonstrated in Figure 2.

In the 190 commercial UHT milks furosine ranged from 13 to 125 mg/L, while HMF values of 0-22 μmol/l (with subtraction of the blank value) and LAL values of 0.5-5.8 μg/m were obtained. About 70% of the milks contained furosine in the range 40-70 mg/L and HMF contents of 10-16 μmol/L. Higher values revealed excessive heating and confirm other findings that thermal processes are often too severe. The results are given in Figure 3.

The main explanation for the differences between direct and indirect heating is that during direct heating saturated steam is injected into the preheated milk, diluting the milk (Andrews 1989; Nangpal & Reuter, 1990) and thus reducing the concentrations of reactants susceptible to heat damage. In addition, it could be assumed that the conditions of heat transfer are different for direct and indirect heating. Steam injection and steam condensation increase the temperature of the directly heated milk almost immediately, whereas this is not the case in the indirect system with heat-exchangers, depending in this case also on the thickness of the layer to be heated.

In order to measure the diluting effect a model experiment with a pilot plant for milk heating characterized by extremely short heating and cooling phases was performed. With this pilot plant it was possible to measure the effects of an indirect heating system that exhibits almost the same temperature-time profile as direct heating (Kiesner et al., 1991). The experimental design and the results of this experiment are given in detail elsewhere (Hewedy et al., 1994).

Figure 4 shows a comparison with the corresponding results of the older studies mentioned above. The results obtained in this special pilot plant are generally higher than the values for the directly heated milk samples, which leads to the assumption that the diluting effect is of large influence. On the other hand, corresponding values of the previous experiments with indirect heating (see Figure 2) are in the same region (although a real comparison is difficult because of the uncertain definition of the effective holding periods for the heat). This stresses the assumption that it is mainly the diluting effect which provides the advantage of the direct heating.

**NUTRITIONAL RELEVANCE OF THE RESULTS**

The losses in available lysine were calculated from the furosine values in the commercial samples. They were between 0.7 and 6.1% of the initial lysine value (72% of the samples were between 1.7 and 3.3%). Considering the high absolute values of lysine in milk the damage to lysine is of only small nutritional significance.

This is also demonstrated by experiments with growing rats, as shown in Figure 5. Rats receiving a diet with a

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*Figure 3: Variance of furosine, HMF or lysinoalanine (LAL) in 190 samples of commercial UHT milk (recalculated from Dehn-Müller et al., 1991; furosine values corrected according to the new furosine standard).*

*Figure 4: Furosine contents in milk treated in a new experimental pilot plant compared with values in milk treated in a conventional pilot plant using direct or indirect heating, operating at 148 versus 142°C, respectively (modified from Hewedy et al., 1994).*
severely heat damaged dried skim milk containing only 2.2% reactive ("available") lysine instead of about 8.5% (flouro-dinitro-benzen method) did not grow sufficiently. A supplementation of lysine restored the protein quality almost to the level of the sample of good quality (Erbersdobler, 1986b). Similar results were obtained in experiments with mildly heated casein-glucose mixtures. The results shown in Figure 5 demonstrate also that the lysine-sugar complexes leading to the decrease in the chemically determined "available" lysine are not or at least very poorly available as sources of lysine. Additionally, it can be seen from the experiments that there was no predominant damage to other essential amino acids nor to protein digestibility. Only cystine exhibited certain losses which is also known from UHT processing, and which is — compared to the scorched dried skim milk of Figure 5 — a milk technology.

It is also known from other similar experiments that this selective lysine damage by derivatization to lactulose-lysine does not impair protein digestibility to a greater extent. It appears that mainly a higher incidence of crosslinking as found in severely heated proteins will negatively influence the overall protein digestion (Erbersdobler, 1989). The small amounts of lysinoalanine, the main marker for protein crosslinking, in the commercial UHT milks show that there is no reason for a negative influence on protein digestibility. This would also be valid for special peptide sequences like the phosphopeptides in casein, as was shown in our own experiments (Meisel et al., 1991). Also, lysinoalanine which is somewhat controversially discussed (see above), appears to be not a problem. Even the highest values found in the commercial milk samples were below the strict regulations in Germany for high quality baby food (Bundesgesundheitsamt, 1986). On the other hand, since its safety is not yet settled, LAL concentration in UHT milk should be low (<2.5 mg/l).

CONCLUSIONS

The results of the present project show that we now have precise indicators of heat damage in UHT milk, like furosine, lactulose, and for screening purposes also HMF. The carboxymethyllysine (CML) or lysinoalanine (LAL) values, on the other hand, appear to be too low for precise testing but can be useful means in cases of overprocessing or incorrect alkali treatment. The losses in available lysine were calculated on the basis of the furosine levels. Supposing an appropriate UHT milk production (furosine values <70 mg/l), the heat-induced lysine losses of 0.4-3.3% of the initial content are of inferior nutritional significance for the consumer considering the high lysine values in raw milk. However, the data may be an aid to good manufacturing practice and could assist in the improvement of processing equipment and in the study of the effect of new processing techniques in general. Although no exact sensorial tests were performed, it was noticeable that commercial milk samples with very low furosine values (direct heating) tasted better, with almost no typical UHT small.

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LITERATURE

Influence of Processing on the Nutritional Value of Milk Proteins

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Abstract

Milk protein comprises various proteins, including casein and whey proteins. Casein, the dominant protein in bovine milk (representing about 80% of the protein), is a very milk-specific protein. The amount of essential amino acids in milk protein is very close to the expected amino acid pattern required by humans. In addition, casein functions both as a protein source and as a source of calcium and phosphorus. Processes utilized for the preparation of dairy products include physical treatments (heating, drying, homogenization), chemical treatments (acid or alkaline solutions, oxidizing agents, solvents or additives) or biological treatments (enzymatic hydrolysis, fermentation). The modifications of milk proteins are classified as physical denaturation, modification or destruction of amino acids, protein-protein interaction or protein and other components interaction (sugar, lipid, additives). According to the modifications, either positive or negative effects can be observed at different levels, including digestion, absorption, excretion, metabolism or retention of the nutrients. The positive effects are usually an increase in amino acid bioavailability. Processing damage to proteins leads to loss of amino acid availability or the formation during processing of anti-nutritive or even toxic substances.

Introduction

Native milk is a complex mixture of macro- and micro-nutrients, the role of which is to provide an equilibrated source of energy, nitrogen and indispensable components, that is, indispensable amino acids, essential fatty acids, vitamins and minerals. Milk protein comprises various proteins, including casein and a whey fraction. Casein, the dominant protein in bovine milk, representing about 80% of the protein, is a very milk-specific protein. It has a high proline content, a high ester-bound phosphate content and a low content of sulfur amino acids, especially cysteine. When compared to whey protein, casein presents a high content of pro-


line and arginine and a low content of cystine, isoleucine, leucine, lysine and threonine. The cystine content of milk is almost exclusively derived from whey protein. The amount of essential amino acids in milk protein is very close to the expected amino acid pattern required by humans though these requirements are still not precisely determined, especially for the adult. In addition, casein functions both as a protein source and as a source of calcium and phosphorus. Due to this high nutritional quality, milk can be used as a source of protein in the human diet, especially in mixed diets to supplement nutritionally other protein sources of inferior quality. Different processes are utilized for the preparation of dairy products, including physical treatments (heating, drying, homogenization), chemical treatments (acid or alkaline solution, oxidizing agents, solvents or additives) or biological treatments (enzymatic hydrolysis, fermentation). Modifications in milk proteins can occur during these treatments since proteins are the most reactive components in milk products. The different types of modifications of milk proteins are classified as physical denaturation, modification or destruction of amino acids, protein-protein interaction or protein and other components interaction (sugar, lipid, additives). These modifications lead to changes in the nutritional properties of milk proteins. According to the modifications either positive or negative effects can be observed at different levels, including digestion, absorption, excretion, metabolism or retention of the nutrients. The positive effects are usually an increase in amino acid bioavailability. Processing damage to proteins leads to a loss in amino acid availability or the formation during processing of anti-nutritive or even toxic substances.

The different treatments, that is, physical, chemical or biological action, produce a series of physico-chemical and biochemical modifications. The incidence of the different types of reaction depends both on the treatment and on the characteristics of the protein. The more frequently occurring modifications include protein denaturation and hydrolysis, modification (racemization, modification, destruction) and interaction between amino acid residues, interaction between protein and sugar, interaction between protein and lipids or interaction between protein and additives (chlorinated-solvents, oxidizing agents, sulfites, nitrates). Each modification can be produced by different treatments.

MODIFICATIONS OF MILK PROTEINS AND AMINO ACID RESIDUES

These modifications originate from reactions in which the sole food constituent involved is protein.

The first type of reactions are protein denaturation and hydrolysis, which can occur during different treatments, usually modify the digestibility of milk proteins, but do not alter the amino acid composition. Protein denaturation usually occurs during moderate heat treatment. This denaturation usually improves the digestibility of proteins which are more easily attacked by the proteolytic enzymes and is especially important for whey proteins. When the denaturation is too prominent it can produce a reduction in protein digestibility and bioavailability. Protein hydrolysis can occur during enzymatic, acid or alkaline treatment, or during milk fermentation. They usually increase protein digestibility. Heat treatment has also been demonstrated to hydrolyse casein partially, especially seryl and phosphoseryl residues. Denaturation and hydrolysis of milk allergens can either reduce or modify the allergenicity of the proteins.

The second type of reactions involves modification and interaction between amino acid residues:

- Racemization occurs mainly in alkaline solution but also during other reactions. D-amino acids are considered to be poorly utilized for maintaining nitrogen balance in man. Alkaline conditions are very effective in promoting racemization of all the amino acids in milk whereas heat treatment mainly produces racemization of aspartic acid. According to different studies, aspartic acid appears as the most sensitive amino acid to racemization. This effect could reduce protein digestibility. Though racemization does not represent a major problem in milk treatment, it can be significant when proteins are in the presence of alkaline solutions.

- Other modifications involve more specific amino acid residues. Different modifications of amino acid residues can occur since the stability of the amino acid side chains are different according to their structure. Aliphatic amino acids (alanine, valine, leucine, isoleucine) do not present reactive side chains. The most reactive amino acid residues in milk proteins include lysine, sulfur-containing amino acids (cysteine, cystine, methionine), tryptophan, threonine, glutamine, asparagine and phosphoserine. Tryptophan is destroyed during heat treatment. Both tryptophan and glutamic acid are able to form mutagen derivatives. Heat or alkaline treatment converts arginine to citrulline and ornithine. Glutamine and asparagine are desamidated, cysteine and cystine are desulfured with formation of dehydroalanine, phosphothreonine and phosphoserine are dephosphorylated with the formation of dehydroalanine or methyldehydroalanine.

- Modified amino acids originating from these reactions are often reactive and can lead to the formation of several types of intra- or interchain cross-links, including lysinoalanine, lantihione, ornithinoalanine and the isopeptides. Deshydroalanine and lysine are highly involved in these protein-protein interactions. Lysinoalanine originates from the reaction between dehydroalanine and lysine, lantihione from dehydroalanine and cysteine, ornithinoalanine from dehydroalanine and ornithine. These reactions can reduce protein digestibility and bioavailability. The physiological and toxicological significance of these products has also to be considered. In addition, during severe heat treatments lysine reacts with deamidated glutamyl or asparagyl residues to form isopeptides glutamyl-lysine and asparagyl-lysine.

INTERACTIONS BETWEEN MILK PROTEINS AND OTHER COMPONENTS

These reactions mainly involve the interactions between proteins and sugar, lipids or additives (oxidizing agents, chlorinated solvents, sulfites, nitrates).

The Maillard reaction, the reaction of proteins with reducing sugars, is an important source of flavour and aromas but is also a major reaction that brings about nutritional damage to food proteins during processing, especially heat treatment, and storage. This reaction is especially important in milk with a high content of both protein and reducing sugars (lactose). The first step of the Maillard reaction is condensation between the carbonyl group of the reducing sugar and the amino group of lysine to the desoxy-ketose compound, or Amadori rearrangement. The major consequence of this initial reaction is a loss in lysine bioavailability. With more severe heating conditions, the reaction further proceeds to different advanced steps, which produce a reduction in protein digestibility, and various com-
Compounds are formed with potential physiological and toxicological effects that remain to be determined.

Oxidizing conditions and agents are responsible for two major areas of damage in milk and other proteins. The first is direct oxidation of methionine, cysteine, cystine and tryptophan. The second reaction originates from the interaction between oxidized unsaturated lipids and proteins. Methionine is oxidized to methionine sulfoxide and methionine sulfone. Cyst(e)ine oxidized to cysteine monosulfoxide, cysteine monosulfone, cysteine sulfonic acid and cystine sulfonic acid. The less oxidized products of methionine and cyst(e)ine are partly utilized whereas the more oxidized ones are unavailable. Several oxidation products of tryptophan have been described, including N-formyl-kyurenine, oxindolalanine, beta-carbolin. The oxidation of unsaturated lipids leads to the initial formation of hydroperoxides and proceeds further to secondary degradation products, including aldehydes, ketones, hydrocarbons, bi- and tri-functional compounds. These secondary products react with other food constituents, especially proteins, to form stable compounds. The bi-functional products may cause cross-linking of proteins. Methionine, lysine and tryptophan are especially involved in these reactions that cause a reduction in the nutritional value of the protein.

Other reactions are caused by the presence of chlorinated solvents, sulfites or nitrates in foods. Chlorinated solvents mainly react with sulfur-containing amino acids. Cysteine reacts with trichloroethylene and trichlorethene to form a toxic product S-(dichlorovinyl)cysteine. Chlorinated derivatives of tryptophan with mutagen activity are also described. Sulfites react with cystine to form sulfonated derivatives. An important toxicological aspect is the addition or presence of nitrates, nitrites and nitrosamines in foods. Nitrosamines are produced by a reaction between secondary amines and nitrite and the majority of them have been found to be carcinogenic. According to different studies, nitrosamines from feeding-stuffs are not recovered in milk. A very small amount of nitrosamine was detected in skim milk powder and none in fermented milk.

CONCLUSION

Protein appears as the most reactive component in milk products. The reactions are especially due to interactions between proteins and other food components. The detrimental physiological effects of protein damage may be overlooked: reduction of protein digestibility, reduction of amino acid bioavailability or appearance of antinutritive or even toxic substances. Detrimental effects on proteins, however, not the only consequences of milk processing, numerous have nutritional or even beneficial nutritional consequences.

TECHNOLOGICAL TREATMENT OF INFANT FORMULA: PROCESSING AND MINERAL CHANGES

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SUMMARY

Minerals are found to be less bioavailable from infant milk formulas than from human milk [1]. Several parameters, related or not related to foods, can affect the absorption and utilization of minerals. It is well known that physiological factors, the amount of minerals ingested and the presence of certain food constituents affect mineral bioavailability [2]. The various processes to which foods are subjected and which may affect the form of minerals and their absorption, has been much less documented.

Peptic and pancreatic hydrolysis of human milk generates peptides that can stimulate duodenal absorption of Ca and its transport into circulation [3]. It is probable that peptides issued from the digestion of infant milk formulas are different and can also be affected by heat treatments or other treatments during processing.

The objective of our research was to determine whether and to what extent each step, during the manufacture of infant milk formulas, affected soluble and lipid-bound Ca and Zn. Samples were withdrawn during the manufacture of an infant milk formula. Soluble and lipid-bound Ca and Zn were estimated after centrifugal separation of in vitro digested samples.

Pasteurization did not significantly reduce solubility of Ca and Zn. Blends forewarmed at low temperature contained a smaller proportion of soluble Ca than skim milk, but Zn was not affected. Forewarning at high temperature reduced solubility of Ca. The solubility of Ca and Zn was lower in sterilized and spray-cried formulas than in blends forewarned at low temperature. Though initial Ca and Zn levels were greater in infant formulas than in human milk, percentages of soluble Ca and Zn were not higher.

Although formulas were supplemented with Ca and Zn at levels higher than those found in human milk, the solubility of Ca and Zn after in vitro digestion was similar in infant milk formulas and human milk. These results suggest research avenues which should help identify factors affecting bioavailability of minerals and better understanding of nutrient interactions.

Literature


It is well recognized that breastfeeding provides the best diet for infants until 4-6 months of life [1]. However, there are circumstances in which full and complete breastfeeding cannot be realized [1-4] and there are also mothers who do not choose to breast-feed [1]. Under such circumstances, the products of the industry have proven essential as substitutes for potentially dangerous preparations that would otherwise be used [1].

The composition of modern infant formulas is oriented on the "golden standard" human milk [5] and are the most highly regulated and controlled of all commercially available foods [6, 7]. Very high demands are put on formulas which remain a major part of the infant's diet up to an age of 12-15 months, which represents a period of rapid growth and development [8]. Milk proteins may be preheated and deaminolized. Ingredients (fats, vitamins, minerals, lactose), approximating human milk composition, are first blended at warm temperatures. This is followed by forewarming the blend at high temperature, adding emulsifiers, stabilizers and heat-stable vitamins, homogenization, and sterilization or spray-drying [9].

Those various processes to which infant milk formulas are subjected may affect the form of minerals and influence their absorption. The bioavailability of some minerals may be reduced by becoming less soluble upon heat treatments [10]. Heating of milk proteins results in an increase of reactivity of specific groups due to the unfolding of
peptide chains. Such protein molecules interact, forming linkages between ionic groups of the proteins, hydrophobic bonds, hydrogen bonds, and disulfide linkages.

Some authors have examined the effects of processing on protein quality in infant milk formulas [11, 12] but few studies have been conducted to evaluate the effect of processing on mineral availability in infant milk formulas and how it compares to human milk. Williamson et al. [13] reported no significant difference in retention of nitrogen, calcium, phosphorus, and sodium between pasteurized and raw human milk. However, all preterm infants gained weight more rapidly when fed raw human milk.

Weeks & King [14] studied the bioavailability to rats of Ca in milk subjected to ultrahigh temperature (UHT) pasteurization compared with the bioavailability of the mineral in raw, high temperature-short time (HTST) pasteurized and stored UHT milk. The intestinal absorption data indicated no significant difference between raw, HTST, UHT and stored UHT milk. The findings were confirmed by fecal yield, which is an index of gastrointestinal absorption. However, Egan & Rennie [15] found that UHT treatment of cow milk, and during the manufacture of dried milk granules for infant milk formulas resulted in a 20% decrease in the absorption of calcium across rat jejunum. Micelles prepared from processed cow milk in infant milk formulas showed an identical rate of calcium absorption to that measured from human milk micelles.

The interaction in milk of casein with simple ions, such as calcium, is complicated by the presence of components in the milk which can compete, under certain conditions, with the calcium ions for the same binding centers on the casein [16]. Heat-induced changes in the original milk alter either the calcium ion concentration or the binding ability of the casein for calcium or both. For Ca in milk, the extent of precipitation to a colloidal form was found to be proportional to the severity of heat treatments between 4 and 90°C [17]. As compared to infant milk formulas, casein in cow milk has not been heat-denatured and may therefore bind iron [18] and copper [19] with a higher affinity. The Malliard reaction which occurs by heat treatment of protein combined with carbohydrate has an inhibitory effect on zinc retention [20].

Nadeau & Clydesdale [21] reported increased calcium, iron and zinc solubility with increasing fat content of processed whole milk in a milk-wheat system and increased solubility due to homogenization. Increased denatured protein surface area following homogenization, as a function of system milkfat content and associated oil-water interfacial area, may be a factor in mineral sequestration. This process may competitively inhibit formation of less soluble mineral complexes with other system components exhibiting cation-binding potential [22].

Storage stability was measured for ready-to-feed milk-based infant formula products prepared with high-heat or low-heat nonfat dry milk or condensed skim milk and processed under commercial conditions with or without UHTST treatment. After 6 months storage at ambient temperature, slight sediment was in all products. Storage-induced sediments were composed, in part, of calcium, phosphorus and nitrogen. It is probable that some solubilization of colloidal calcium occurred during storage of these samples [23].

Calcium and zinc in human milk are mainly bound to whey protein and to low-molecular-weight compounds, whereas in cow milk they are bound mainly to casein. Significant proportions of calcium and zinc are in the fat fraction of human milk. Almost none of either is found in the fat fraction of cow milk [24]. The amount present in the fat portion of infant formulas is unknown, and the significance of lipid-binding on the bioavailability of calcium and zinc has not been established.

The objective of our study [25] was to determine, by an in vitro digestion method, whether and to what extent each step, during the manufacture of infant milk formulas, affected soluble and lipid-bound calcium and zinc. Specific objectives were to determine the proportions of soluble and lipid-bound calcium and zinc throughout the manufacture of powdered and liquid infant milk formulas, and to compare the proportions of soluble and lipid-bound calcium and zinc in various milk samples to those in human milk.

In brief, samples were withdrawn during the manufacture of powdered and liquid infant milk formulas to determine steps which may affect soluble and lipid-bound calcium and zinc. The solubility of calcium and zinc was estimated as the ratio of soluble calcium and zinc in the supernatant after centrifugal separation of samples subjected to in vitro peptin, pancreatin, and lipase digestion, to the total content of calcium and zinc in milk or formula. Those results were compared to human milk in which the solubility was estimated by the same technique.

No reduction in calcium solubility occurred due to pasteurization of skim milk used for production of either liquid or powder formulas. After adding various ingredients making the blend and forewarming at low temperature, the solubility of calcium was lower than in skim milk. The ingredients added (demineralized whey protein, oils, lactose, vitamins, minerals, emulsifiers, stabilizers) may have influenced the solubility of calcium in specific ways. Corneau et al. [26] reported that the chemical form of calcium added to a formulation influenced calcium solubility.

The solubility of calcium decreased upon subsequent forewarming at high temperature but did not decrease further after the final sterilization or spray-drying. This was probably caused by calcium and phosphate transfer to the colloidal phase which reduced calcium solubility. Though human milk received no heat treatment, calcium solubility was not different from sterilized or spray-dried infant milk formulas and was lower than in cow milk. Heating is not the only factor involved in calcium solubility. Milk constituents have an important effect. In our study, 37.5% of calcium in infant milk formulas was provided by calcium salts in the forms of CaCl₂ and calcium citrate in the sterilized formula and calcium hydroxide and CaCl₂ in the spray-dried formula. However, those salts have a low solubility in order to keep protein stability upon heating [27].

The fraction of calcium bound to lipids was not different in the blend forewarmed at low temperature, at high temperature, or in sterilized or spray-dried infant milk formulas. Infant milk formulas do not contain notable amounts of milkfat globule membranes. Fats are added in the form of oils to provide a fat composition closer to the fatty acid composition of human milk [28]. From our results in the preparation of infant milk formulas, where oils were used, the distribution of calcium in the fat fraction of liquid and spray-dried infant milk formulas was not significantly higher than in human milk.

The markedly lower calcium solubility of infant milk formulas compared to skim milk is noteworthy. According to Ashmead et al. [29], some unsaturated fatty acids can form calcium salts which are poorly absorbed. Thus, the absorption of calcium can be depressed in the presence of high levels of certain fats. Reykda & Lee [22] reported high calcium solubility when milk was concentrated to whole milk. They attributed this effect to the formation of calcium soaps and to interactions with digestion products.
According to Irving [30], calcium soaps may be solubilized by the bile in the intestine, thus facilitating calcium absorption. Therefore, the significance of lipids on the bioavailability of calcium to infants cannot be predicted.

Pasteurization of skim milk used for production of both liquid and powdered infant formulas did not affect zinc solubility. After adding various ingredients making the blend and forewarming at low temperature, the percentage of soluble zinc was not lower than in the skim milk used for the formulation of infant milk formulas. The percentage of soluble zinc decreased by 33-50% in liquid infant formulas upon subsequent forewarming at high temperature. Final sterilization or spray-drying did not reduce zinc solubility as compared to infant formulas forewarmed at high temperature. However, zinc solubility in the spray-dried formula was lower than for the blend forewarmed at low temperature. Although zinc concentration was almost three times higher in sterilized or spray-dried infant milk formulas than in human milk, the percentage of soluble zinc was not different from human milk.

A severe heat treatment, such as forewarming at high temperature, seemed to lower zinc solubility. Protein solubility decreases upon severe heating. Probably zinc, attached mainly to casein in cow milk, would also become less soluble after forewarming at high temperature. The major factor affecting zinc solubility in infant milk compared to human milk, would again be the fraction to which it is bound. Also, not only is the casein content different between human milk and cow milk, but so is the composition of casein. This can affect its ability to bind metals [31]. The amount of calcium and iron, and the presence of zinc-binding compounds in milk, such as casein and citrate, can affect zinc absorption [32].

Notable amounts of zinc were found in the lipid fraction at all steps in liquid and powder infant milk formulas. Forewarming the blend at high temperature increased the percentage of lipid-bound zinc for liquid and powder infant milk formulas as compared to infant formulas forewarmed at low temperature. The percentage of zinc in the fat fraction was not different between sterilized or spray-dried formulas and human milk. Cunnane [33] proposed that the higher amount of essential fatty acids in human milk (compared to cow milk) could partly explain the greater zinc absorption by infants fed human milk, by affecting the intestinal brush-border lipid composition, thereby altering permeability of the membrane. According to Singh et al. [34], the lower bioavailability of zinc from cow milk may be caused by high casein content, since bovine casein is poorly digested by infants. Further disturbances of zinc absorption could also be caused by phosopholipides formed by the action of trypsin and chymotrypsin which retain their high zinc-binding capacity. The apparent higher absorption from formula, compared to cow milk, may be due to the adjustment in protein ratio, which is approximately one-half that of cow milk [35]. However, it would most likely be due to the fatty acid profile differences since infant milk formulas are made to resemble, as closely as possible, the fatty acid profile of human milk.

The solubility of minerals may only be one predictive factor of their potential bioavailability. There are physiological factors promoting mineral absorption which cannot be reproduced in vitro. Amounts of calcium and zinc higher than normally present in human milk were purposely added to infant formulas to compensate for the hypocalcemic effect of phosphate load in cow milk-derived formulas [36]. More research is needed to better understand how various processes affect mineral bioavailability and mineral interactions in infant milk formulas.

**LITERATURE**

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EFFECTS OF MICROWAVE HEAT TREATMENT ON MILK

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In the last 5-6 years it was postulated that microwave heat treatment, especially of milk, can be a possible serious public health problem. Results of experiments on amino acids in milk formula, heated by microwave, showed the formation of D-amino acids compared to conventionally heated samples [1], and changes in the blood of experimental subjects who had consumed microwave-treated foods have been found [2].

PRINCIPLES OF MICROWAVE ENERGY

The microwave oven first appeared in 1946 [3]. In recent years, the popularity of the microwave oven has increased and found wide applications in the household. Most consumers use it for reheating meals or warming cooked and chilled food systems. Compared to conventional electric oven heating, the primary advantage of microwave heating is a large saving in time. In the food industry, the microwave is applied for thawing and temperature control, cooking, drying, pasteurization and, less successfully for sterilization [4].

Microwaves are part of the electromagnetic spectrum. They comprise wavelengths in the range of millimeters and decimeters and frequency in the range 300 MHz-300 GHz. Lower frequencies are in the range of radio wavelengths while higher frequencies correspond to heat radiation (infrared) and light (visible, ultraviolet). Frequencies in the microwave range are used in communications (television broadcasting, radar) and in medicine (microwave therapy). In medical usage, heat is generated in deep-seated human tissues. A frequency of 2450 MHz (wavelength of 12 cm) was reserved for the industrial and medicinal use of microwaves (including household equipment). Microwaves are non-ionizing forms of radiation.

Microwaves are generated in a microwave oven by a magnetron, which is supplied with direct current that originates on an electrode with usual mains voltage of 220 V and 60 Hz and is converted to high-frequency microwave energy. This energy is sent as oscillating waves over an antenna to the wave guide and enters the oven cavity. Microwaves show the following properties:

- they are reflected by electrical conductors (metals, for example steel, aluminium or copper);
- they penetrate electrical isolators (glass, porcelain, ceramics, stoneware, plastics; the view window in the door is equipped with a metallic grating to prevent an escape of microwaves);
- they penetrate substances of low electrical conductivity such as foods and are absorbed by these, generating heat. Production of heat arises from two mechanisms: ionic polarization and dipole rotation. The ionic polarization, that is, the ions in a solution responding to an electric field, is less important for the heating of foods than dipole rotation which depends on the existence of polar molecules in foods in the form of water. When an electrical field changes, the polarity also varies, which happens permanently with high frequency microwaves; the dipole molecules then try to align with the changing field and this rotation of the molecules generates heat in foods.

TEMPERATURE COURSE DURING MICROWAVE HEATING OF MILK

Microwaves are reflected by the oven sides and floor. Because of the penetration power of microwaves and the differential heating behaviour of food components, it must be expected that the temperature in microwave-treated foods depends on the size and shape of these foods. Large nonuniformity of temperature has been found in microwave-treated refrigerated and frozen foods. Experiments were performed with milk in different vessels in a microwave oven equipped with three temperature sensors located inside the vessel under the surface, in the centre and at the bottom of the milk [5]. A temperature of 78°C was intended in the centre of the vessels. The geometry of the vessel had a great influence on the temperature distribution inside the different vessels. Boiling occurred at the surface before the temperature in the centre reached the intended 78°C and at this time the temperature at the bottom and in the centre of a 900 ml vessel reached nearly 50°C. Microwave heating of milk required more time than water, for which the presence of milk minerals may be responsible, analogous to mashed potatoes with different ion concentrations.
Also, an uneven temperature profile was found in 120- and 240-ml nursing bottles following microwave heating of infant formulae for 40 or 60 s, regardless of the bottle type used [6]. The temperature at the top was significantly higher than at the bottom, middle or side of all bottles, with all coloured plastic bottles being significantly hotter than either glass or clear plastic.

HAZARDS OF UNEVEN TEMPERATURE DISTRIBUTION IN MICROWAVE-TREATED INFANT FORMULAE

There are several reports that warming of infant formulae in microwave ovens is hazardous or sometimes dangerous for infants. Up to now, three reports on the hazards of the warming of infant formulae have been published. In one case scald burn injury to a 1-week-old infant related to improper use of a microwave oven was reported. The formula was heated for 1 min under the highest setting [7]. In two other cases fatal burns on the oropharynx and palatal lesion were reported in healthy 3- and 4-month-old infants, respectively, who were fed with microwave-heated formula [8, 9].

INFLUENCE OF MICROWAVE HEATING ON MICRO-ORGANISMS IN MILK

Many studies have been undertaken on microwave heating of milk, in particular: to investigate pasteurization of milk using microwave ovens, to examine the possibility of extending the average shelf life of pasteurized milk, to use microwave energy to inactivate different pathogens in milk, to assess the influence on the milk nutrients or the uneven temperature distribution due to the microwave treatment. Publications differ in the following parameters: type of container, volume, initial temperature and time of microwave exposure, power of the oven and the parameters used. Parameters such as manipulation of heated milk, minimum temperature after heating and composition of the milk must also be taken into account.

Microwave heat treatment of milk is effective in reducing bacterial counts. Microwave heat treatment of milk for 2.5 min killed 97.7% of bacteria [10]. After storage for 14 days at 8°C, the total colony count had doubled and reached more than 10^6 cfu/ml. Hamid et al. [11] found a killing rate of > 99.9% after microwave treatment of milk for 12 s or 65 s. In microwaved goats' milk there was on average a 5-log reduction of the bacterial contamination of 10^6 organisms per ml [12].

Experiments to inactivate Yersinia enterocolitica, Campylobacter jejuni and Listeria monocytogenes in milk by microwave energy have been reported. Sterilized whole milk was inoculated with 10^6-10^7 cells of these bacteria/ml. Milk samples (20 ml) in glass vials were placed in water and heated for 1–20 (Y. enterocolitica, C. jejuni) or 60 min (L. monocytogenes) with a microwave oven set at 71.1°C. Y. enterocolitica was inactivated completely after 8 min of heating and C. jejuni after 3 min [13]. Heating for 10 min inactivated all L. monocytogenes cells in milk [14]. Cold enrichment tests (after storage at 4°C for 15 days and then incubated at the same temperature for 2 months) for Y. enterocolitica and L. monocytogenes were negative for up to 8 weeks of incubation [13, 14].

EFFECT OF MICROWAVE HEATING ON MILK NUTRIENTS

Heat treatment of milk influences some nutrients. The following discussion is confined to the effects of microwave heat treatment on some anti-infective factors, vitamins, other nutrients and flavour in human and cows' milk and especially to the question of the formation of D-amino acid in microwaved milk.

Anti-infective factors in human milk

The usual pasteurization or ultra-high temperature heat treatment of cows' milk inactivates more or less or destroys different milk enzymes and immunoglobulins. Human milk is normally not heatable, except for high-risk premature infants. To provide a continuous human milk supply, many intensive-care nurseries freeze fresh human milk for proper storage. These infants need human milk because they are very susceptible to infection and have a relatively immature digestive system. For more rapid accessibility, frozen human milk is thawed using microwave ovens. The influence of this treatment on anti-infective factors has been studied by Quan et al. [15]. Aliquots (2 ml) of human milk were thawed at room temperature and these samples served as controls. Two other samples were exposed in glass test tubes in the microwave oven for 30 s at a low or high power setting. In the first case the mean temperature was 33.5°C (range 20–53°C) and in the second 90.5°C (range 72–98°C). The microwave-treated samples contained significantly lower lysozyme activity and IgA directed against E. coli O antigen 06 than the controls. Total IgA and IgA directed against E. coli O antigen group 01 and 04 were affected only by the high microwave treatment.

Sigman et al. [16] also analysed frozen human milk (30 ml), from 20 lactating mothers, treated in a microwave oven for 50 s at 70 watts, and compared it with 30 ml aliquots of frozen milk placed in a refrigerator (10°C) overnight for 16–18 h, defrosted under running water (44–49°C) until an endpoint temperature of 37°C was obtained, or placed in a water bath (62.5°C) for 30 min (= pasteurization). Microwave-treated human milk showed percentage losses of IgA of 30.5 ± 32.6% compared to 18.2 ± 21.3% in pasteurized milk, whereas the two other milks were not significantly different from the control.

Vitamins

The influence of microwave heating on vitamins in milk has been studied several times.

Vitamin A: Demel et al. [10] found no loss of vitamin A and 8-carotene in microwave-heated milk. Medrano et al. [17] were in agreement in the case of whole milk; however, a small loss was observed in low-fat milk. In the same order of magnitude a loss of vitamin A was detected in milk (1.5 or 3.5% milkfat) microwaved for 4.5 min and heated to 80–90°C on a hotplate [18] and in pasteurized and UHT microwave-heated treated milk [19]. However, losses of vitamin A of 27% in raw microwave-heated-treated milk have been established by the latter authors.

Vitamin E: A loss of approximately 17% for vitamin E was found in microwave-treated pasteurized milk by Demel et al. [10] and in low-fat milk by Medrano et al. [17]. These latter authors found only a small loss in whole milk.

Vitamin B12: After the report of Vidal-Valverde & Redondo [20] it seems that thiamin is a special case. They found that the content of vitamin B12 was significantly reduced in microwave-treated UHT whole, low-fat and skim milk samples subjected to the two treatments of 2 and 4 min. Compared to the control, the loss of more than 50% occurred on treatment for 4 min. These high losses of vitamin B12 contrast with other results on microwave-heated-treated milk and with most data for pasteurized and UHT milk in which practically no loss of vitamin B12 occurred [21]. In an earlier study we found no losses of vitamin B12 in the upper and lower parts of microwave-treated milk and in stirred or unstirred milk [23]. Also Demel et al. [10] found only a loss of 4%. In a recently completed work on the influence of microwave heat treatment on different vitamins of milk we did not find any losses of thiamin and could not confirm the data of Vidal-Valverde & Redondo [20].
Vitamin B\textsubscript{2}. After the same groups the riboflavin content of milk was practically not modified after microwave heat treatment. Also no significant reduction in vitamin B\textsubscript{2} has been established following microwave heating of infant formulae in 120- or 240-ml bottles [6].

Folic acid. The folic acid content of microwave-treated milk was reduced compared to raw milk, but was only slightly lower than in pasteurized milk [2].

Vitamin B\textsubscript{17}. Steiner et al. [22] heat-treated milk and cheese by microwave and conventional methods and found lower vitamin B\textsubscript{17} losses in the microwaved foods than in the conventionally heat-treated foods.

Vitamin C. Domel et al. [10] found a loss of 36% for vitamin C in microwaved pasteurized milk. We also found some losses of vitamin C in the upper and lower parts of microwave-treated milk and in stirred or unstrirred milk [23]. However, no significant reduction in vitamin C has been established following microwave heating of infant formulae in 120- or 240-ml bottles.

Formation of D-amino acids in microwave-heated milk

In 1989, Lubec et al. [1] stated that milk formulae heated in a microwave oven for 10 min contained cis-3-hydroxyproline, cis-4-hydroxyproline at concentrations of 1–2 mg/ml milk, and also D-proline. According to the authors the latter amino acid should be nephrotoxic, neurotoxic and hepatotoxic. In this letter neither the microwave power used nor the final temperature were indicated, neither an intensive data presentation nor an exact instruction on the experimental realization. There was only a description of the conventional heating which was performed in a water bath at 80°C. Later, the precise conditions used by these authors were indicated as the following: The samples were heated under pressure at 174–178°C. It is not surprising that fundamental chemical changes in a food were caused by such drastic conditions, far removed from kitchen practice. Lubec stated in a letter to the German Federal Health Office that the data contained in the letter to "The Lancet" should not cause concern. The presence of D-amino acids in foods has been reviewed by Zagon et al. [24].

Data for the influence of microwave heating on the isomerization and racemization of amino acids have been published in recent years. Aqueous solutions of L-proline, L-lalanine or L-glutamic acid were heated for 30 min in a microwave oven (mean temperature: 98°C, maximum: 104°C) or by conventional heating (mean temperature: 100°C, maximum: 104°C). These amino acids were not modified by these treatments and the D-antimer was not produced [25]. Experiments on milk were performed by three groups. The group of Fay et al. [26] used 150 ml UHT milk and reconstituted infant formulae (protein fraction consisted of a mixture of whole milk and demineralized whey [A], enzymatically hydrolyzed whey protein [B] and a mixture of hydrolyzed animal and soy protein [C]). They microwave-heated the milk and the infant formulae in open glass baby bottles for 3 min at 600 W (final temperature, 82–93°C) or for 20 min at 70 W (final temperature, 58–66°C). After these heat treatments, the amino acids were determined after acid hydrolysis of samples. According to these authors, there were only very small differences between untreated and treated samples. The isomerization of proline and hydroxyproline in formula C was not affected by the microwave treatment. A reduced isomerization of valine and isoleucine was found in microwave-treated UHT milk, together with slightly higher isomerization levels for proline, asparagine, glutamine and phenylalanine. The latter amino acids showed an opposite trend in formulae A and B. Marcholli et al. [27] heated reconstituted infant formula in a conventional oil bath (20 ml; 10 min at 80°C), in a microwave oven (100 ml; 75 s, final temperature, 80–90°C) or in a wave-guide device (20 ml; 5 min at 120°C). They found no significant racemization in the protein under all conditions examined. Also, D-proline was not present in the protein, at least not above 1 mg/ml, or in free amino acids (< 20 g/l). Hydroxyproline was not detected. Petrucelli & Fisher [28] heated whole and skim milk samples for 10 min in a microwave oven at medium power or in a hot water bath at 80°C and determined D-aspartate and D-glutamate. They found no significant difference in the concentrations of these D-amino acids between the conventionally heated and microwave heated milks.

L-Amino acids dominate in nature. However, D-amino acids have been detected as common components of many food products and are consumed daily in varying amounts. In humans the conversion of D-amino acid via the enzyme D-amino acid oxidase is possible, but seems to be restricted. Until now, no adverse effects from the consumption of D-amino acids in foods have become known [24].

Other nutrients in milk

The concentrations of total fat, dry matter, protein and reactive lysine were not changed by microwave heating of milk. The activities of peroxidase and xanthine oxidase were very low in microwaved and stored milk. Comparable to pasteurization, the concentration of whey protein was reduced compared to raw milk which can be shown with the measurement of non-casein-nitrogen concentration. Non-casein-nitrogen concentration decreased in the upper 2 cm layer more than in the lower parts of 1000 ml skim milk which was microwave treated until (for 11 min) the temperature at the center reached the intended temperature. Reactive lysine was not altered. Stirring of microwave-treated milk after reaching boiling on the surface caused a lower loss of non-casein-nitrogen than in the unstrirred samples. The extent of denaturation in the latter samples was a little higher [23].

Flavour

Microwave heating of food or mixtures of nutrients can generate different new compounds, as do all heat treatments. In a model mixture consisting of 150 g glucose, 350 g casin and 500 ml water, furosin was produced in the same amount as by conventional heating [23]. The concentrations of hydroxymethylfurural, furose and lactulose in microwave-heated at 80 or 90°C for 0.5–7 h did not differ significantly from conventionally heated milk [30, 31]. In a triangle taste panel test with 27 panelists, 10 detected a difference between microwave-treated and pasteurized (62.8°C for 30 min) milk [32].

**BIOLOGICAL EXPERIMENTS**

The nutritive value of a casein solution heated by microwave or conventionally to a temperature of 80°C for 2 min was determined in a 10-day feeding study with rats. Their net protein utilization, digestibility and biological value were comparable [33].

Besides the publication of Lubec et al. [1], data of Blanc & Hertel [2] caused a sensation, especially in Switzerland. These authors administered 400 ml of raw, pasteurized (76°C, 18 s), boiled (hot plate, boiling time 150 s) or microwave-treated (boiling time 300 s at ca. 98°C) milk and also vegetables to 8 people (7 macrobiotics) per day on an empty stomach. Blood samples were taken 15 min before and after the feeding as well as 2 h later. The blood was analysed for erythrocytes, haemoglobin, average haemoglobin concentration, leucocytes, lymphocytes, iron, total cholesterol, HDL- and LDL-cholesterol and bioluminescence. According to the authors, there were signs of an anemic disposition in blood which they consid-
ered as a possible beginning of a cancerous process.

CONCLUSION
The composition of microwave treated milk does not differ very much from pasteurized milk. Based on present knowledge it can be concluded that intake of milk microwaved under household conditions is no threat to human health. Fears about the formation of D-amino acids in microwaved milk can be ignored, because their formation results from extreme heat, and household equipment does not utilize these conditions. Only heating of infant formulae using a microwave oven can be hazardous due to the uneven temperature inside the bottle used.

LITERATURE

THE IMPACT OF CHROMATOGRAPHIC LACTOSE SEPARATION

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ABSTRACT
Milk consists of four main components — fat, protein, lactose and minerals. While there have been methods to separate the other components of milk, a specific separation method for lactose has been missing. We have developed a chromatographic separation method, which can separate milk into two fractions. The first is a lactose-free milk, the other a lactose solution. It is a physical separation method using only water as eluent.

The appearance of lactose-free milk is like normal milk. The casein is in micellar form and it contains all the minerals of milk. The sweetness can be regulated to the original level by adding a small amount of some sweeteners.

The nutritional benefits of the lactose-free milk compared to caseinates and milk protein concentrates are:
- the taste and appearance are as good as normal milk;
- it contains all the minerals of normal milk;
- the lactose content can be freely regulated until zero;
- the energy value of it is less than half of normal milk.

Two years ago Valio Ltd launched a new Hyla Diet-ice cream line, which is based on the use of lactose-free milk. The products are aimed at lactose-intolerant, diabetic and calorie conscious consumers.

INTRODUCTION
Cheese production is based on the separation of proteins from milk using enzymatic coagulation. Cream and butter productions are based on the separation of fat from milk using gravity or a centrifuge. The minerals of milk can be separated by ion exchange or electrodialysis. Lactose, the main component of milk, is the only component which has not had any specific separation method.

Lactose malabsorption has been found to be very common in the adult population of the world [1]. The lactase deficiency has a genetic background. Milk has traditionally been an important part of the diet in Northern Europe, where lactose malabsorption is uncommon compared to other parts of Europe.
(Figure 1). In Finland only 17% of the adult population are lactase deficient. However, in Finland the symptoms of lactose intolerance are quite common because of the high milk consumption. Therefore there is a need for low-lactose or lactose-free milk products.

HYDROLYSIS OF LACTOSE AND HYLAMILK PRODUCTS

The commercial lactase enzymes isolated from yeasts and moulds have been available already for 20 years. The lactose content of milk can easily be reduced to less than 1% by incubating the milk together with the enzyme. The main problems of the hydrolysis are high costs and the proteolytic side effects of the lactase preparations.

Valio launched the lactose hydrolyzed skim milk powder in 1980. Since then the HYLAMILK-product line has grown in Finland each year [2]. The consumers demand a HYLAMILK-version of each milk product. Today Valio is selling about 50 different lactose hydrolyzed milk products. In HYLA-products at least 80% of the original lactose is hydrolyzed. These products seem to be well tolerated by most lactose-intolerant consumers.

Lactose hydrolyzed milk still has some problems. Glucose and galactose are sweeter than lactose. Many consumers claim that the HYLAMILK is too sweet. Lactose hydrolyzed milk is not very suitable for diabetics, because the monosaccharides are quickly absorbed into the blood. Some people are still worried about galactose in the diet, even if the safety of lactose hydrolyzed milk has been shown quite clearly [3]. Hydrolysis of lactose in milk does not reduce the energy value of milk; in fact, the lactose malabsorbers absorb more calories of it than of normal milk. These problems can be avoided, if lactose is separated from milk instead of being hydrolyzed.

CHROMATOGRAPHIC SEPARATION OF LACTOSE

Chromatographic separation has been used in the sugar industry for 30 years in order to separate sucrose from molasses. It is also used for separation of fructose and glucose in the production of high-fructose sweeteners. Chromatographic separation is a physical separation method based on the molecular sieve effect and ion exclusion. Only water is used as an eluent. No regeneration is needed for the special type of cation exchange resin used as separation medium.

We have developed new dairy applications for this method [4], which has proved to be useful both in milk and whey processing. Milk can be separated to a lactose-free milk and a lactose fraction. Ultrafiltration permeate or permeate mother liquor can be separated to a mineral and a lactose fraction.

LACTOSE-FREE MILK AND HYLAMILK PRODUCTS

The first chromatographic separation column (30 000 l of resin) was installed in our Joensuu lactose plant in 1988. The aim was to increase the yield in lactose manufacture. The production

Figure 1: Prevalence of adult-type hypolactasia in various European countries and populations (small number = prevalence in a population, large bold number = average prevalence in a country) and hypothetical isograms for the frequencies of the hypolactasia gene [1].
of lactose-free milk started in 1992. It solves all the problems of lactose hydrolyzed milk. Its sweetness can be regulated to the same level as normal milk. Its energy value is less than half of that of skim milk and it does not contain any lactose or galactose. Such a milk is beneficial for elderly people and for patients with sugar disorders [5].

Lactose-free milk is used as the main component of HYLA-Diet-ice cream products. The products are made for lactose-intolerant persons but also for diabetic and calorie-conscious people. These ice creams have been a success and the variety is growing every year.

In future the lactose-free milk powder may be an important product in international trade – it contains all the valuable proteins and minerals of milk (Table 1); freight costs are only 40% compared to skim milk powder; it does not cause problems to lactase-deficient populations; the other fraction from the process is a rather pure lactose solution; it can be utilized easier than ultrafiltration permeate.

LACTOSE SEPARATION FROM PERMEATE AND SUVAL WHEY SALT

Nowadays ultrafiltration is widely used to produce whey protein concentrates. Normally there is a problem in the utilization of the permeate (lactose and minerals of whey). Crystalline lactose can be produced from permeate, but in that case a new side product, permeate mother liquor, is produced. It is very difficult to dry it because of its hygroscopicity, and its high salt content limits its use in animal feeding. Chromatographic separation of permeate mother liquor produces two fractions. The mineral fraction can be easily dried to a whey salt powder. The lactose fraction can be recycled to the crystallization in order to increase the yield of lactose. Valio launched the SUVAL whey salt in 1994. Its mineral composition is very physiological (Table 2). Another advantage is that it is a natural salt and not a mixture of chemicals. It can be used to replace sodium chloride in many applications. It contains the small peptides and amino acids of whey. They give some flavour enhancing properties to the whey salt. Thus it is possible that also the mineral fraction of whey could be widely utilized as a profitable product in its own right.

| Table 1: Chemical composition of HYLADiet lactose-free skim milk powder |
|-----------------------------|---------------------|
| Proteins                    | 77%                 |
| Minerals (Ash)              | 13%                 |
| Fat                         | 2%                  |
| Lactose                     | 1%                  |
| Organic acids               | 4%                  |
| Moisture                    | 3%                  |
| Calcium                     | 29 g/kg             |
| Potassium                   | 27 g/kg             |
| Sodium                      | 9 g/kg              |
| Magnesium                   | 3 g/kg              |
| Phosphorus                  | 20 g/kg             |
| Chloride                    | 21 g/kg             |

<table>
<thead>
<tr>
<th>Table 2: Chemical composition of SUVAL whey salt</th>
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<tbody>
<tr>
<td>Minerals (Ash)</td>
</tr>
<tr>
<td>Peptides and amino acids</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Organic acids</td>
</tr>
<tr>
<td>Moisture</td>
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<tr>
<td>Potassium</td>
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<td>Sodium</td>
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<td>Calcium</td>
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<tr>
<td>Magnesium</td>
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<tr>
<td>Chloride</td>
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<td>Phosphorus</td>
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LITERATURE
DAIRY PRODUCTS: IS THERE A FUTURE FOR NUTRITION AS A MARKETING TOOL?

MARVELY PRODUCTS: IS THERE A FUTURE FOR NUTRITION AS A MARKETING TOOL?

DES ALLEGIATIONS NUTRITIONNELLES AUX ALLEGIATIONS “SANTE”

M.-O. Gailing
Nestlé France S.A.

Les allégations sont, d’après la définition du CODEX, “tout message ou représentation qui énonce, implique, suggère qu’une denrée alimentaire possède les caractéristiques particulières se rapportant, notamment, à la nature, l’identité, les qualités, la composition, la durabilité, l’origine, la provenance, le mode de fabrication ou d’obtention en vue d’en promouvoir la vente”.

C’est donc un domaine extrêmement vaste de la communication sur l’aliment qui est ouvert ici.

Dans cet exposé, nous nous limiterons aux allégations dites nutritionnelles et aux allégations “Santé”.

Si les allégations nutritionnelles sont relativement clairement définies, nous allons voir qu’il n’en est pas de même des allégations “Santé” et que la différence entre ces deux types d’allégations n’est pas facile à faire et arrive à des situations ambiguës.

Les allégations nutritionnelles, telles qu’elles sont définies dans la directive 90/496/CEE, se limiteraient à “toute représentation ou tout message publicitaire qui énonce, suggère ou implique qu’une denrée alimentaire possède des propriétés nutritionnelles particulières:
- de par l’énergie qu’elle fournit, fournit à un taux réduit ou aucun, ne fournit pas.
- de par les nutriments qu’elle contient en proportion réduite ou aucune ou ne contient pas”.

Si cette définition est prise dans son sens le plus restrictif, il ne s’agit, ici, que de ce que l’on appelle couramment les allégations nutritionnelles quantitatives absolues ou comparatives, sans que, dans ce texte, ne soient précisés de critères de composition contrairement aux dernières propositions du CODEX.

Il faut, d’ailleurs, sur ces critères de composition permettant d’alléguer sur un nutriment, se poser un certain nombre de questions. Quels sont les nutriments à prendre en compte ? Peut-on aussi considérer, ici, les constituants de ces nutriments ? Quel est le référentiel à utiliser 100 g, 100 kcal, la portion, la densité nutritionnelle et énergétique variant considérablement pour chaque type d’aliment ?

En ce qui concerne les allégations nutritionnelles quantitatives comparatives, quel seuil doit-on accepter ? ± 20, ± 25, ± 30% ? N’est-il pas plus intéressant de réduire la teneur en matière grasse de 20% pour un aliment qui représente un apport important dans l’alimentation plutôt que de réduire de moitié la teneur d’un aliment qui en apporte peu ? Comment peut-on rester informatif et cohérent dans le message nutritionnel pour le consommateur ?

Si malgré toutes les questions que l’on se pose encore sur ces allégations nutritionnelles quantitatives et comparatives, la situation est relativement claire.

Entre ces allégations nutritionnelles et les allégations purement “Santé” se trouve un “no man’s land” actuellement occupé par les allégations dites fonctionnelles.

Il s’agit, en fait, de communiquer sur la fonction d’un nutriment telle qu’elle pourrait être décrite dans un manuel de biochimie. Là, encore, les questions affluent. Quel sort les nutriments à prendre en compte ? (caux avec AJR ? les essentiels ?)… Faut-il considérer certains constituants de nutriments (acides aminés, acides gras ?)…

Comment doit-on communiquer sur ces fonctions ? Peut-on les simplifier ? Les dissociar ? Peut-on communiquer sur une fonction pharmacologique d’un nutriment alors que ce dernier ne représente que 20% des AJR d’un produit donné ? Sur quelles bases réglementaires cette communication peut-elle se faire ? (liste positive ou dossier ?)

Les allégations fonctionnelles ne représentent-elles pas le premier pas vers les allégations “Santé” ? Les allégations “Santé” qui mettent en évidence un lien entre l’alimentation et la santé et aussi entre l’aliment et la santé, commencent à faire, dans de nombreux pays et aussi au CODEX, l’objet de discussions soutenues. Certains états comme les USA et le Japon se sont, d’ailleurs, déjà dotés de législations dont la philosophie est, d’ailleurs, très différente.

Bien entendu, si le consommateur attend de l’industrie Alimentaire la mise en évidence de bénéfices des aliments sur sa santé, il faut que cette dernière accorde tout le sérieux nécessaire aux recherches et à la communication qu’elle pourrait faire sur ces bénéfices et qu’elle tienne compte du fait que les produits ainsi présentés doivent s’intégrer dans une alimentation équilibrée, variée et diversifiée.

Pour cela, des règles précises doivent être définies prévenant une relation claire entre les différents acteurs et tenant compte de la possibilité de protection des résultats des recherches entreprises par les uns et les autres dans des domaines précis.

MARKETING NUTRITION MESSAGES IN NEW ZEALAND

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ABSTRACT

Around the world, consumers accept that the food they eat can influence their short- and long-term health. This long recognized concept is integrated into the many daily decisions we make about the food we eat. Hence nutrition is often used to add value to branded food concepts. However, underlying the consumer’s perceptions
of the nutritional value of the unmodified foodstuff or base food groups, sustaining high perceived nutritional value of dairy products and removing attitudinal barriers to choice of dairy products based on health concerns is an important platform for improved dairy products consumption.

Building strong health values into dairy products requires marketing of generic nutrition messages.

Effective programmes involve more than a didactic nutrition education approach. There is often a mismatch between what nutritionists think people need to know, what will motivate change in behaviour and what is a current area of consumer concern.

In New Zealand ongoing monitoring of consumer attitudes and trends is used as the base to target multi-disciplinary nutrition promotions that are changing nutrition attitudes to dairy products and providing opportunities for brand marketers to reinforce dairy positive messages.

NUTRITION LABELING AND HEALTH CLAIMS IN THE US: IMPLICATIONS FOR MARKETING DAIRY PRODUCTS

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2 San Luis Obispo, CA, USA

ABSTRACT
In January of 1993, the United States Food and Drug Administration issued final regulations on nutrition labeling and health claims. The regulations constitute a major overhaul and modernization of nutrition labeling in the US. Manufacturers were given until May of 1994 to comply. With few exceptions, all foods must carry the basic nutrition labels; strict rules were laid out for optional label statements related to nutrition and health. Key parts of the new labeling regulations include a) the basic label requirements; b) the definitions and allowed uses of nutrient descriptors (for example, “reduced in fat,” “rich in calcium,” “low sodium”); and c) criteria for use of health claims (for example, “diets rich in calcium help build and maintain healthy bones and may reduce risk of osteoporosis”). The dairy industry in the United States has an opportunity to take advantage of the labeling regulations by using various nutrient descriptors and/or health claims on the many dairy products that qualify, or even by re-formulating some higher-fat or higher-sodium products to assure that desirable nutrient descriptors and health claims can be used.

BACKGROUND: THE NEW LABEL REQUIREMENTS

Historical perspective
The US Food and Drug Administration (FDA) first published regulations for nutrition labels on foods in the 1970s. If a nutrition label appeared on a food package, it had to include information on serving size, number of servings per container, calories, protein, total carbohydrate, total fat, vitamin A, vitamin C, thiamin, riboflavin, niacin, calcium, and iron. However, the decision to use a nutrition label on a food package was voluntary, with two exceptions: addition of nutrients to foods (fortification, such as the addition of vitamins A and D to milk) and nutrient claims on packages (for example, “good source of vitamin C”) mandated use of nutrition labels.

There were a few small changes to the regulations over the years; for example, FDA added a requirement that sodium be included on nutrition labels. Yet during the 1980s, consumers, the food industry, and health professionals all agreed that the regulations were badly in need of major revision. Nutrients such as saturated fat, dietary fiber, and sugar were of increasing interest but were not listed on labels; on the other hand, nutrients such as thiamin and riboflavin were required on nutrition labels but posed no problem for the general US population. The US Congress got involved, passing the Nutrition Labeling and Education Act which was signed into law in November 1990. The NLEA required the FDA to propose sweeping new regulations within a year. Proposed regulations were published in 1991; comments from consumers, industry, health professionals, and others were received and taken into consideration; and final regulations were published on 8 January 1993. Manufacturers had an additional period of time of about a year and a half before compliance with the new regulations was mandatory. The rest of this paper will be devoted to a brief description of the new regulations and how they pertain to marketing dairy products.

A look at the label
The 1993 regulations require that nearly all packaged foods in the US carry nutrition labels. There are a few exceptions—foods with no nutritional value, such as plain coffee or tea and herbs; foods from very small companies; very small packages which instead must give a phone number or address for more information— but most food packages must now carry nutrition labels. An exception relevant to the dairy industry is cheese cut from a larger block and sold to consumers at delicatessen counters in grocery stores. Cheese and other foods sold in a similar manner do not have to carry nutrition labels.

Following is a brief overview of some key parts of the nutrition labeling requirements.

Format. There is a prescribed format for all nutrition labels. The basic “Nutrition Facts” label is shown in Figure 1; whole milk is the example food. It includes a title (“Nutrition Facts”) and information about serving size; calories and calories from fat; key macronutrients and subcategories of interest; two vitamins and two minerals (vitamin D is added on this example label for reasons discussed later); and

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serving Size</strong></td>
</tr>
<tr>
<td><strong>Servings Per Container</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount Per Serving</th>
<th>Calories</th>
<th>% Daily Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Fat</strong> 8g</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td><strong>Saturated Fat</strong> 5g</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td><strong>Cholesterol</strong> 35mg</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td><strong>Sodium</strong> 115mg</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td><strong>Total Carbohydrate</strong> 11g</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td><strong>Dietary Fiber</strong> 0g</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Sugars</strong> 11g</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong> 8g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vit. A 6%</th>
<th>Vit. C 4%</th>
<th>Calcium 30%</th>
<th>Iron 0%</th>
<th>Vit. D 25%</th>
</tr>
</thead>
</table>

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

<table>
<thead>
<tr>
<th>Calories</th>
<th>Total Fat</th>
<th>Saturated Fat</th>
<th>Cholesterol</th>
<th>Sodium</th>
<th>Total Carbohydrate</th>
<th>Dietary Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,000</td>
<td>20g</td>
<td>5g</td>
<td>300mg</td>
<td>2400mg</td>
<td>25g</td>
<td>12g</td>
</tr>
<tr>
<td>2,500</td>
<td>25g</td>
<td>6g</td>
<td>350mg</td>
<td>2600mg</td>
<td>25g</td>
<td>15g</td>
</tr>
</tbody>
</table>

*Calories per gram: Fat D 4 Carbohydrate 4 Protein 4

Figure 1: “Nutrition Facts” label.
an information panel in small print which looks exactly the same on every nutrition label and is intended to help consumers understand what the label information means to them.

Flexibility and alternative presentations. The label format shown in Figure 1 can be modified for some foods. Oddly-shaped or small packages can use approved abbreviated formats, as can certain foods (for example, soft drinks) with insignificant amounts of seven or more nutrients mandated on labels. Numerous dairy foods such as small packages of cheese might be able to use modified formats because of small package size. Butter is an example of a food that could use a simplified format because it contains insignificant amounts of seven nutrients mandated on food labels (specifically, total carbohydrate, dietary fiber, sugars, protein, vitamin C, calcium, and iron).

The Daily Values. Daily Values are reference numbers on the new labels. As the label footnote says, the percent Daily Values are based on a 2000 calorie diet, so consumers are advised that their own needs could be higher or lower depending on their energy needs. The Daily Values are derived from one of two places. For vitamins and minerals (except sodium and potassium), they are based on the old US Recommended Daily Allowances used in the previous nutrition labeling regulations — which, in turn, were derived from the 1974 edition of the Recommended Dietary Allowances for Americans. (It is expected that the Daily Values for vitamins and minerals will be updated in the future.)

For the other nutrients (fat, fat subcategories, cholesterol, sodium, potassium, total carbohydrate, dietary fiber, sugars, and protein), the Daily Values are based on recommendations from the National Research Council’s 1989 publication, Diet and Health: Implications for Reducing Chronic Disease. They are consistent with recommendations from a wide range of nutrition organizations, health professionals, etc., as well. The old nutrition labeling regulations did not have reference numbers for these nutrients with the exception of protein.

Required and optional nutrients. Required nutrients are shown in the Figure. Note the emphasis on macronutrients and “problem” nutrients in US diets, such as fat and saturated fat (which we tend to overeat) and fiber and calcium (which we tend to undereat). In addition to the mandatory nutrients, labels can also include information on calories from saturated fat, amount of polyunsaturated and monounsaturated fat, potassium, soluble and insoluble fiber, sugar alcohols, and a host of other vitamins and minerals. If a nutrient content claim is made for any of the “voluntary” nutrients, or if a food is fortified with any of the “voluntary” nutrients, then nutrition information on these components becomes mandatory. For example, most milk in the US is fortified with vitamin D, so the nutrition label must include information on this “voluntary” nutrient (as shown above in the example label for whole milk).

NUTRIENT DESCRIPTORS

Overview
Nutrient descriptors, also called nutrient content claims, are words or phrases which describe the amount of a nutrient in a serving (and in a standard reference amount) of a food. There are no claims about how the nutrient affects health — simply the amount of a nutrient present. The nutrient descriptors are strictly defined by FDA. Examples of nutrient content claims include “low in fat,” “rich source of calcium,” “reduced in sodium,” and “cholesterol free.”

Categories. There are three general categories of nutrient descriptors. Absolute claims directly describe the amount of a nutrient in a food. Good examples are use of the words “free,” “low,” “rich in,” or “good source of” to describe individual nutrients. The second general category is for relative claims, wherein comparisons of the nutrient level in one food to another are made. Examples of comparative claims are use of the words “less,” “reduced,” “more,” and “light.” A final category of nutrient content claims is called implied claims, meaning that if a food is described in a way that implies a certain content of a nutrient, then the implied level of the nutrient must be met. For example, calling a breakfast cereal “rich in bran” implies that the cereal is rich in dietary fiber, so it must meet the FDA definition for a fiber-rich food.

Highlighting what is in the product. Some nutrient content claims are designed to highlight essential nutrients that are present in a serving and standard reference amount of a food. “Good Source,” “Contains,” and “Provides” are synonymous and can be used for nutrients that are present at 10-19% of the Daily Values per reference amount. “High,” “Rich in,” and “Excellent source of” are also synonymous and require that the food has at least 20% of the Daily Value of the nutrient per reference amount. To use comparative claims such as “more,” “fortified,” or “added,” the reference serving must have at least 10% more of the Daily Value than an appropriate comparison food.

Highlighting what is not in the product. Most nutrient content claims highlight nutrients that are missing from a product — nutrients that US consumers are advised to eat less of. “Free” is defined for calories (less than 5 per reference amount), fat (less than 0.5 g), saturated fat (less than 0.5 g), cholesterol (less than 2 mg, and 2 g saturated fat or less), sodium (less than 5 mg), and sugar (less than 0.5 g). “Low” is also defined for all of the same nutrients except for sugar (calories — 40 or less; fat — 3 g or less; saturated fat — 1 g or less; cholesterol — 20 mg or less; and sodium — 140 mg or less). The term “reduced” can be applied to all of the above nutrients and means that there is a reduction of at least 25% compared to the original product or to a standard product. The word “light” can be used to describe products that have a third fewer calories or 50% less fat than the original or standard product.

Potential applications to dairy foods
In scanning the grocery store shelves, it is apparent that relatively few dairy foods take advantage of nutrient content claims on their labels unless they are fat-modified products. Yet there is a golden opportunity to highlight the nutritional properties of many milk products. While each product must be evaluated individually, following are some examples of how dairy products could take advantage of nutrient descriptors.

Highlighting what is there: calcium, selected vitamins. Many dairy products qualify as “good” or “excellent” sources of several essential nutrients. All fluid milks, many cheeses (for example Cheddar, Parmesan, Provolone, and others), and yogurts should qualify as “excellent” sources of calcium. Fluid milks fortified with vitamin A qualify as “good” sources of the vitamin, and those fortified with vitamin D according to government regulations qualify for “excellent” claims. Fluid milks and yogurts are “excellent” sources of riboflavin and phosphorus and “good” sources of potassium and protein. Cottage cheese is a “good” source of riboflavin and an “excellent” source of protein; other cheeses are generally “good” sources of protein. Reduced-fat ice creams and frozen yogurts may qualify as “good” sources of calcium and riboflavin. Some solids-fortified milks and yogurts may qualify as “good”
sources of magnesium or possibly zinc. It is clear from these examples that there is ample opportunity to exploit the labeling regulations by telling consumers about essential nutrients — especially calcium — in dairy foods. Some calcium-fortified breakfast cereals, a popular brand of chocolate covered granola bars — calcium-fortified, of course — and calcium-fortified orange juices are just a few products proclaiming themselves as good or rich sources of calcium. Why, then, don't more dairy products which are naturally rich in calcium make proclamations on their labels?

Highlighting what is not: sodium, fat, cholesterol, sugars. Several dairy products may qualify for "low sodium" claims, including yogurts, sour cream, ice cream, fluid milks, frozen yogurts, creams, Swiss cheese, and cream cheese products. The "low fat" descriptor applies to lowfat milks (see comment below), lowfat cottage cheese, and lowfat yogurts. "Low cholesterol" applies to nonfat dairy products, cottage cheeses, lowfat yogurt, and 1% lowfat milk.

Use of claims such as "sodium reduced," "fat reduced," "reduced sugars," etc. obviously, would apply to any reformulated dairy food meeting the 25% reduction in the nutrient per reference amount. The term "light" could be applied to a product with a 50% reduction in fat. At the current time, FDA is reviewing a petition to rename nonfat (skim) and 2% lowfat fluid milks to make the names more consistent with the new regulations. Specifically, nonfat milk would be renamed "fat-free milk" and 2% lowfat milk — which actually does not meet the definition for low fat but was exempted because of tradition and standard of identity requirements — would be renamed "reduced-fat milk."

HEALTH CLAIMS
A health claim differs from a nutrient descriptor in that an actual statement about the relationship of a nutrient to a disease or health-related condition is made. FDA conducted extensive literature reviews to determine what health claims were appropriate to make. Only eight diet- or nutrient-health relationships were determined to be supported by enough evidence to allow health claims on food labels. Strict criteria were established for use of each health claim, and model health claim statements were provided by FDA.

Approved health claim topics
There are eight areas in which health claims are permitted: 1) calcium and osteoporosis (the food must meet the high calcium requirement); 2) fiber-containing grain products, fruits, vegetables and cancer (food must naturally be a good source of fiber and be low fat); 3) fruits and vegetables and cancer (product must be low fat and a good source of vitamin A, vitamin C, or fiber); 4) fruits, vegetables, and grain products that contain fiber and coronary heart disease (product must be low fat, low cholesterol, low fat, and have naturally at least 0.6 g soluble fiber); 5) fat and cancer (product must be low fat); 6) saturated fat and cholesterol and coronary heart disease (food must be low fat, low saturated fat, and low cholesterol); 7) sodium and hypertension (food must be low sodium); and 8) folate and neural tube defects (food must be a good source of folate). Any additional diet-disease relationships will have to undergo intense scrutiny by the FDA before they will be approved for use as health claims on food packages.

FDA's suggested wording of health claims includes language that highlights the overall diet or lifestyle of an individual. For example, the model health claim for calcium and osteoporosis is, "Regular exercise and a healthy diet with enough calcium help teen and young adult white and Asian women maintain good bone health and may reduce their high risk for osteoporosis later in life."

Disqualifying nutrients and potential applications of health claims to dairy foods
As mentioned above, there are stringent standards for the level of a nutrient about which a health claim is made. In addition, there are "disqualifying" levels of certain nutrients which were deemed to make the product unsuitable for a health claim. Specifically, a serving and reference amount of the food cannot exceed 13 g total fat, 4 g saturated fat, 60 mg cholesterol, or 480 mg sodium. These disqualifying nutrients mean that many dairy (and other) foods cannot carry health claims even if they meet the levels of the health claim nutrient. Consider a food that is low in sodium but high in fat or saturated fat: it could not make a health claim about sodium and hypertension. It could, however, carry the "low sodium" nutrient descriptor as long as the label also states, in smaller print, that information on fat and other nutrients is listed on the nutrition label.

Beyond disqualifying nutrients there is also a stipulation that a food carrying a health claim must have at least 10% of the Daily Value for at least one of the following: vitamin A, vitamin C, iron, calcium, protein, or fiber. This is to prevent non-nourishing foods from carrying health claims. For example, soft drinks are fat-free and low sodium but are not able to carry a dietary fat and cancer claim or a sodium and hypertension claim because of this stipulation. Most dairy foods easily meet this stipulation, however.

Calcium and osteoporosis. Lowfat and nonfat milks, lowfat and nonfat yogurts, buttermilk, lowfat chocolate milk, and some light or lowfat cheeses should qualify to use the calcium and osteoporosis health claim. Whole milk and most cheeses exceed the disqualifying levels of saturated fat. It is possible but only speculative that using a heavier blend of nonfat and lowfat fluid milks but not on whole milk could be confusing to consumers (since all fluid milks have about the same amount of calcium per serving) or could be detrimental to sales of whole milk.

Sodium and hypertension. Again, because of disqualifying levels of saturated fat, many dairy products cannot carry a claim about sodium and hypertension. But lowfat and nonfat fluid milks, lowfat yogurt with fruit, and reformulated products may qualify.

Fat and cancer and fat, cholesterol, saturated fat and coronary heart disease. Nonfat and 1% lowfat milks, nonfat yogurts, and some other fat-modified dairy foods may meet requirements for this health claim. However, few traditional dairy products can meet the stringent requirements for low fat and/or saturated fat.

Future possibilities for additional health claims. It is not unreasonable to expect that, sometime in the future, additional health claims relevant to dairy foods could be approved for food labels in the US. Possibilities include: a) lower risk of hypertension with diets rich in calcium, potassium, and magnesium; b) lower risk of colon cancer with diets rich in calcium; c) improved lactose tolerance from milk products made with certain live, active dairy cultures; and d) protection against dental caries by several hard cheeses. The dairy industry can help make these possibilities a reality by continuing to support research on these and other diet-health relationships. Still, the disqualifying levels of fat and especially saturated fat will continue to make it difficult for many dairy foods to take advantage of health claims on food labels. It appears that in the US, relating dairy foods to good health will remain an important task to be accomplished through other nutrition education channels.
CONCLUSIONS

The 1993 nutrition labeling regulations offer both opportunities and challenges to dairy processors. The opportunity to use nutrient descriptors to highlight milk, yogurt, and cheese as good or excellent sources of calcium and protein. For fat-modified dairy foods, manufacturers must take the opportunity to let consumers know their products are especially designed for people who are fat- and calorie-conscious. In addition, use of the Food Guide Pyramid on dairy product labels would highlight the need to eat 2-3 daily servings of milk and milk products (a goal Americans, on average, fail to meet). The Food Guide Pyramid is a frequent sight on breakfast cereal boxes or loaves of bread packages of pasta, yet dairy manufacturers have largely ignored the positive and simple message it can offer consumers.

Health claims offer more of a challenge to dairy manufacturers because disqualifying levels of fat and saturated fat prevent use of health claims on many traditional dairy products. Nevertheless, fat-modified dairy foods may qualify for use of health claims and it makes sense to consider their use when possible. Why let manufacturers of other food products lay exclusive claim to capitalizing on the 1993 labeling regulations?

Selected key literature


Food Labeling Update for Wisconsin Dairy Manufacturers and Marketers (published periodically from 1994 - present). Dairy Council of Wisconsin, 999 Oakmont Plaza Drive, Westmont, IL 60559.


THE NUTRITIONAL MESSAGE ON A NEW PRODUCT FOR INFANTS

C. Bouley

Groupe Danone, Direction Recherche et Développement Produits Frais, 15 Avenue Gallié, 92350 Le Plessis Robinson, France

ABSTRACT

Fresh dairy products (yogurt and Petit Suisse) are very often used for beikost by infants in spite of the fact that paediatricians recommend the consumption of infant formula for the first year or more, thereby delaying the introduction of cow’s milk.

Faced with this situation, Danone has set up a new fresh “cheese” specifically adapted to infants and young children. With a good taste, and an appropriate texture, it contributes to the food intake of iron and essential fatty acids: one serving (50 g) contributes to 25% of EC labeling requirements of iron for infants (0.55 mg) and represents 270 mg of linoleic acid and 35 mg of linolenic acid. Its consumption could improve the food intake of lipids and iron. Compared to the traditional fresh cheese (Petit Suisse) its caloric value is reduced, with a repartition of the caloric value very close to that of an infant formula: less proteins and lipids, a reduction of 30% of sucrose content. A new process guarantees a high calcium level, necessary for bone mass construction. The bacteria used for fermentation have been selected to produce L. lactis acid alone, which is easily metabolized by infants.

To conclude, this new fresh “cheese” is specifically adapted for beikost. Its formula is adapted to the needs of infants. It guarantees a significant intake of iron and essential fatty acids, and its consumption allows the discovery of a new taste and texture by infants and young children.

A recent study on food purchased for infants in France pointed out a high penetration rate of fresh dairy products and desserts: Petit Suisse is the main dairy product bought, with a penetration rate of 91% for 7-12 month-old infants and for yoghurt the figure is 77%. From 10 to 12 months of age, products containing fruit (Petit Suisse, yoghurt) are bought to a larger extent than plain products and concurrently the penetration of small glass jars is decreasing (SOFRES study on 1000 mothers with infants from birth to 2 years of age, 1992). Fresh dairy products are very often used for beikost by infants in spite of the fact that paediatricians recommend the consumption of infant formula until 1 year old or more, thus delaying the introduction of cow’s milk. Therefore, Danone has identified the need for a product specifically adapted to infants and young children and introducing the advantages of a follow-up formula. A new product has been developed taking into account paediatricians’ recommendations and French regulations.

INFANT NEEDS AND REQUIREMENTS FOR IRON AND ESSENTIAL FATTY ACIDS

Infant growth

During the first 3 years of life, growth is extremely rapid:
- height gain is maximal during the first months after birth: 20-25 cm during the first year and 20 cm from 10 months of age to 3 years;
- body weight gain is 30 g/day during the first 2 months, then 20 g/day up to 6 months of age and 12.5 g/day from 6 to 12 months of age;
- brain weight gain is 2 g/day during the first year of life and the brain reaches nearly 70% of its final weight by the age of 3 years.

Growth requires energy and it has been estimated that 3-5 kcal are required for each gram of body weight gained. The dairy energy requirement for infants aged 6-12 months is 100 kcal/kg of body weight, and the mean value during the first year is 108 kcal/kg (Dupin H., 1992).

Iron needs and deficiency/requirements

Iron is essential to the important increase in quantities of haemoglobin, myoglobin and enzymes during the first year of life. The need for iron during this period is 200-270 mg (depending mainly on birth weight) and consists of the desirable increment in total body iron plus the amount of iron needed to replace dermal and gastrointestinal losses (62 mg and 29 mg, respectively). The estimated requirement for absorbed iron are approximately 0.55-0.75 mg/day (Fomon S.J., Nutrition of normal infants, 1993).

Considering the bioavailability of iron, the requirement is 10 mg per day from birth to 3 years of age (CNSFP, Nutrition Committee of the French Paediatrics Society, 1992).

Anaemia due to the lack of iron is considered to exist among persons who have a concentration of haemoglobin inferior to 11 g/dl together with a deficiency in iron which is characterized by at least two abnormal values among the independent factors (erythrocyte proto-
porphyrin, serum ferritin, transferrin sat-
uration and mean corpuscular volume).

The consequences of iron deficien-
cy and anaemia are numerous and
include impaired motor development
and coordination, psychological and
behavioural effects, decreased physical
activity.

French surveys showed that among
groups of infants 6 months to 4 years of
age in the Paris region the frequency of
abnormal values of haemoglobin is sig-
nificantly high at early ages and differs
between children with native born par-
ents (17% at 10 months of age, 9% at
2 years, 4% at 4 years) and children
with parents born in the DOM-TOM or
enigrants (30 and 29% at 10 months and
2 years, 7% at 4 years) (Rossignol
C., 1990). Iron deficiency is frequent
during rapid growth: 29% for infants
6 months to 2 years of age, 13.6% 
between 2 and 6 years. Frequency of
anaemia was 4.2% for infants 6 months
of age to 2 years and 2% at 2–6 years
old (Herberg S., 1990).

Essential fatty acids needs and defi-
ciency/requirements

Fatty acids of the N-6 series (linoleic
acid and arachidonic acid) cannot be
formed from oleic acid in the human
body and cannot give rise to the N-3
series such as α-linolenic acid which
therefore must be supplied by food.
Linoleic acid and α-linolenic acid are
essential components of cell mem-
branes and are precursors of
prostaglandins. They are necessary for
proper infant growth, muscle structure
and brain phospholipids constitution.

Essential fatty acid deficiency in
infants can lead to non-optimal growth,
skin changes, hair loss and increase in
metabolic rate.

Dupin recommends that 3.5–5% of
energy comes from linoleic acid and
0.5–1% from linoleic acid, with a ratio
of 4:6. The Joint FAO/WHO Codex
Alimentarius Commission and the ESP-
GAN Committee on Nutrition recom-
end a minimum level of 300 mg of
linoleic acid per 100 kcal. This is also
stated in EC labelling requirements.

INFANT DIET BALANCE

Deheeger and Rolland-Cachera
studied the diet of a French popula-
tion of 2-year-old infants in 1973 and
1986. They observed an insufficient intake of
iron and essential fatty acids by com-
monly fed foods despite a satisfactory
diet balance: iron intake was
5.96 mg/day in 1973 and 6.94 mg/day
in 1986; essential fatty acid intake was
3.9 g in 1973 and 4.4 in 1986 (Deheeger
& Rolland-Cachera, Annals of Nutrition

Concerning dairy products in the
diet, the main results were as follows:
– dairy product intake in the infant
total diet provides 30% of the total
energy intake, 45% of proteins, 33%

<table>
<thead>
<tr>
<th>Table 1: “Petit Gervais Croissance aux fruits” – nutritional content</th>
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<tbody>
<tr>
<td><strong>Protein (g)</strong></td>
</tr>
<tr>
<td>4.9</td>
</tr>
<tr>
<td><strong>Linoleic acid</strong></td>
</tr>
<tr>
<td>540</td>
</tr>
<tr>
<td><strong>Linolenic acid</strong></td>
</tr>
<tr>
<td>70</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
</tr>
<tr>
<td>16.3</td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td><strong>Calorie value (kcal)</strong></td>
</tr>
<tr>
<td>130</td>
</tr>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
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<td>2</td>
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</tbody>
</table>

* CNSFP: Nutrition Committee of French Paediatrics Society.

<table>
<thead>
<tr>
<th>Table 2: A comparison of “Petit Gervais Croissance aux Fruits” with traditional and recent traditional fresh fruit cheese</th>
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</thead>
<tbody>
<tr>
<td><strong>Amount per 100 g</strong></td>
</tr>
<tr>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>15</td>
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<tr>
<td>16</td>
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<tr>
<td>6</td>
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<td>130</td>
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</table>
of lipids, 37.5% of sucrose and 80% of calcium,
- their contribution to iron and essential fatty acid requirements is low: 0.86 mg iron out of the 10 mg required for this age.

Another study showed that 70% of 10-month-old French infants have a daily iron intake less than the recommended amount and this figure rises to nearly 95% at the age of 2 years (Deheeger, Arch. Fr. Pediatr. 1990; 47: 531–7).

Vitamin D is also a key nutrient for infant growth (bone mass construction) and deficiency has been frequently observed. Human milk and cow's milk are relatively poor in this nutrient. Despite the supplements given by doctors, vitamin D intake for infants is not sufficient.

In spite of follow-up infant formula commercialization and use, food commonly fed to infants does not provide sufficient amounts of iron and essential fatty acids. Further, these nutrients are not found during food diversification.

Therefore, Danone has set up a new fresh "cheese" specifically adapted for infants and young children: "Petit Gervais Croissance". Its consumption should limit the risks of iron and essential fatty acid deficiencies.

"PETIT GERVIAI CROISSANCE"

Nutritional aims
The main objectives were as follows:
- to reduce the risk of iron deficiency during infancy and early childhood,
- to provide the essential fatty acids mentioned above,
- to provide a pleasant taste and texture despite the addition of iron,
- to select optimal fermentation bacteria,
- to contribute to the nutritional balance adapted to this period of infant growth.

Formulation and nutritional balance
Compared to the traditional fresh cheese, "Petit Gervais Croissance" caloric value is reduced (130 kcal per 100 g) with a reparation of the caloric value very near to that of an infant formula: less protein (15%) and lipid (34%) and a reduction of 30% of the sucrose content (carbohydrates: 51%).

One serving (50 g) of "Petit Gervais Croissance aux fruits" contributes to 25% of the EC labelling requirements of iron for infants (0.55 mg) and represents 270 mg of linolenic acid and 35 mg of linoleic acid, with the goal of improving the intake of iron and lipids from food.

In addition, a new process guarantees a high calcium level which is necessary for bone mass construction.

Due to regulations, it is not possible to add vitamin D to the product.

In order to meet the infants' needs for iron and calcium, Danone has also conducted and sponsored research on the bioavailability of these nutrients in "Petit Gervais Croissance". Results showed that in 150 g "Petit Gervais Croissance", 8% of iron (ferrous lactate) was absorbed (Hulberg L., Göteborg, Sweden, 1994). Bioavailability of calcium was not affected by the new process used or by the addition of iron: the fractional calcium absorption from 100 g of "Petit Gervais Croissance" was 38.8% and measured using a double isotope extrinsic labelling technique (W. van Dokkum, TNO Nutrition and Food Research Institute, Zeist, the Netherlands, 1994). In addition, simultaneous ascorbic acid intake improves the bioavailability of iron.

Equivalence with other milk-based products:

<table>
<thead>
<tr>
<th>100 g of &quot;Petit Gervais Croissance&quot;</th>
<th>Follow-up infant formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>180 ml 210 ml</td>
</tr>
<tr>
<td>Calcium</td>
<td>160 ml 140 ml</td>
</tr>
<tr>
<td>Iron</td>
<td>100 ml ++</td>
</tr>
<tr>
<td>Essential fatty acids</td>
<td>125–225 ml 600 ml</td>
</tr>
</tbody>
</table>

Selection of optimal fermentation bacteria
Fermentation bacteria were selected based on their ability to produce only L. lactis acid which is easily metabolized by infants. Further, the bacteria chosen result in a little post-acidification and thus contribute a pleasant taste.

Product taste and texture
"Petit Gervais Croissance" has been tested among many infants and children. The product has a good taste and an appropriate texture.

CONCLUSION
"Petit Gervais Croissance" is a new fresh cheese specifically adapted for belkost and infant needs. It guarantees a significant intake of iron and essential fatty acids and its consumption allows the discovery of a new taste and texture for infants and young children. It can be considered a pleasant option for children discovering new foods, providing advantages of follow-up infant formula: 100 g of "Petit Gervais Croissance" provides as much iron and essential fatty acids as at least 130 ml of follow-up infant formula (Tables 1 and 2).

LE MARKETING ALIMENTAIRE ET LA SANTÉ
1985–1995
Y. Boutonnat
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LA SANTÉ OU LES SANTÉS?
Les produits santé envahissent les rayons des GMS. Certains se rapprochent des médicaments, d'autres sont protocole ou rendent beaux.

La santé dans l'alimentaire devient un phénomène complexe puisque l'on ne trouve pas un produit qui fait du bien à la santé, mais des produits qui touchent différents concepts de santé.

Dans la décennie 1985/1995 se succédèrent 3 courants majeurs de santé.

- Le concept de santé devient l'expression des styles de vie et de mentalités d'une époque. Schématiquement:
  1985/1989 Le besoin d'équilibre
  1989/1993 Les besoins d'allégement et de nutrition
  1993/… Le besoin de vie saine
Les nouveaux produits santé qui réussissent sont ceux qui vont définir leur concept et/ou leur communication publicitaire en fonction des besoins de santé du moment.

1985/1989 Le besoin d'équilibre
Il succède au besoin de vitalité du début des années 80, où le dynamisme et le tonus étaient la preuve de la santé.
La recherche d'équilibre consiste à être moins superficiel et moins mécanique dans son alimentation. La seule absorption de produits vitaminés et la seule élimination des impures n'ont plus suffisamment.

Les produits alimentaires équilibrés sont ceux qui vont à la fois fournir au corps les éléments indispensables à son entretien et aussi apporter un bénéfice mental et psychologique, comme l'épanouissement ou l'image de soi.

L'équilibre alimentaire apporte l'harmonie du corps et de l'esprit.

1989/1993 Les besoins de nutrition et d'allégement
C'est le retour à une démarche de santé basique

Basique

<table>
<thead>
<tr>
<th>J'ajoute</th>
<th>J'arrête</th>
</tr>
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<tbody>
<tr>
<td>Besoin de nutrition</td>
<td>Besoin d'allégement</td>
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</tbody>
</table>
**Le besoin de nutrition**

Prise de conscience que, dans nos sociétés modernes, l'industrialisation de l'alimentation peut aboutir à un appauvrissement des produits. Préoccupation de la dimension qualitative de la nutrition face à une vie plus irrégulière et stressante pouvant aboutir à des carences. Recherche de produits comportant véritablement les ingrédients utiles à la santé, comme: les minéraux, les vitamines, les oligoéléments, le fer... La dimension nutritionnelle garantit la richesse d'ingrédients positifs pour la santé.

**Le besoin d'allègement**

C'est une préoccupation des formes. Il incarne la demande superficielle d'une silhouette fine et aérée. Cette recherche de santé par l'allègement cherche à relier les contraires. En effet, le schéma n'est plus: "Il faut souffrir pour être beau/boîte" mais "je veux tout". Les produits innovants performants ne passent plus par le régime et la tristesse. Les produits allégés proposent le paradoxe: légèreté + plaisir gourmand.

**1993/... Le besoin de vie saine**

C'est le besoin d'individus qui s'orientent de plus en plus dans leur vie, vers la recherche des sens. Dès lors, les produits santé ne doivent plus se restreindre à une fonction: relendre le corps et l'esprit, être un apport d'ingrédients ou permettre la minceur... Ils doivent prioritairement assurer que leurs conceptions sont honnêtes, qu'elles restituent les qualités originales des produits possédant les meilleures vertus naturelles. Une vie saine pour des consommateurs en quête de clarté, de vérité et de bien-être naturel.

**En synthèse**

Une analyse qualitative en matière de nouveaux concepts produits et de leurs communications qui fait apparaître deux règles majeures:
- la santé n'est pas un concept en soit mais fluctue en fonction des mentalités et des époques,
- la seule valeur santé ne suffit pas pour être attractive. Il s'agit d'y associer une valeur positive et déroutante qui vienne contrebalancer l'image un peu sérieuse et médicale de la santé.

**BILAN DE LA DÉCENNIE 1985–1995**

"Une perte d'engagement pour l'hypermédicalisation alimentaire":
- un pic dans les investissements et dans les innovations produits en 1991,
- une nette tendance à la baisse depuis 1991.

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**Figure 1: Évolution des investissements publicitaires des produits santé (en MF).**

**Figure 2: Investissements publicitaires des deux concepts produits santé (en MF).**

**Figure 3: La part des investissements publicitaires des produits santé par rapport au total alimentaire (en %).**

**Figure 4: Nombre de lancements par an.**

**Les investissements publicitaires**
- Une baisse des investissements qui n'est pas le seul fait de l'effondrement du "light".
- Les produits à caractère nutritionnel voient également leurs investissements baisser (Figure 2).
NUTRITION 94/95: WHAT IS NEW FOR THE DAIRY SECTOR?

THE RECOMBINANT
TASTE OF CHEESE

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Genétique Microbienne, Institut National de la Recherche Agronomique, 78352, Jouy en Josas, cedex, France

An important objective in industrial fermentation by lactic acid bacteria is the production of reliable, good-tasting products. The use of recombinant DNA technology in achieving this goal is limited by a negative public image, and concerns that the product would somehow be marked as 'recombinant'. Nevertheless, genetic techniques have potentially much to offer in the construction of reliable strains, especially as properties of the lactic acid bacteria are being elucidated.

Some problems faced in industrial fermentation are:

1. Phage sensitivity of fermenter strains;
2. Irreproducible start-up of fermenter cultures;
3. Strains which grow well but give poor taste;
4. Strains which give good taste but grow poorly.

The present industrial approach to address these problems relies partly on luck. It is considered ethical to isolate a strain at random which is, for example, phage resistant or a high protease producer. The random approach to find strains with improved characteristics can be fruitful, but their useful properties remain a mystery, and may even be lost by subculturing.

A strain with desired characteristics may also be isolated by a directed, genetic approach. To this end, we have constructed genetic tools which can alter strain characteristics. Our genetic tools can be used in a wide variety of lactic acid bacteria, including those which are poorly transformable. It should be made clear that an "engineered" strain can be constructed which is identical to a strain isolated at random. The engineered strain has the advantage that its improved properties are well defined, and thus reliable, and can be reproduced.

We also developed a system to generate new strains with altered characteristics. Two advantages of our system compared to the current random method of screening for good strains are:

1. The new strain is perfectly food grade;
2. The particular improvement made can be identified, and eventually transferred into other strains used for fermentation.

Using the recombinant approaches which will be discussed here could lead to a systematic improvement of starter strains whose only trace of "recombinant genetics" is the simplicity of their application. Use of recombinant methods will come into acceptance by improving public information and by associating their use with a healthy, reliable and good-tasting product.

Cheesemaking is both an industry and a biological science. The precise steps taken to produce a particular product are in fact biological processes, enzymatic fermentations, which alter particular components of milk to give a desired taste and texture to the final product. Initially, bacteria found naturally in milk (the Lactic Acid Bacteria, or LAB) were used for fermentation, and gave rise to quite diverse products; more than 200 different types of cheeses made from raw milk are produced in France alone. As bacteriological methods improved, the types of bacteria which ferment milk to give a particular type of cheese were identified. This allowed industrial fermentation, where the types of bacteria could be defined and cultured in the laboratory.

In the last decade, molecular biology and biochemistry have taken the science of cheesemaking further: The particular enzymes that give flavour and texture to cheeses have been at least in part defined; they comprise proteases that break down proteins, and enzymes which hydrolyze sugars or synthesize complex sugars. Certain bacteria grow...
better than others; we know now that bacteriocins may be responsible for good survival against other bacteria, that abortive infection mechanisms improve survival in case of phage, and that enzymes, for example catalase, help bacteria to survive when toxic products accumulate at the end of a culture.

**THE INDUSTRIAL APPROACH** (viewed by a scientist). An important industrial objective at present is to select for those LAB which have the right combination of enzymes, and which are resistant enough to survive in culture. They must also survive against other bacteria and bacteriophages which could destroy a 'good' culture in a matter of hours. Although some of the desired properties are well defined, the selection itself remains empirical; strains are screened for the desired characteristics, and the one which best meets these requirements is retained.

Chemical mutagenesis can be employed to accelerate the selection of variant strains. This treatment is not considered recombinant, but it does cause the accumulation of mutations which could later be screened and selected. The disadvantage of such an approach is that the mutations which improve the fermentation properties of the strain remain unknown; thus, the improvement cannot be transferred, for example, to another strain.

**THE SCIENTIFIC APPROACH.** Bacteria naturally gain, eliminate, and modify genetic information, as exemplified below:

1. Many types of cells have a natural state in which DNA is taken up from the environment. This DNA can then be assimilated into the chromosome by recombination enzymes.

2. Short sequences, called Insertion Sequences (IS), are present in most natural strains. IS elements carry the genetic information to integrate into the chromosome at different positions; the insertion results in a mutation.

3. Bacteria themselves encode the information to be 'mutators'. When cells are in resting, or stationary phase, their own enzymes will favour mutations to occur. These mutations may permit a better survival, or confer other, random properties.

4. Plasmids exist in numerous organisms, and often carry useful genetic information. Many of them are capable of self-transfer to other strains, which then allows the new strains to adopt information.

It is partly for these reasons that certain 'variants' arise which are better adapted to their environment.

Our approach is based on the natural ability of bacteria to alter their genetic content. We use our understanding of these properties to force certain events to occur.

We developed two approaches to modify genetically strains which can lead to non-recombinant variants. In the first approach, a thermosensitive plasmid we call pG-host is used to exchange the genetic information on the chromosome with the information carried by the plasmid. Use of pG-host for this purpose has succeeded where other approaches have failed. The modified strain has no trace of the plasmid; only the desired modification is present in the strain. The genetic exchange can result in (i) introduction of new genes, (ii) alteration of an already present gene, (iii) removal of genetic information. In each case the change is stable and permanent. The resulting strain is not recombinant if it involves an alteration or rearrangement of DNA already present in the strain. In fact, the identical strain could be obtained using pG-host or using a random screening method, except that the precise change introduced is known when using pG-host.

Our second approach makes use of a genetic 'tag' which inserts randomly in the bacterial chromosome. The 'tag' is an IS element (described above) found naturally in some strains of Lactococcus lactis. The IS tag is cloned on pG-host. By raising the temperature of a bacterial culture containing the tag on the plasmid, we can select for its insertion in the chromosome. In this procedure, only the tag remains; the plasmid is eliminated, leaving no trace. This system can be used to select for variants with particular properties because insertion of the IS element can give rise to a strain with altered properties. The resulting strains are all food grade, because they contain no non-lactococcal DNA.

What are some rational objectives for the use of recombinant technology? Some bacterial variants which are better adapted for cheesemaking have defined characteristics (for example more of one type of protease, good resistance to phages, etc.). However, the right combination of characteristics may not be present in the same organism. Using a genetic approach, some of the desired characteristics can be combined in one organism. This may involve the transfer of a trait from one lactic acid bacteria to another, or even the elimination of an undesirable trait: in these cases, the "new" variant is not, formally, recombinant.

Both approaches described above can also be used to address an industrial need which has potentially increasing importance; the identification of their own strains. Both approaches described above can be used to give strains a 'signature'. Using the first approach, addition or removal of even a dozen nucleotides from the DNA genome is enough to distinguish a particular strain. With the second approach, the IS tag can insert somewhere on the DNA where it does not necessarily alter strain properties. The site of insertion will be unique. Strains can be readily identified by PCR techniques. A particular strain used for industrial fermentation can be earmarked in these ways.

**Perspectives.** Industrialists are interested in producing a reliable safe product, and at the same time want to improve techniques to overcome problems. The recombinant DNA approach, while potentially a powerful means to attain these goals, has a negative public

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**NO TO RECOMBINANT CHEESE!**

Hey, what IS recombinant cheese, anyway??

Uhhhh, To tell you the truth, I don't know exactly...
and industrial image because it implies the creation of monster strains. By working together, we can define the real problems involved in fermentation, and provide solutions which do not involve the transfer of 'foreign' genes, but rather a rearrangement of genes already present in the lactic acid bacterial repertoire. In this way the bacteria work for us. Whether it will be this year or in 10 years, this approach will surely be employed. Recombinant cheese may have a taste surprisingly similar to cheese itself.

DAIRY BACTERIAL STRAINS WITH PROBIOTIC PROPERTIES: CRITERIA FOR SELECTION

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ABSTRACT

Since Metchnikoff at the turn of this century, much research has been performed on the probiotic properties of lactic acid bacteria. Moreover, in the past decades, many results have been obtained, showing potential health beneficial properties of lactobacilli. However, published results are often ambiguous or conflicting. The reason for such discrepancies may reside in the fact that all strains of lactobacilli are not equivalent in terms of biological properties. This raises the question of defining criteria for the selection of the best strains.

As an example of strain selection, this paper deals with the scientific results obtained on the L. acidophilus LA1 strain in the Nestlé Research Center, in collaboration with an INSERM laboratory. First, data on the selection of the strain on the basis of its adhesion to (cultured) human enterocytes are given. Thereafter, in vitro data on the competitive inhibition of various enteropathogens by LA1 are presented. Finally, results on human studies on gut colonization, immunomodulating effects and anti-pathogenic properties of the strain are given.

The use of lactobacilli for milk fermentation has been known since Antiquity. Fermentation was primarily a means of preserving foods by acidification. The possibility that lactic acid bacteria, and especially lactobacilli, could exhibit beneficial health effects in consumers was first suggested by E. Metchnikoff in the late XIX century. Until now, many probiotic effects of LAB have been proposed (Bourlioux, 1994).

- Regulation of intestinal transit
- Degradation of undigested carbohydrates
- Barrier effects against enteropathogens
- Improvement of lactose intolerance
- Immune modulation....

Among the 200 genera and species constituting the normal human flora, lactobacilli and bifidobacteria are of special interest since they are economically important for the food industry. Although the fact that fermented milks have beneficial health effects is generally well accepted by consumers, the scientific data supporting the claims are often controversial if not lacking (Sanders, 1993). This may be due to the fact that in a given genus or species, all the strains are not equivalent in terms of probiotic activities. For example, it is well known by scientists, producers, and even consumers that depending on the bacterial strains used to produce fermented milks, the organoleptic properties are totally different (acidity, aroma, texture...). This is probably true for probiotic properties linked to particular strains. This clearly addresses the question of the selection of the best probiotic strains (O'Sullivan et al., 1992; Klaenhammer, 1992; Reid et al., 1990). Some criteria are rather obvious - non-pathogenic behaviour, resistance to gastric acidity and bile toxicity, resistance to technological processes - but they are not sufficient to imply biological activities. Thus, other criteria should also be considered, such as the ability of human enterocytes, ability to compete with enteropathogens, good gut colonization capacities, immune modulating properties (Marteau & Rambaud, 1993).

ADHESION OF LACTOBACILLI TO HUMAN ENTEROCYTES CELL LINES

Recently, two models of differentiated human intestinal epithelial cells became available (HT-29 and Caco-2 cells) (Pinto et al., 1983; Zweibaum et al., 1985). Because they express morphological and physiological characteristics of normal human enterocytes, they are considered as valuable tools for studies of bacterial adhesion. Indeed these models have been extensively used and validated in studies dealing with adhesion of enteropathogens (Gailard et al., 1985; Neesser et al., 1989; Mounier et al., 1992). More recently, they have been used to examine adhesion of LAB, particularly lactobacilli (Cocconier et al., 1992; Chauvière et al., 1992; Elo et al., 1991).

About 30 lactobacilli (all from the Nestec collection) were tested for their ability to adhere to Caco-2 cells. Only 3 to 4 were adherent and the L. acidophilus LA1 strain was the most adherent. (Bernet et al., 1994). The results obtained are summarized in Table 1.

The results indicate that among the strains tested only LA1 was highly adherent, LA3 was classified as moderately adherent whereas LA10 and LA18 were non-adherent. Upon examination of the adhesion mechanism several features appeared important. The effect of EGTA suggested that adhesion of LA1 was partially Ca-dependent. As for other strains (Conway et al., 1989; Chauvière et al., 1992), the adhesion of LA1 was primarily suspected to be mediated by an extracellular secreted protein because of the needs for the bacterial culture supernatant in the adhesion assay and its sensitivity to trypsin. However, recent works allow us now to rule this hypothesis. We have found that LA1 is able to adhere to the same extent to Caco-2 cells in the

<table>
<thead>
<tr>
<th>Lactobacilli strains</th>
<th>Adhesion Without EGTA</th>
<th>Adhesion With 20mM EGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus LA1</td>
<td>155 (26)</td>
<td>63 (5)</td>
</tr>
<tr>
<td>L. acidophilus LA3</td>
<td>66 (21)</td>
<td>18 (9)</td>
</tr>
<tr>
<td>L. acidophilus LA10</td>
<td>18 (3)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>L. acidophilus LA18</td>
<td>23 (7)</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>

Results are the means of three different experiments: number of lactobacilli (+/ SEM) adhering to 100 Caco-2 cells.
The absence of culture supernatant (acetate and phosphate-based buffers). As recently underlined by Greene & Klaenhammer (1994), in vitro adhesion of lactobacilli to enterocytes is influenced by pH. Indeed, we obtained better adhesion scores for LA1 in acidic conditions compared to neutral pH. However, a non-adherent strain (LA10 for example) remained non-adherent whatever the pH. It appears therefore that although the pH of the assay is important for adhesion studies, it is not the sole criterion. In addition, even if the global luminal intestinal pH is around neutrality, we cannot rule out that due to metabolic activity (proton pumps for example) of the enterocytes, their local pH is more acidic. Thus, adherent strains might be exposed to lower pH because of the close vicinity of intestinal cell membranes. Another consideration is the possible metabolic activity of the bacteria itself in the intestine. This could create an acidic micro-environment also favouring adhesion. Obviously, more work is still needed before firm conclusions can be drawn.

**EFFECT OF ADHERENT L. ACIDOPHILUS ON GASTRO-INTESTINAL PATHOGENS**

Anti-diarrhoeal effects of lactobacilli have often been claimed and reported in the literature. Some convincing data have been obtained (Isolauri et al., 1991; Kaila et al., 1995) using carefully selected strains. We undertook a study to test the ability of the most adherent strain, LA1, to inhibit competitively adhesion of various pathogens to both Caco-2 and HT-29 cells (see Table 2).

The data clearly indicate that LA1, by competing with pathogenic bacteria, limited the adhesion of the latter to the epithelial cells. However, LA1 was most effective when incubated either before or at the same time as the pathogens. A dramatic loss of efficiency was observed when the pathogens were pre-incubated with the cells.

We then studied the capacity of LA1 to protect Caco-2 cells from invasion by enteroinvasive pathogens (Table 3).

As shown previously, LA1 inhibited attachment of enteroinvasive pathogens and consequently their capacity to invade the cells. In addition, the effect was dose-dependant (see 10^8 versus 10^7 cfu/ml) (Figure 1).

Another interesting effect of LA1 was observed with *Helicobacter pylori*. This Gram-negative pathogen is often cultured from gastric biopsies of patients suffering from gastric ulcers. Infection by *H. pylori* is a risk factor for further development of gastric ulcers and even gastric cancers. We investigated the effect of LA1, the non-adherent LA10 and a *L. bulgaricus* on adherence of this microorganism to HT-29 cells (Figure 2).

HT-29 alone did not express urease (Control). When cells were incubated with *H. pylori*, the activity was maximum (Hp). Competition with LA1 lead to a strong inhibition of urease activity (Hp/LA1), whereas the non-adherent LA10 and *L. bulgaricus* were almost inactive (Hp/LA10 and Hp/LJ5, respectively). In fact, further experiments demonstrated that the anti-*H. pylori* effect was not due to LA1 itself but rather to a secreted factor, since the same effect was obtained with a spent supernatant irrespective of the presence of LA1. A double-blind controlled study with *Hp* positive patients is currently underway.

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**Table 2: Competitive inhibition of diarrhoeagenic E. coli by LA1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>ETEC CF1A (% inhibition)</th>
<th>DAEC C-F1845 (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation together with LA1^b</td>
<td>80 (5)</td>
<td>85 (8)</td>
</tr>
<tr>
<td>Pre-incubation of LA1^b</td>
<td>77 (7)</td>
<td>81 (3)</td>
</tr>
<tr>
<td>Post-incubation of LA1^c</td>
<td>22 (3)</td>
<td>45 (5)</td>
</tr>
</tbody>
</table>

^a 35S-labelled E. coli (10^6 cfu/ml) and LA1 (5 x 10^6 cfu/ml) were incubated together on Caco-2 cells.

^b LA1 (10^6 cfu/ml) for 30 min then 35S-labelled E. coli (10^6 cfu/ml) for 60 min.

^c 35S-labelled E. coli (10^6 cfu/ml) for 30 min, then LA1 (5 x 10^6 cfu/ml) for 60 min. Results are expressed as the mean (+/-SEM) of three experiments on three successive passages of Caco-2 cells. ETEC: enterotoxigenic E. coli. DAEC: diffusely adhering E. coli.

**Table 3: Inhibitory effect of LA1 on enteroinvolut bacterial invasion of Caco-2 cells**

<table>
<thead>
<tr>
<th></th>
<th>EPEC (JPN-15)</th>
<th>Y. pseudotuberculosis (YPIII)</th>
<th>S. typhimurium (SL 1344)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell invasion (% of incubated bacteria)</td>
<td>1.5 (0.4)</td>
<td>8.5 (0.9)</td>
<td>8.0 (3.0)</td>
</tr>
<tr>
<td>Inhibition of cell invasion (% of inhibition)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA1 10^8 cfu/ml</td>
<td>95 (2)</td>
<td>64 (19)</td>
<td>37 (4)</td>
</tr>
<tr>
<td>LA1 10^7 cfu/ml</td>
<td>31 (9)</td>
<td>15 (7)</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>

Invasion was measured after extensive washing of the monolayers and incubation in the presence of gentamycin to kill non-internalized pathogens. Pathogens and LA1 were incubated together.
IN VIVO HUMAN EFFECTS OF FERMENTED MILKS CONTAINING LA1

A critical feature in determining the biological effects of LAB at the gut level is their ability to survive the gastric acidity and bile toxicity. It is well known in this respect that _L. bulgaricus_ has poor survival (1/10 000 remains alive in the small bowel). _L. acidophilus_ has a better survival rate and is recovered from the faeces of volunteers fed fermented milks containing strains of this species (Marteau et al., 1992; Schiffrin et al., 1995) (Figure 3).

LA1 was further tested for its ability to increase the blood phagocytic capacity in humans after consumption of a fermented milk for 3 weeks. Results showed that both the monocytes and granulocytes from peripheral blood were activated and able to phagocytose an opsonized _E. coli_. Moreover, the effect on phagocytosis lasted for at least 6 weeks after intake of the LA1-containing fermented food had ended.

No changes were seen in the percentages of other blood leucocyte subsets (Schiffrin et al., 1995).

Finally, we assessed the immunoadjuvant properties of a fermented milk containing LA1. Human volunteers received an oral vaccine (_S. typhi_ Ty21A) with or without consumption of a fermented milk. The results revealed a significant increase of blood IgA response to the _S. typhi_ LPS in the group receiving the fermented milk (Link-Amster et al., 1994) (Figure 4).

Although the immunoadjuvant effect was evident in this study, it was difficult to attribute this effect to LA1 itself because of the presence of other bacterial strains in the tested product. However, very recently another study was performed where LA1 itself was shown to have immunoadjuvant effect on total serum IgA. In this work, LA1 alone (no other associated bacteria and no concomitant vaccination) was given in a fermented milk to volunteers (Marteau et al., submitted).

CONCLUSION

Although probiotic properties of fermented dairy products have been suspected for centuries, actual scientific proof of these effects is lacking (Sanders, 1993). This may be due to the fact that probiotic properties are not exhibited by all strains. This highlights the importance of strain selection. In this paper, we report data showing that adhesion to human intestinal cells can be a valuable criteria even if the underlying mechanisms are not clear (Greene & Klaenhammer, 1994). Other criteria should also be considered such as origin of strains, immune properties. Thus to obtain the desired effects, more attention should be paid to the choice of the bacterial genera used in fermented foods. On the basis of our work with the LA1 strain, it appears that the main effects observed (adhesion, exclusion of pathogens, immunoadjuvant effects, increase of phagocytosis) are taking place in the small bowel and the stomach rather than in the colon. This could mean that lactobacilli are more likely to induce effects in the small bowel, which is normally a poorly colonised organ. The intake of carefully selected lactobacilli through fermented milks ingestion could create a local, transient but important presence of Gram-positive, non-pathogenic bacteria provide a barrier against pathogens and a stimulation of the small bowel associated immune tissue. In the colon, the situation is probably completely different since this organ is highly colonised, it would be difficult to significantly modify the ecology with fermented milks. In any case, biological activities should be examined in the human, through controlled studies involving industry, academic scientists and clinicians. Such strategies will probably help to make the probiotic concept acceptable to the scientific community, regulating bodies and consumers.

LITERATURE

FERMENTED MILKS AND HEALTH BENEFITS

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ABSTRACT
Fermented milks have been consumed for several centuries and health benefits have often been associated with them. Since the beginning of this century, we have seen increasing interest in this food from the scientific community. Some health advantages of fermented milks, like improvement of lactose digestion in lactose deficient individuals and reduction of certain types of diarrhea, are now well documented. Several studies have indicated positive effects on maintenance of the natural balance of the intestinal flora and on immunomodulation. Action of fermented milks on reduction of colon cancer and treatment of urogenital tract infections have been noted but need further investigation. Microorganisms found in fermented milks, mainly lactic acid bacteria, have been shown to provide this variety of health benefits, as a result of their growth in milk and/or simply their presence in high numbers. In this paper we discuss the different parameters which we think should be considered in studies dealing with fermented milks, like growth and survival of the various strains as well as their metabolic and structural properties. Strain specificity and the effect of combining several strains on the final effect are also addressed. This discussion is supported by a few examples from the literature or from the studies undertaken with Danone.

INTRODUCTION
Fermented milks have been consumed for thousands of years, and various health benefits have been associated with these products for hundreds of years. Matchenko in the early 1900s with his book "The Prolongation of Life" encouraged other researchers to pursue studies on fermented milks [1]. The first "curdled milk" appeared in 1906 in France and was prepared with pure lactic acid bacteria cultures according to a protocol proposed by Matchenko [2]. In the 1970s, the possible probiotic effects related to the consumption of fermented milks saw increased scientific interest.

To prevent contamination and improve storage properties, milks were historically fermented with traditional cultures composed of a mixture of unidentified bacteria and yeasts found naturally in the environment and giving rise to a variety of products like yogurt, kefir, koumis and leben. These cultures are of considerable diversity and provide a variety of fermented milks with different tastes and textures. During the last century many lactic acid bacteria have been isolated and characterized and strains are now carefully selected for the health benefits they demonstrate, to provide the basic for "novel" fermented milks produced in a modern and scientific way that assures a more consistent food, a product of improved quality, and complies with more and more of consumers' needs seeking a good, nutritional and healthy diet.

We will discuss here some of the data available in the literature on health benefits of fermented milks and present results obtained with Danone. Only lactic acid bacteria presently utilized in milk products are considered here.

GENERAL CONSIDERATIONS
Today, health is a major concern and to answer the consumers' expectations, the food industry must: (i) collect and generate information on health benefits of fermented foods, (ii) look for the mechanisms leading to the observed benefits, and (iii) run clinical trials with the final products under a statistical procedure that will ensure the reality of any health attribute.

Health benefits of fermented milks may be attributed to the milk, to the bacterial cell components or to the live microorganisms, to the metabolites produced in the course of the fermentation or of the digestion of the product and finally to the action of the microorganisms during their passage through the gastrointestinal tract. Fuller [3] defined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Nowadays, the term probiotic is often used for live microorganisms which have an action not only on the intestinal flora, but also on other parts of the body. A broader definition could be envisaged for the term probiotic as "a live microbial feed supplement with a beneficial effect on the host". Milk components, dead bacterial cells, and fermentation and digestion products can be referred to as prebiotics as they concern a set of lifeless molecules. Fermented milks are always a combination of probiotic and prebiotic actions.

While dead bacteria sometimes show positive effects in the host, in most cases intact live cells give a broader effect. For an optimal action, LAB must be present in high numbers and survive the acidity of the stomach.
and the bile acids in the intestines. As a first step in fermented milk development, good growth and survival of the various bacterial strains in fermented milks and during the gastrointestinal transit must be assessed to ensure adequate activity. Of course, only strains which have a history of safe use (for example isolated from traditional lactic cultures) or supported by toxicological studies will be kept for further developments.

Depending on the health benefits studied, the second step in the selection of specific strains can be based on metabolic potentialities (production of peptides, polysaccharides, acids, bacteriocins, enzymes, ...), structural properties (for example cell wall composition) or interaction with host cells (transient contact or adhesion) and with the intestinal flora. Before any of these activities can be utilized in the selection process, correlations between the particular property and a specific health benefit must be proven in vivo.

Combinations of bacteria, the most elaborate of which is symbiosis (mutually beneficial association of two species), add another dimension to health benefits of fermented milks, as they can produce new molecules. Traditional fermented milks in the world constitute an immense source of strains or microbial combinations with probiotic potential.

**SOME FACTS ABOUT FERMENTED MILKS AND HEALTH**

Those species that have been extensively studied so far, with several experimental data on man, are the two yogurt bacteria *Streptococcus (S.) thermophilus* and *Lactobacillus (L.) bulgaricus*, *L. casei* and bifidobacteria. *L. acidophilus* has received important scientific interest, with however very few human studies. We will present here major proven health benefits of milks fermented with those bacterial species, and discuss whenever possible the impact of the specific selection and utilization of particular strains.

**Yogurt**

The most factual health benefit linked with yogurt consumption is the reduction of lactose malabsorption [4]. This effect is endorsed by both observation and clinical trials, but the mechanism is not yet fully understood. Some specificity among lactic acid bacteria species has been shown, with *L. bulgaricus* giving the highest activity, bifidobacteria being on the low activity side [5]. Heat-treated yogurt will not show the same level of activity toward lactose digestion as live yogurt [6].

The first mass production of yogurt was started by the pharmacist Isaac Carasso, with the goal of combating intestinal infections. This idea utilized common knowledge in European and Asian populations. A few studies give statistical data on the effect of yogurt on the reduction of diarrhea in man [7, 8]. In their last study, Saavedra et al. [8] tested a yogurt with both *S. thermophilus* and *Bifidobacterium bifidum*. Whether one or both bacteria were responsible for the observed effect cannot be assessed.

Yogurt bacteria show some influence on immunological parameters. Consumption of yogurt was associated with a decrease in allergic symptoms in the elderly [9]. Pottier et al. [10] recently demonstrated the adjuvant effect of yogurt and *L. casei* D-114 001 (formerly called 114 002) fermented milk on the vaccinal anti-cholera immunity response in mice. This effect was obtained even with heat-treated yogurt, but not with milk alone, suggesting that cell components or fermentation products rather than active organisms were needed for this specific action.

**Milks fermented with bifidobacteria**

Certain strains of bifidobacteria survive the intestinal transit [11] and reach the colon in significant numbers [12]. Because they are one of the major species present in the colon, bifidobacteria can influence the endogenous intestinal microflora, which results in such physiological effects as altering enzyme activities of the microflora [13], and regulating gut transit [14].

Bifidobacteria help in some diarrhea [8, 15] or constipation [16]. A recent crossover and double blind study conducted on 36 women has indicated the specific action of milk fermented with yogurt bacteria and *Bifidobacterium* Bio strain D-173010, a strain selected for its good survival in fermented milks and throughout the gastrointestinal transit, on the reduction of the sigmoidal transit time compared to yogurt [17]. It had previously been shown that this effect was obtained with live bifidobacteria cells only [14].

Bifidobacteria are reported to improve digestive parameters [18] and demonstrate some antipathogen actions in man [19]. They also exert an action on parts of the immune system [20, 21].

Several strains reduce the levels of some colonic enzymes implicated in the conversion of procarcinogens to carcinogens in humans [13, 22]. Fermented milks with viable yogurt strains (D-540 083), *L. helveticus* (D-119 028) or *Bifidobacterium* sp (D-173 010), but not with *L. acidophilus* (D-112 023) have shown an effect on colon cancer cell growth and differentiation with cocultures in vitro. *Bifidobacterium* sp, along with *L. helveticus*, were most effective in reducing human HT-29 cell growth rate and in increasing the activity of dipeptidyl peptidase IV, a specific marker of cell differentiation [23]. These results are indicative of some specificity among various lactic acid bacteria.

Major effects have been found with populations of bifidobacteria in fermented milks of $10^8$ cells/g [8, 14, 17]. Bifidobacteria are not well adapted to fermented milks and suffer in the presence of oxygen. Therefore, important selection criteria for specific strains is growth and survival in acidified and partly aerobic conditions.

Some studies involve milks fermented with a combination of bifidobacteria and *L. acidophilus*. Whether or not this combination gives additional or altered health benefits is not known.

**Milks fermented with *Lactobacillus casei***

*L. casei* is mainly found in the small intestine in man. Some strains of *L. casei* can survive the transit through the stomach [24] and reach the ileum in sufficient quantities to have a physiological effect. In a recent in vitro study, we showed that the ileum survival rate of several strains of *L. casei* was 5–10% (except for one strain : 0.5%) compared to Bifidobacteria (10–30%) or *S. thermophilus* (0.5%) if associated with *L. casei* [25].

Among LAB, *L. casei* is the most studied species for its effects on the reduction of incidence and duration of several types of diarrhea and its capacity to modulate the immune system. It can aid the prevention and treatment of antibiotic-related diarrhea [26] and travellers' diarrhea [27], and have a strong effect on infantile diarrhea [28–30]. *L. casei*‘s ability to ameliorate or prevent certain types of diarrhea seems related to its ability to alter the activity of the intestinal microflora thus preventing the development of potentially undesirable microorganisms. But the real mechanisms for specific actions are complex and not fully understood. Studies on animals have demonstrated both a stimulation of the non-specific and specific immunity system. Kaila et al. [31] found increased levels of several immunoglobulins in their study of children with acute diarrhea when treated with *L. casei* strain GG. In this study a dosage of $10^6$ *L. casei* per day was utilized. The effect was not observed with heat-treated fermented milk. We
studied resistance to *Salmonella* infection on mice in a random study. The survival yield after *Salmonella* infection was 90% with a milk fermented with yogurt bacteria and *L. casei* D-114 001, 75% with *L. casei* D-114 001 alone, 50% with Yakult, *L. casei* strain B or yogurt and only 10% with milk [32]. This observation demonstrates some level of strain specificity and shows that a combination of different strains can give additional benefits. A requirement for adhesion of the bacterial cells to intestinal cells to get an effect on diarrhea or on the modulation of the immune system has been postulated but is so far not proven.

*L. casei* has been shown to decrease the activity of enzymes related to the risk of colon cancer [33], and to inhibit mutagenicity [34]. *L. casei* was also found to decrease the recurrence of superficial bladder cancer in humans. The mechanism of action may be associated with both a reduction in the formation of carcinogenic compounds in the intestines and an immunomodulating effect [35]. Additional studies will be needed to further investigate the effect of *L. casei*, as well as that of Bifidobacteria and other lactic acid bacteria, in reducing the risk of cancer.

**Milk fermented with Lactobacillus acidophilus**

*L. acidophilus* is one of the *Lactobacillus* species that survive well the gastrointestinal transit. Like *L. casei*, it is most often found in the small intestine. It also constitutes the dominant flora of the urinary tract in women. Among possible involvements of *L. acidophilus* in human health, one can cite the reduction of blood cholesterol [36] and fight against vaginal *Candida* infections [37]. Additional carefully controlled clinical studies are needed to confirm these observations.

Many *L. acidophilus* strains do not grow well in milk and survive poorly in fermented products. Selection of specific strains must consider this point.

**CONCLUSION**

While studies on the effects of consuming fermented milks with live bacteria are more and more numerous, more rigorous well-structured, double-blind trials with appropriate controls are needed to guarantee the reality of health benefits. For a better selection of specific strains and a better understanding of interactions between food, microorganisms and the host, more studies are needed to establish the mechanism(s) by which these bacteria exert a pro- or prebiotic effect and determine which properties are responsible for this activity. The presence of viable bacteria in large numbers, together with good survival throughout the gastrointestinal transit, are very often a prerequisite for optimal activity. All the other health oriented properties of microorganisms utilized in fermented milks stay at the level of working hypothesis as no correlation between a specific structural or metabolic property and a health benefit has so far been clearly shown in man.

Due to difficulties in carrying out properly controlled clinical studies, strain specificity has rarely been proven in vivo. Nevertheless, some strain specificity has now been shown in vitro or with animal studies. In addition to growth and survival of each strain, the organoleptic properties resulting from the fermentation of milk must be considered in the process for strain selection, as fermented milks must stay as always a good food. Traditional fermented milks are so numerous in the world that they constitute an immense source for the search of appropriate bacteria with specific properties.

The food industry already uses the specificity of lactic acid bacteria strains to improve existing fermented products and to develop new ones. Strain specificity can be used for food products with specific health benefits which will answer consumers’ needs.

**LITERATURE**


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**POSITION AND ACTIONS OF EDA ON PDCAAS**

**T. van Hooydonk**

Belgium

The nutritional evaluation of dietary proteins has a long history of research and debates on its methodology. This is not surprising because of the complex nature of protein absorption and utilization and the far-reaching implications with respect to the nutritional image of a protein and its impact on nutritional, agricultural and regulatory policies.

The biological assays such as PER, BV and NPU were the methods of choice in the past but, on the recommendation of the FAO/WHO Expert Consultation (1990), have now been replaced by a more chemical assay called PDCAAS (Protein Digestibility Corrected Amino Acid Score).

In essence the nutritional score of a protein depends with this method on the limiting essential amino acid compared with a reference pattern. Indeed an easy, simple and cost effective method but also with serious limitations.

The Technical Subgroup of EDA has clearly addressed these shortcomings in publications and during special workshops.

Our main conclusions are:

1. The basic knowledge about human protein absorption and utilization is still inadequate to estimate the protein and amino acids needs precisely which weakens the robustness of the reference patterns used to calculate the score.

2. The PDCAAS method inherently neglects physiological effects such as curd formation in the stomach, the contribution of proteins on the bio-availability of minerals, bio- active peptides and anti-nutritional factors present in most vegetable proteins. These still poorly understood attributes may be implicitly evaluated in a bio-assay.

3. The PDCAAS by definition does not discriminate between the nutritional value of relatively rich proteins because the maximum score is set at 1. In this way valuable information on protein complementation is lost.

In a special workshop of the Utrecht Group organized by the Dutch Foundation of Dairy, Nutritional and Health the experts unanimously formulated the following resolution:

"...The advantages and limitations of the PDCAAS method for assessing the comparative nutritional quality of food proteins was discussed. It was the consensus of the workshop that the convention of rounding down to 1.0 PDCAAS values that are greater than 1.0 does not adequately credit the ability of proteins of high quality, such as those of cow's milk, to complement the nutritional value of dietary proteins of lower quality. It is strongly recommended, therefore, that the primary PDCAAS value also be used as a basis for assessing the value of high quality proteins".

Another action of EDA is the execution of a research project at the University of Limburg (the Netherlands). The aim is to compare the difference in utilization of dietary protein, using an advanced pig model capable of measuring the fluxes of each amino acid at relevant areas in the pig’s body. The first results indicate a remarkable function of intestinal cells on amino acid release. These findings and first conclusions are presented in the following paper.
HEALTH ASPECTS OF BST MILK

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2 University of Cambridge, Department of Anatomy, Downing Street, Cambridge CB2 3DY, UK

ABSTRACT
In theory, employment of BST in dairying might have two possible types of adverse effect on the health of consumers — those resulting from consumption of the milk and those resulting from milk avoidance. In the latter case, because milk is a valuable source of nutrients, the predicted significant reduction in milk consumption were BST to be licensed in the EU might exacerbate deficiency diseases, such as osteoporosis. But the main focus of this paper is on the doubts which have been expressed over the safety of consuming milk from BST treated cows, in which the concentration of insulin-like growth factor 1 (IGF1) is increased.

Official statements (such as those of the USFDA) have claimed that IGF1 in milk of BST treated cows is: (i) not substantially increased in concentration; (ii) undergoes digestion in the gut lumen; (iii) is, in any event, biologically inactive in the consumer at such low concentrations. The paper examines such claims in the light of the recent publication of several scientific reports and expert review articles.

There is now evidence for the following: (i) milk IGF1 concentration in milk can increase substantially (up to five-fold according to one of the manufacturers) in response to BST injections; (ii) the milk protein casein protects IGF1 from proteolysis (consistent with the biological role of IGF1 in the neonate); (iii) at the concentration of IGF1 in milk, even of non-BST treated animals, it exerts mitogenic effects in the ileum of rats. However, the claim that IGF1 is likely to cause breast cancer in consumers appears to be without scientific foundation.

Safety concerns thus relate to possible effects of raised IGF1 concentrations in milk on the gut tissues of the consumer. Conceivably, such effects might be beneficial. But sufficient uncertainty appears to exist as to suggest the need to explore the issue more fully in the period before the EU next considers licensing of BST. In particular, research is recommended on the effect of IGF1 on gut cytokinetics, studies which have not featured in previous safety evaluations.

SUMMARY OF RECOMMENDATIONS
It is recommended that the safety of milk and milk products from cows treated with BST be re-examined in the light of recent reports which suggest that insulin-like growth factor 1 (IGF1) in such milk is both bioactive in intestinal tissues and protected from degradation by casein in milk.

INTRODUCTION
In theory, employment of bovine somatotropin (BST) in dairying might have two types of adverse effect on the health of consumers — those resulting from milk avoidance and those resulting from consumption of milk which has been subject to compositional change.

With respect to the former possibility, a communication from the European Commission to the Council and the European Parliament in 1993 stated:

- According to the Information and Forschung Institute (IFO) study the proportion of consumers which would favour a total boycott of products associated with B.S.T. would lead to a drop in consumption of some 11% [1].

Because of the dietary importance of milk, such a reduction would seem likely to have a significant adverse effect on public health. For example, in the UK, milk and milk products supply almost 60% and 20%, respectively, of dietary calcium and protein, and are important sources of vitamins and trace elements [2]. Consequently, the approximately 30% reduction in per capita intake of calcium from milk and milk products which occurred between 1980 and 1990 [3] has undoubtedly contributed to the increasing rates of osteoporosis, a disease of which there are currently 140,000 new cases per annum among postmenopausal women in the UK [4] and which costs the Health Service £500 million per annum [5].

Further reductions in the intake of calcium, which would be predicted from an increase in milk avoidance due to BST, could only exacerbate this trend.

There has been much debate concerning the second possibility, namely, that consumption of milk from BST treated cows might present a health hazard to the consumer. However, it is widely recognized that the BST which enters milk is, itself, unlikely to pose any risks, for severe reasons, viz: (i) the quantities present are small; (ii) it is probably biologically inactive in humans; (iii) it is destroyed by pasteurization; and (iv) it is largely digested in the gut. But BST is not the sole concern: the physiological process by which injection of BST induces an increase in milk yield also has the effect of increasing the concentration in milk of insulin-like growth factor 1 (IGF1) [6]. This is, at least, a theoretical hazard, because (i) bovine IGF1 and human IGF1 are identical molecules [7], so that IGF1 (which is a potent inducer of cell proliferation) in bovine milk may be bioactive in humans; and (ii) IGF1 is not destroyed by pasteurization.

Official statements, for example those of the UK Government's Ministry of Agriculture, Fisheries and Food [8], claim that IGF1 in the milk of cows treated with BST poses no threat to consumer health because: a) the milk concentration of IGF1 is not substantially increased by BST; b) IGF1 undergoes digestion in the gut of the consumer; and c) IGF1 is biologically inactive in humans at such low concentrations.

The object of this paper is to subject these claims to rigorous examination, since it would clearly be in no one's interests for any loophole to be overlooked when we are considering a food product of such nutritional, social and economic importance. In examining this issue it is important to note that while the official human safety evaluations of BST were conducted some years ago, there have been several very recent reports which have a bearing on the safety question. We thus need to consider whether these new findings necessitate a reappraisal of the evidence on which the original safety claims were made.

THE INCREASE IN MILK CONCENTRATION OF IGF1 IN BST MILK

The earliest report, in a referred academic journal, of an increase in the concentration of IGF1 in milk of treated cows showed a 3.6-fold increase over a period of 7 days, during which the cows received daily injections of BST [9]. Subsequent reports of the IGF1 concentration in milk of BST-treated cows have suggested two- to five-fold increases, depending on dose, with some elevations being statistically significant and others not, for example [10]. But many such reports have appeared in unrefereed publications; for example, a recent definitive review [11] article cites only four reports of IGF1 concentrations, all of this type. Thus there appears to be a paucity of information in peer-reviewed articles on this important question.

However, two statements made by manufacturers of BST in their applications for marketing authorization to the
CCE Committee for Veterinary Medicinal Products are instructive. Lilly Industries claimed that the milk of cows treated with their BST product (Optiflex B40): is unlikely to contain more than 50 ng/ml IGF1 [12]. This compares with a mean figure for bulked milk of 4.32 ng/ml (range 1.27–8.10) reported by the USFDA [13]. Moreover, in their equivalent application for marketing authorization, Monsanto claimed in respect of studies with their BST product, Somatech: the IGF1 levels went up substantially [about five times as much] [14].

Hence there seems to be no doubt that the concentration of IGF1 in milk is increased by BST treatment, although the extent of increase appears variable. It is often claimed that the increase in IGF1 concentration in milk caused by BST is within the normal physiological range, and it is certainly true that the concentration varies with stage of lactation, being particularly high (greater than 150 ng/ml) in colostrum [15]. However, this point is not strictly relevant because colostrum is not marketed. Moreover, if concentrations are naturally higher, for whatever reason, BST will tend to increase them still further.

Another important issue is the accuracy with which the concentration of IGF1 in milk is assayed. Many studies employ an acid-ethanol extraction procedure to remove the IGF1 binding protein (IGF1BP), which otherwise interferes with the determination of IGF1 by radioimmunoassay. However, the technique is inefficient and back-binding of the IGF1 to IGF1BP occurs following neutralization of the acid-ethanol extracts, thus leading to underestimation of IGF1 [11].

Uncertainty also exists about the extent to which IGF1 in milk of BST-treated cows exists in the form of des(3N)IGF1, a truncated form of the molecule with more potent bioactivity. Following concerns earlier expressed by one of us [16], Monsanto Company scientists developed a procedure to isolate des(3N)IGF1 from milk of cows treated with BST. Their analysis of a single bulked milk sample from six cows treated with BST suggested that des(3N)IGF1 accounted for less than 3% of the total IGF1 present [17]. The authors conceded that the method was not strictly quantitative... making it difficult to precisely calculate the concentration of IGF1, but even so the presence of this quantity might represent a 30% increase in bioactivity, since des(3N)IGF1 has been shown to be up to 10 times more potent than IGF1 itself.

DIGESTION OF IGF1 IN THE GUT
The MAFF report states: IGF1 is degraded in the gut in the same way as other proteins including BST [6].

There is now considerable evidence challenging that claim. In experiments reported by the USFDA [13] in which rats were fed IGF1 by gavage, there were significant increases in body weights, liver weights and tibial lengths of animals receiving 2.0 mg/kg body weight. As these are all systemic effects it is clear that IGF1 escaped digestion and was absorbed into the general circulation in amounts sufficient to induce anabolic effects. In fact, certain other systemic effects were observed at 1/100th of that dose level (20 μg/kg) but dismissed by the authors as sporadic results. (It is of course questionable whether short-term experiments on rats provide data relevant to the safety of long-term dietary habits of humans.)

But direct evidence for the protection of IGF1 from digestion by the milk protein casein has also recently been obtained. Xian et al. [18], who were investigating the possibility of using IGF1 therapeutically to promote tissue growth and repair in the gut, discovered that while IGF1 administered alone was rapidly degraded in the stomach and small intestine, when administered with casein it was substantially protected from proteolysis. For example, 80–90% of IGF1 survived 60 min incubation in rat stomach or duodenal luminal flushings, when in the presence of casein at concentrations at which it is present in milk (30 mg/ml).

Such findings, which are paralleled by those indicating a similar protection of epidermal growth factor by casein [19], are consistent with the postulated role of IGF1 present in milk in stimulating intestinal development in the neonate [20].

BIOACTIVITY OF IGF1 IN THE HUMAN GUT
According to the MAFF report [8]: Exposure of the gut lining to ingested IGF1 would not have any effect on the rate of cell division of the epithelial cells, as the receptors on these cells are restricted to the cell surfaces which are not in contact with the gut contents.

This claim, which appears remarkably categorical, is called into question by a number of observations and expert opinions. According to a recent expert review, IGF1: binds to the GI tract, upregulates its own receptor, and may stimulate cellular proliferation [20]. Indeed, it has been known for some time that type 1 IGF1 receptors are present on the luminal surface of the gut epithelium [21]. Moreover, experiments on rats showed that local intraluminal infusion of IGF1 at concentrations equivalent to those in normal milk induced polyamine synthesis in mucosal tissue, a process linked to cell proliferation and indicative of IGF1's trophic action on mucosal cellularity [22]. In another study, suckling rats which were administered IGF1 orally showed significantly increased specific activities of the following jejunal enzymes: maltase, lactase, alkaline phosphatase and amino peptidase [23]. Moreover, in vitro studies on tissue from the human gut have shown that IGF1, albeit to date at concentrations higher than those in BST-milk, stimulates cell division [24].

Prestigious medical committees have expressed doubts about the effects of increased concentrations of IGF1 on the consumer. Thus, a committee of the American Medical Association considered that: Further studies will be required to determine whether ingestion of higher than normal concentrations of bovine insulinlike growth factor is safe for children, adolescents and adults [25]; while a US National Institutes of Health assessment panel recommended determination of: acute and chronic local actions of IGF1, if any, on the upper intestinal tract [26]. However, the evidence on safety of IGF1 in milk employed by the USFDA [13], while it referred to measurements on a number of organs of rats fed high concentrations of IGF1, did not examine local effects on the gut or, specifically, gut cytokinetics. It is for this reason that Burton et al. [11], in a review article, concluded:

Therefore it could be considered an oversight for Juskevich and Geyer [of the USFDA] to suggest that ingested IGF1 is inactive. Many more potential effects of ingested IGF1 on the gastrointestinal tract and the local immune system of the gut need to be explored.

Subsequently, these authors pointed out that the concentrations of other bioactive proteins in milk might be altered by BST treatment, and suggested the need to conduct the following experiments:

1) full characterization of hormones and bioactive substances in milk of... treated cows, including... prostaglandins, erythropoietin, progesterone, prolactin, thyroid hormones, gonadotropin-releasing hormone, thyrotropin-releasing hormone, growth hormone-releasing factor, vasoactive intestinal peptide, epidermal growth factor, estrogens, relaxin,... etc. etc.
2) the feeding of milk from... treated cows to neonatal primates to determine effects on gastrointestinal tract development, absorption and function.

3) the effects of consumption of milk from... treated cows on immune system function in the gut.

Other reviewers [27], after emphasizing the physiological effects of IGF1 in bovine colostrum in stimulating intestinal development in the calf, concluded with the following remarkable statement: mature cows' milk... may be a more perfect food than it is usually thought because even very low concentrations of IGF-1 in milk may have a positive biological action in the consumer. This shows a touching faith in the benevolence of Nature but it must be questioned whether constant exposure of the consumer's gut to a factor which stimulates intestinal development is necessarily always a 'positive' influence.

From the above, there seems little doubt that IGF1 in milk is bioactive at the level of the gut epithelium. Conceivably, as Baumrucker & Blum [27] imply, the effects might be beneficial: but it seems imperative that the issue is investigated more fully to establish precisely what effects IGF1 has on gut cytokinetics.

EPSTEIN'S CLAIM THAT CONSUMERS ARE AT RISK OF BREAST CANCER

Epstein [28] has claimed that IGF1 in milk might promote development of breast cancer. This suggestion would seem to be unwarranted for several reasons [29]. Thus: 1) the normal concentrations of circulating IGF1 are of the order of 100 ng/ml in adults and up to 500 ng/ml in pubertal adolescents [30], so that the impact of ingested IGF1 on adult serum concentrations would be negligible; ii) although many breast carcinoma cell lines are dependent on IGF1 for in vitro growth (a property they share with countless other normal and transformed cell types) there is no evidence, biological or genetic, that systemic IGF1 can contribute directly to the development of human breast cancer [31]; if Epstein were to be correct, an increased incidence of breast cancer should be evident in clinical conditions in which circulating IGF1 concentrations are elevated, but this is not the case; for example with acromegalics, whose IGF1 levels may be elevated substantially and in whom a significant proportion of that IGF1 is free in the serum [32], there is no increased risk of breast cancer, the most marked tumour excess being evident in the colon [33].

SUMMARY

The recent reports of IGF1's protection from proteolysis by casein and its ability to induce cell proliferation in the gut epithelium, together with significant doubts about the degree to which its concentration in milk is increased by BST treatment suggest the need to examine its effects on gut cytokinetics. The areas of uncertainty identified by a number of expert reviewers underline this recommendation. However, the claim that BST might, through the agency of increased milk IGF1 concentrations, be instrumental in causing breast cancer in consumers seems to be without scientific foundation. Quite apart from such considerations, it is a matter of concern that were BST use to result in widespread milk avoidance, there might be significant adverse effects on public health.

LITERATURE


26. National Institutes of Health. NIH tech-

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2 TNO Nutrition & Food Research, P.O. Box 360, 3700 AJ Zeist, the Netherlands

In 1990/1991 FAO/WHO/UNU issued an evaluation system of dietary proteins based on true digestibility and its amino acid composition resulting in the Protein-Digestibility Corrected Amino Acid Score (PDCAAS). In this scheme the biological value of a dietary protein is calculated from the first limiting amino acid as resulting from the comparison of the amino acid composition with the metabolic need for preschool children. The biological value as computed by this approach is regarded by this panel to be valid for all age groups except for infants [1].

IDF’s Nutrition Week in Helsinki in June 1994 devoted two sessions to the critical judgement of the consequences of this new PDCAAS evaluation scheme. Professor Vernon Young from the Laboratory of Human Nutrition at MIT, Cambridge, USA, contributed by lecturing on “Protein Quality and Evaluation”. Moreover, a round table discussed further the implications of the PDCAAS procedure. This round table was composed of the organizer Professor Barth, DHE Potsdam/Germany, Dr Gertjan Schaafsma, TNO Zeist/Netherlands, Dr Rolof van der Meer, NIZO Ede/Netherlands, Professor Dr Helmut Erbersdobler, University of Kiel/Germany, and Professor John Milward, University of Surrey/UK. The essence of the different contributions was outlined in the Nutrition Newsletter [2].

“Milk protein will continue to hold a singular position amongst other dietary proteins, though Table 11 of the dietary recommendations issued by FAO/WHO/UNU attributed a biological value to milk protein which is far less in excess than that of legume protein (for example: soy protein isolate) when compared to former FAO/WHO evaluation schemes.”

The key arguments for these conclusions are:
First, milk protein is virtually devoid of antinutrients.
Secondly, its rich content of lysine and sufficient content of sulfuric amino acids make it particularly suited to complement other proteins lacking sufficient amounts of these amino acids.
Thirdly, the biochemical knowledge on which the new reference pattern is based is everything else but firmly established. This holds to the fact that the data are too scarce up to now and have to be complemented by further research.
Fourthly, the other nutritional attributes, which are more and more emerging like hormone-like activities, and binding and transport of nutrients also have to be taken into consideration.”

The main arguments forwarded by Dr Schaafsma at the Nutrition Week in Helsinki, in a position paper by TNO Nutrition and Food Research Institute of February 1994 [3], and further at the Nutrition Week in June 1995 in Paris can be summarized as follows.

(1) Reference pattern
Reference patterns for the computation of PDCAAS are very critical for the outcome of the biological value of a given protein. One has to keep in mind that this reference pattern has been obtained in a small number of malnourished children in Central America. Moreover, they have never been published in a critically reviewed international journal. Therefore, this reference pattern may be regarded as preliminary, and the number of children studied was too low.

(2) True digestibility
This parameter is equally critical, because PDCAAS values are based on true digestibility. This value may be falsely low, because of the unknown fraction of nitrogen appearing in the faeces and originating from colonic metabolism. Therefore, true digestibility should be replaced by more physiological and recently established methods to calculate absorbed amino acids, for example the homogarine method developed by Erbersdobler and co-workers [4]. In any case, prececal digestibility is more appropriate to use than the outdated method to measure true digestibility.

(3) Cutting of PDCAAS
It is not appropriate to cut PDCAAS values exceeding the value of 1. This is very important, because primary values of PDCAAS may be significantly higher than 1.0. Such values above 1.0 translating a particular rich content of essential amino acids, for example lysine, may be important to take the fact into consideration that such a protein can complement other proteins deficient in such amino acids. Insofar, the synergistic effect of different dietary proteins may be incorrectly neglected.

All these arguments led the scientists, who got together on the occasion of an EDA meeting in Utrecht in March 1995, to reach the following consensus:
“The advantages and limitations of the PDCAAS method for assessing the comparative nutritional quality of food proteins was discussed. It was the consensus of the workshop that the convention of rounding down to 1.0 PDCAAS values that are greater than 1.0 does not adequately credit the ability of proteins of high quality, such as those of cow’s milk, to complement the nutritional value of dietary proteins of lower quality. It is strongly recommended, therefore, that the primary PDCAAS value also be used as a basis for assessing the value of high quality proteins.”

LITERATURE
PROTEIN QUALITY MEASUREMENT BY THE PDCAAS TECHNIQUE

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ABSTRACT
The use of the Protein Digestibility Corrected Amino Acid Score (PDCAAS) technique is of scientific and commercial importance. It provides food formulators with the ability to distinguish the relative protein quality among protein foods that have amino acid profiles which are unsuited to the growth and maintenance needs of the human. It is of only limited value to distinguish among high quality proteins. Because protein sources are usually combined with another in dietary use, protein quality assessment must recognize the superiority of the strongest proteins that are able to complement the quality of other proteins in the diet. The “score” reported by the PDCAAS recognizes adequacy of the protein to support growth and maintenance needs. It does not recognize the presence of amino acids that are surplus to requirement, but which are nutritionally valuable and potentially superior proteins.

By choosing to move away from the assessment of protein quality using biological techniques, to the PDCAAS technique, the food formulator does not receive information about:
- the presence of anti-nutritional, nor
- the level of availability of essential amino acids, nor
- the ability of the body to assimilate the protein, nor
- the presence of beneficial bioactive components associated with the protein.

The PDCAAS procedure is itself limited because:
- amino acid analytical techniques are imprecise,
- rat faecal digestibility measures are of limited value,
- individual amino acid digestibilities are not measured, and
- the recommended daily requirements of amino acids are under debate.

Alternative measurement procedures that demonstrate the quality of proteins of animal origin are desirable.

For many years protein quality has been measured using animal models. Of high commercial importance has been a test that measures growth response in the rat fed on a base diet plus the test protein. The result, known as the Protein Efficiency Ratio (PER), is a measure of grams body weight increase/grams protein consumed. The technique became the basis for determining the “Required Daily Amount” (RDA) figure that is used in the Nutritional Labeling laws of the USA and for those in a number of other countries. Dairy proteins were recognized as being superior to vegetable proteins with the consequence that the food formulator could include less in the formulation to achieve the RDA level desired. This justified a price premium for the best quality proteins.

Regrettably the PER test proved difficult to standardize between laboratories and the rat proved to be an imperfect model for the human. In particular its dietary requirement for the sulfur-containing amino acids is higher than is appropriate (FAO/WHO, 1990). An alternative test was devised which is now known as the Protein Digestibility Corrected Amino Acid Score (PDCAAS). The score intends to measure those dietary essential amino acids that are absorbed by the rat. It then compares the dietary pattern of digestible amino acids to the pattern deemed to be required by the pre-school child. If the needs of that child are met then the “score” is reported as 1.0. Consequently it is inferred that there is no need to consider quality beyond the point of sufficiency (FAO, 1990).

The soy industry supplies protein materials that provide (by most measures) the best quality among the vegetable proteins (Table 1).

Table 1: PDCAAS data for vegetable proteins

<table>
<thead>
<tr>
<th>True protein (%)</th>
<th>PDCAAS digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy protein isolate*</td>
<td>98</td>
</tr>
<tr>
<td>Kidney beans*</td>
<td>81</td>
</tr>
<tr>
<td>Pinto beans*</td>
<td>79</td>
</tr>
<tr>
<td>Rolled oats*</td>
<td>91</td>
</tr>
<tr>
<td>Whole wheat**</td>
<td>91</td>
</tr>
<tr>
<td>Wheat gluten*</td>
<td>96</td>
</tr>
</tbody>
</table>

* Sarwar (1989)
** Sarwar (1987)

Indeed the best soy protein products are adequate in meeting the amino acid

Table 2: Examples of misinterpretations of published PDCAAS data

<table>
<thead>
<tr>
<th>Statement</th>
<th>Source</th>
<th>Affiliation of author</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;All proteins with a PDCAAS of 1.0 are high quality proteins and are equivalent in protein quality.&quot;</td>
<td>Henley, E.C. &amp; Kuster, J.M. Food Technol. 48 (4): 76 (1994).</td>
<td>Protein Technologies International (USA)</td>
</tr>
<tr>
<td>&quot;There is absolutely no nutritional advantage to consuming proteins with scores greater than 1.0, since excess amino acids are not utilised by the body, per se.*&quot;</td>
<td>Weeks, P. Food Technol. NZ 29 (5): 4 (1994).</td>
<td>Protein Technologies International (NZ)</td>
</tr>
<tr>
<td>&quot;Supro proteins offer comparable protein quality versus milk proteins plus significant advantages in terms of health benefits and cost-effective functionality.&quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: The nutritional quality of milk and soy proteins (as measured by PER or PDCAAS)

<table>
<thead>
<tr>
<th>Protein</th>
<th>PER</th>
<th>PDCAAS primary value</th>
<th>PDCAAS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein concentrate*</td>
<td>3.0</td>
<td>1.08</td>
<td>1.0</td>
</tr>
<tr>
<td>Milk protein isolate*</td>
<td>2.8</td>
<td>1.27</td>
<td>1.0</td>
</tr>
<tr>
<td>Casein*</td>
<td>2.5</td>
<td>1.08</td>
<td>1.0</td>
</tr>
<tr>
<td>Soy protein isolate**</td>
<td>2.1</td>
<td>0.94</td>
<td>0.92</td>
</tr>
</tbody>
</table>

* Data from NZ Dairy Research Institute unpublished reports.
** Sarwar [1987].

needs of the growing child and so they score well in the PDCAAS measure. The measure is, however, a "score" in which the primary values are truncated to "1.0". Consequently those proteins that have a primary value greater than one appear to be of very similar quality to soy protein. In commercial terms the concept of superiority can be buried by misrepresenting the PDCAAS data. This has indeed happened (Table 2).

Adequacy is misrepresented as "equality". Superiority is ignored. This is surely an outcome that was not intended by the FAO/WHO group. In a real situation no person (except some infants) receives only a single protein in any meal. Normally the amino acids of one protein that are surplus to requirement complement the amino acids that are insufficient in other proteins. The diet is therefore balanced by the contribution of superior proteins to the amino acid patterns of inferior proteins.

In fact, the PDCAAS value and the biological testing procedures (PER as an example) provide different information (Table 3).

While the PDCAAS score recognizes adequacy, the complementary value of superior proteins is not demonstrated. A second major problem with the PDCAAS is that the values for individual ingredients are not necessarily additive for dietary formulation. The value of milk proteins in supplementing other proteins is demonstrated by considering the value of milk protein in combinations with cereal (Table 4).

Table 4: Complementarity: the inadequacy of the PDCAAS for single proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>PDCAAS of the protein alone</th>
<th>Calculated PDCAAS of a 50:50 mix with wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>0.97</td>
<td>0.78</td>
</tr>
<tr>
<td>Casein</td>
<td>1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

For any given level of a protein mixture in a diet, proteins of animal origin improve the nutritive quality of proteins of vegetable origin. The efficiency with which a human body can use a diet of mixed proteins to build its own protein is significantly enhanced when milk proteins are included. It is not possible however to predict the value of the mixture from the PDCAAS scores of the single proteins. The limiting amino acid of wheat protein is lysine but soy protein is not an adequate source to supplement the lysine levels in the manner that milk proteins will do.

This effect, known as complementarity, cannot be demonstrated by use of the PDCAAS score. This is partially because primary values of greater than one are not reported and partially because the score is calculated from the level of only the limiting amino acid. The data have use in ranking proteins but the scores for the protein sources are worthless for calculating the protein quality of mixed protein diets. Further, the truncation of the PDCAAS score at 1.00 for the limiting amino acid presupposes a level of confidence in the WHO/FAO accepted requirements for humans which is unrealistic as this has been a matter of debate and the issue has not yet been resolved. Therefore truncated PDCAAS scores may be misrepresenting the protein quality of single protein sources giving an unfair advantage to lower scoring proteins.

Other considerations are relevant to the use of the PDCAAS procedure. These are:

Table 5: Criteria assessed by protein quality tests

<table>
<thead>
<tr>
<th>Criteria</th>
<th>PER</th>
<th>PDCAAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment of anti-nutritional?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recognition of unavailability of amino acids</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recognition of hunger suppressants</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Assimilation of digested amino acids</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

a) The value of a biological test

1) In discardung the full biological tests (for example PER) in favour of analytical testing (for example PDCAAS) valuable information has been lost (refer Table 5).

The PDCAAS score gives only limited assessment of anti-nutritional compounds. For example, the anti-nutritional compounds in unheated soybean cause a reduction in nutritional quality which is not necessarily reflected in impaired digestibility values. The growth rate of rats is decreased by the presence of anti-nutritional compounds including trypsin inhibitors and lectins. (Table 6). The PER assay could distinguish the protein quality of diets containing these anti-nutritional compounds but the PDCAAS score cannot. Moreover, most commercial soy products contain some active anti-nutritional compounds.

The effect of these anti-nutritional compounds is partially related to hunger suppressant activity. Hunger suppressants can also be produced by over-processing. For example, overheating of soybean flour results in a dramatic decrease in the amount of diet eaten by chicks (Skrede & Krogdal, 1985).

The PDCAAS score is further flawed by its dependence on amino acid analysis which cannot distinguish unavailable forms

Table 6: The effect of anti-nutritional compounds upon protein quality

<table>
<thead>
<tr>
<th>PER</th>
<th>Digestibility (% rat faecal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy flour protein extract</td>
<td>1.4</td>
</tr>
<tr>
<td>Soy flour protein extract - trypsin inhibitor free</td>
<td>1.9</td>
</tr>
<tr>
<td>Soy flour protein extract - heated</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Kakade et al. (1973).
of amino acids that can be produced by harsh processing. Three examples illustrate this point. First, all D-amino acids co-elute with L-amino acids and therefore harsh alkali treatments will result in unavailable amino acids which will not be assessed by the PDCAAS. Second, oxidizing conditions may convert methionine and cysteine to unavailable oxidized forms which will not be detected by amino acid analysis because performic acid pretreatment results in the same oxidation reaction. Third, unavailable Amadori rearrangement products of lysine and carbohydrates break down under acid hydrolysis and yield a high proportion of lysine, which will be analyzed and reported as being bioavailable.

Additionally the PDCAAS does not measure the level of assimilation of digested amino acids into animal/human protein which may be greatly decreased by harsh processing conditions (Batterham et al., 1994).

b) Accuracy of the PDCAAS

(1) Inadequacies of the amino acid analytical technique

The amount of variation between laboratories conducting amino acid analysis has been defined in an interlaboratory study conducted by Sarwar et al. (1983). (Table 7). Assuming that all laboratories will produce results within ± 2 standard deviations of the mean value of all laboratories, it can be shown that the level of a particular amino acid may vary by large amounts between laboratories, for example for the analysis of a soy protein sample, by 47% of the mean value for tryptophan and by 23% of the mean value for cysteine and methionine. Other protein samples have similar levels of variability between laboratories. The degree of variability increases slightly if only single analyses are performed. It is disturbing to note the degree of accuracy with which PDCAAS scores are regarded when the amino acid analysis from single laboratories varies to such an extent.

(2) The measurement of "digestibility"

The determination of protein or amino acid digestibility is an integral component of any protein quality evaluation system. Faecal protein digestibility is commonly used in determining the PDCAAS. Both true and apparent faecal digestibility data are misleading however due to the interference caused by hindgut microbial metabolism. The inadequacy of faecal digestibility data is shown by the fact that 60% of faecal protein is in fact bacterial protein. The composition of that protein bears no direct relationship to undigested food protein. The inherent problems with faecal digestibility measurement are well recognized and the measurement of amino acid digestibility at the terminal ileum has been proposed as an alternative. Observations from simple stomached animals such as the pig and rat indicate that ileal digestibility coefficients are sensitive in detecting differences in digestibility among different protein sources and are accurate indicators of amino acid digestion and absorption (at least for proteins which have not been subjected to damage during processing).

A new method (feeding the test animal an enzyme hydrolysed casein based diet and with ultrafiltration of the digesta collected) for accurately determining ileal endogenous amino acid loss under physiological conditions, has recently been developed (Moughan et al., 1990; Butts et al., 1993). Application of this method demonstrates that the presence of dietary peptides in the gut supports a much higher ileal endogenous amino acid flow in comparison to unphysiological protein-free feeding (the traditional approach).

The new enzyme hydrolysed casein method for determining endogenous amino acid loss (Moughan et al., 1990) has been applied to commercial food samples and the true digestibility coefficients obtained are significantly greater in magnitude than their apparent counterparts (Table 8).

This new method for determining endogenous loss requires some further validation, but offers much promise for application to dairy products. The approach should be able to be

Table 7: Degree of error (between laboratories) involved in the amino acid analysis of a soy protein sample

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Variability (2 standard deviations from mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>±18.8%</td>
</tr>
<tr>
<td>Histidine</td>
<td>±24.6%</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>±6.6%</td>
</tr>
<tr>
<td>Leucine</td>
<td>±9.4%</td>
</tr>
<tr>
<td>Lysine</td>
<td>±11.6%</td>
</tr>
<tr>
<td>Methionine</td>
<td>±22.6%</td>
</tr>
<tr>
<td>Cysteine</td>
<td>±22.8%</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>±13.8%</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>±8.8%</td>
</tr>
<tr>
<td>Threonine</td>
<td>±17.6%</td>
</tr>
<tr>
<td>Typtophan</td>
<td>±47.4%</td>
</tr>
<tr>
<td>Valine</td>
<td>±13.6%</td>
</tr>
</tbody>
</table>

* Sarwar et al. (1983).

Table 8: The true and apparent ileal amino acid digestibility (%) determined with the growing pig and using the enzyme hydrolysed casein, ultrafiltration technique to determine endogenous amino acid flow at the terminal ileum (1)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>True</th>
<th>Apparent</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>96</td>
<td>88</td>
<td>*</td>
</tr>
<tr>
<td>Valine</td>
<td>98</td>
<td>94</td>
<td>*</td>
</tr>
<tr>
<td>Cysteine</td>
<td>89</td>
<td>81</td>
<td>*</td>
</tr>
<tr>
<td>Methionine</td>
<td>100</td>
<td>98</td>
<td>*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>98</td>
<td>94</td>
<td>*</td>
</tr>
<tr>
<td>Leucine</td>
<td>99</td>
<td>96</td>
<td>*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>99</td>
<td>96</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>98</td>
<td>96</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>99</td>
<td>96</td>
<td>NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>99</td>
<td>97</td>
<td>NS</td>
</tr>
</tbody>
</table>

(1) S.M. Rutherford, P.J. Moughan, unpublished, Lactic casein

* P < 0.05.
** P < 0.01.
applied to provide accurate "state of the art" information on dairy products, possibly provid-
ing a marketing edge over compet-
itors. The FAO/WHO (1989) urged that in nutritional labeling the use of digestible amino acid values may be preferred to a statement on the protein digestibility - corrected amino acid score. Indeed, it is easy to conceive of many situations whereby the purchaser of dairy products may require accurate absolute data on the digestible amino acid composition. Where such data are needed, true ileal digestibility coefficients should lead to a more accurate definition of protein quality. However, it should be noted that true ileal digestibility data may not necessarily lead to higher PDCAAS values, as true ileal protein digestibility coefficients may not be numerically higher than true faecal digestibility coefficients. Nevertheless, the dairy industry, in developing and promoting new and better methods of protein digestibility measurement, should bear in mind not only the need for absolute accuracy but also the need to demonstrate the relative advantages of milk proteins over vegetable proteins. For the purpose of valid relative comparison, the use of apparent digestibility measures or the correction of apparent ileal digestibility values for a standard endogenous excretion (for example, a constant endoge-
nous factor determined using the EHC method) would appear to be justifiable and may be com-
mercially advantageous. True (EHC method) ileal digestibility coefficients should be sensitive in detecting differences in amino acid digestibility between soya and dairy products.

c) The presence of bioactive materials
Evidence is growing that fragments of proteins known as peptides can modulate the digestive process (Schlimme et al., 1988). Also pep-
tides can influence metabolic processes.

CONCLUSION
The PDCAAS technique for measurement of protein quality has assisted in ranking the quality of vegetable pro-
teins. Its effect has been to level down the apparent quality of animal proteins through truncating the primary values as determined.

As a first and early step the technique would be improved by reporting primary values. Subsequently substan-
tial revision of the technique is needed to improve the scientific precision of the methodology. One approach could be the use of true ileal digestibility, appropri-
ately accounting for endogenous amino acid loss.

Eventually test procedures that rec-
ognize the occurrence of factors such as anti-nutrients, the unavailability of amino acids, the occurrence of hunger suppressants, and the efficiency of assimilation of digested amino acids will need to be created.

Because we can believe that the value of milk proteins is very high, the world-wide dairy industry can confident-
ly support improvements in the scientific worth of such methods of assessment.

LITERATURE
Sarwar, G., Christensen, D.A., Finlayson, A.J., Friedman, M., Hackler, L.R.,
Sarwar, G., Plant Foods Hum. Nutr. 39: 23-

A CASEIN-BASED PROTEIN MEAL INDUCES A HIGHER GUT PROTEIN SYNTHESIS RATE THAN A SOY-BASED PROTEIN MEAL

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Department of Surgery, University of Limburg, Maastricht, the Netherlands

ABSTRACT
The purpose of the study was to assess the quality of Casein- and Soy protein by studying the contribution of individual amino acids to tissue formation in gut and muscle tissue. In 36 g cold-in pigs (20-25 kg), a primed constant infusion protocol (PH-
PHE) was used to measure protein syn-
thesis and degradation of portal-
drained viscerum (+ gut) and hindquarter (+ muscle) in the fasted and fed state at steady-state. Isotopogenous meals were given at a constant rate and consisted of Maltodextrin with Casein (CAPM) or Soy protein (SOPM). True digestibility was comparable between CAPM and SOPM. Gut protein synthesis was 3-fold higher in CAPM. It was calculated that 45% of protein taken up from the CAPM meal is used for protein synthesis within the gut, while only 15% in SOPM. Also, the data indicated that the liver extracts more amino acids in SOPM, probably for urea synthesis. No differences were observed in muscle metabolism.

The present study indicates that the difference between a Casein-based protein meal and a Soy-based protein meal is the amount of amino acids retained in the gut wall during the meal. This observation is in line with the hypothesis that the labile protein pool is located in the gut. Increasing this labile pool during the meal probably has a positive effect on 24 h protein utilization.

INTRODUCTION
Interpretation of studies dealing with amino acid requirements is subject to discussion [1, 2]. Induction of the enzymes of amino acid oxidation on high protein diets, the amount of dispensable amino acids within the meal, diurnal cycling and many other factors, all influence estimates of amino acid requirements [1-4]. Measurement of amino acid kinetics in vivo is essential to understand the role of each of these factors.
For the definition of optimal protein requirements, the labile protein reserves are important [3]. This labile protein pool could play a significant role in the post-absorptive phase to provide essential amino acids to the body free amino acid pool. The ability of different protein sources to increase the labile protein reserves might serve as one of the criteria of protein quality [3]. Our own studies have indicated that the labile protein pool indeed is important in amino acid kinetics during a meal and that this pool is located within the gut [5–7].

The major purpose of the present pilot experiments was to assess the quality of Casein- and SOY protein by studying the contribution of individual amino acids to tissue formation in gut and muscle tissue.

Here we used a pig model to calculate protein synthesis and degradation in gut and muscle. Both nutrition and radioactive tracers were infused at a constant rate to achieve steady-state conditions.

**MATERIALS AND METHODS**

The multicatheterized pig model [5, 8] and specific activity measurement technique [9, 10], developed in our laboratory, were used in the pilot experiments. In the conscious pig (20–25 kg), a primed constant infusion of radioactive tracers (3H-PHE and 3H-Leucine) was used to calculate protein turnover (protein degradation = (PHE production) and synthesis = PHE disposal) of gut and muscle in the fasted state. A defined isonitrogenous enteral meal[6] was given at a constant rate until steady-state absorption was obtained[9]. Then, again measurements were done. Using PDV production of unlabelled PHE, true digestibility[11] of PHE was estimated[12]. To calculate arterial concentrations, blood flow[13] and net balance of the individual amino acids, glucose, lactate, ammonia, and urea, their concentrations were measured in the arterial and venous blood supply of gut and muscle. Because of the low number of pigs studied, the data presented are descriptive, and statistical analysis is omitted.

**RESULTS**

Test meal is infused via the gastric catheter at a constant rate. It is assumed that digestion and absorption in the small intestine of its constituents also is constant. Uptake of amino acids from the digested protein by the intestinal cell, therefore, should be a parameter of digestion and absorption. Using PHE as indicator, production during the meal was comparable in both groups (Figure 1A). Correcting for the amount of PHE in CAPM and SOPM and PHE production from protein breakdown, true digestibility was also comparable (92% and 102%, respectively). However, PHE disposal (Figure 1B) was 3-fold higher in CAPM. This indicates that 45% of PHE taken up from the CAPM meal is used for protein synthesis within gut tissue, while only 15% is used in SOPM. The lower net balance (Figure 1C) in CAPM is in line with this calculation.

Maltodextrins within the meal are broken down to glucose and subsequently taken up PDV production is a parameter of absorption. No difference was observed[15], but arterial glucose was higher in CAPM[16].

Amino acids, escaping protein synthesis within the gut pass through the liver. As a result, with SOPM portal PHE concentrations are higher than in CAPM (148 ± 11, 105 ± 11 μM). Whole body PHE Ra[17] is the result of splanchnic[18] production and protein breakdown in other organs. However, no difference was observed between CAPM and SOPM (2.9 ± 0.2 and 3.1 ± 0.3 μmol/kg/bw/min). Also, arterial PHE concentrations were comparable (94 ± 13 and 108 ± 13 μM). Therefore, it appears that the liver extracts more PHE in SOPM. As liver metabolism was not assessed by the present experimental setup, this remains to be investigated.

Amino acid delivery to the peripheral tissue, for example muscle, thus seems to be comparable between CAPM and SOPM. PHE disposal and production, however, were lower in SOPM (Figure 2A, B), but PHE net balance was comparable (Figure 2C). Only 30% of PHE absorbed by the gut is taken up by muscle, indicating about 20% uptake in CAPM and 85% uptake in SOPM by non-muscle tissue[20].

Calculating net balance across the gut of other essential amino acids like histidine, valine, isoleucine, leucine, methionine and lysine and correcting this balance for the amount in CAPM and SOPM, shows that about 50% in CAPM and 85% in SOPM of enteral intake is delivered to the liver, comparable to the amount of PHE delivered (55% and 85%, respectively)[21].

**DISCUSSION**

This pilot study indicates that the difference between a Casein-based protein meal and a SOY-based protein meal is the amount of amino acids retained in the gut wall during the meal. This means that protein amino acid scoring patterns do not reflect in vivo organ responses [2].

The results of this pilot study are in line with the hypothesis that the normal response during the meal is to enhance gut protein synthesis to expand its labile protein pool. When this is the case, then the better protein will result in a lower portal appearance of amino acids. This will cause a reduced delivery of amino acids to the liver (urea synthesis) and peripheral tissues (oxidation) and (wasteful) breakdown of amino acids, which can not be used in protein synthesis, is minimal. When uptake of the meal is finished, delivery of amino acids by the gut to these organs continues at a low rate by protein breakdown of the labile protein pool, and amino acid breakdown consequently is low. As a result, 24-h protein utilization is more effective in the case of a protein meal that is temporarily taken up in substantial amount by the gut.
Figure 1: PHE production, disposal and net release by the gut.

In line with the hypothesis, it remains to be established whether the amino acids, not used for protein synthesis within the gut and thus delivered to the liver, are used within the liver for protein synthesis or for urea synthesis.

In conclusion, these pilot experiments suggest that Casein-based protein meals have a higher biological value than SOY-based protein, because:

(a) Retention in the intestinal cells is 3 times higher (45% versus 15%), allowing release according to the body’s needs
(b) More amino acids are taken up by the liver, probably indicating less effective use of ingested proteins.

Figure 2: PHE production, disposal and net uptake by the muscle.

LITERATURE


CORONARY HEART DISEASE AND THE FRENCH PARADOX

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The major causes of death in affluent societies – cardiovascular disease, cancers and digestive disorders – show markedly different incidence rates in different European countries. It has been proposed that different dietary patterns in Europe could account for the differences observed. However, these relationships have never been fully documented. In particular, the classic "l lipid hypothesis" (that high consumption of fat, particularly of saturated fatty acids, leads to high risk of CHD through its effect on plasma cholesterol) alone, fails to explain the differing rates of coronary heart disease (CHD).

The low rates of coronary heart disease (CHD) in France are difficult to explain in terms of conventional risk factors: the French do not eat less saturated fat than the British and they have similar smoking habits, serum cholesterol levels and blood pressure.

Among men, death from coronary heart disease is up to 10 times more common in some parts of the world than others. The highest rate of all is in the Czech Republic. The lowest rates among men are in Japan, China and France.

Interestingly coronary mortality has been decreasing worldwide over the past 30 years except in ex-eastern black countries and surprisingly Spain (+ 12%). In France, CHD mortality dropped by 26% in spite of the fact that in France, the fat intake has not changed at all during the past 25 years.

The French Paradox, as the Americans call it, is a good illustration of the weak predictive value of blood cholesterol on a country scale.

The Paradox is that we, the French, eat as much fat as the Americans, more saturated fat – the P/S ratio is 50% higher in the US, where mean cholesterol level is much lower than in France, and ironically coronary mortality is nearly three times higher in the US. (A high P/S ratio is said to be a good protection against CHD, but this is yet to be proved.) Clearly this shows that fat intake is not the major issue.

Yet dietary fat and cholesterol are generally considered to be major determinants of CHD risk. In most prospective studies, the fat intake and the rate of CHD are not correlated. When an association is found, its size is very small: only 1-2% more fat in CHD patients as compared with subjects with no CHD. Surprisingly in some ongoing studies there is either no difference or even a slightly lower intake in people with CHD.

Several explanations have been put forward to account for the protective effects of wine. Among others, wine intake has been associated with increased average concentrations of HDL cholesterol and non-alcoholic, phenolic substances present in red wine have been shown to have potent antioxidant properties. In one study these phenolic components of red wine inhibited the oxidation of LDL even more effectively than the nutrient antioxidant, vitamin E.

Fruit and vegetables are a good source of antioxidant nutrients and the consumption of these foods is higher in France than in most countries.

The WHO-Monica study has shown that improved plasma vitamin status is strongly correlated with lower rates of death from CHD.

The LRC-GPPT study has confirmed the protective effect of high levels of serum carotenoids: it is an important finding as serum levels could be influenced by a high intake of fruit, vegetables and dairy products. As you know, in western diets, dairy products provide up to 26% of carotenoids.

Two prospective studies from Harvard have clearly shown that vitamin E consumption – regardless of the source – was highly protective against CHD.

There is a 35% reduction in risk in men with the highest intakes. The results in women are similar.

More recently, an analysis of the diet in 17 European countries has confirmed the inverse relationship between the levels of antioxidant vitamins in the diet and the risk of CHD.

Antioxidants are a very important issue because LDLs containing oxidized polyols are very atherogenic.

Last year it was shown that the levels of polyunsaturates in the adipose tissue - which reflects the dietary intake - are strongly correlated to the amount of these fatty acids in the plaque of atheroma.

The problem of polyols is that because of their double-bonds, they are easily oxidized. This is a major concern since it has been shown that the diet is the major antioxidant source, which is positively associated with the severity of CHD in patients undergoing coronary angiography.

For all these reasons there is today a global consensus recommending a balanced nutrition with a diet of natural foods rich in antioxidant vitamins, particularly fruit and vegetables, sunflower oil, butter and other dairy products.

Recent results from a major epidemiological study have shown that trans fats acids may have adverse effects on health. This study reported the dietary intake of trans fats acids and the risk of CHD. Subjects with the highest intake of trans fatty acids from vegetable sources had a 3-fold increase in risk. There was no association between intake of trans fatty acids from animal fat and CHD.

The average intake of trans fatty acids in France is 3 g/day compared to averages of 5 g/day in Germany and about 10 g/day in the USA.

Trans fatty acids, among the various fatty acids, have the most negative effects on the serum profile (increase of cholesterol and LDL, decrease in HDL levels).

From the Framingham study, it has been perfectly demonstrated that the lower the level of HDL, the higher the risk of CHD in men as well as in women and that the protective influence of HDL was nearly 3 times stronger than the atherogenic effect of LDL.

As a consequence, any dietary recommendations which could lower the HDL-cholesterol levels or increase the cholesterol/HDL ratio should not be encouraged in the general population.

Two recent studies conducted in Paris on healthy young medical students (Hôpital Bichat) and in elderly people (Hôpital Sainte Perine) have shown that substituting margarine rich in polyunsaturated to butter actually decreases HDL levels and increase the LDL/HDL ratio, which is undesirable with regard to CHD risk in healthy subjects.

In conclusion, there remain many anomalies with regard to relationships between lifestyle factors and CHD. However, it is becoming increasingly clear that CHD will continue to appear paradoxical if we retain the fixation that dietary fats play a major cauative role. In addition to the dietary factors described, which challenge the lipid hypothesis, other lifestyle factors have to be considered.

Current epidemiological evidence suggests that the most important dietary advice to decrease the risk of CHD is to have a varied and balanced diet with moderate consumption of wine and a high consumption of fresh fruit and vegetables.

The apparent contradiction of high saturated fatty acid consumption but lower CHD in countries like France, may simply reflect the fact that this association is small or not causal.
ANNUAL REVIEW ON CULTURED MILKS AND PROBIOTICS

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SUMMARY
Recent advances in cultured milk and probiotic research have proceeded fast to confirm the health benefits of some probiotic strains. This year we have received new published material from a number of members allowing us to complete the annual task for the IDF and Group F20. Simultaneously the working group for our symposium has discussed the meeting in Potsdam and prepared background data for it. The aim is to provide a new update on cultured milks and health as well as probiotics for the symposium.

EFFECTS ON GUT MICROFLORA
Secondary bile acids, especially deoxycholic acid (DCA), are implicated in the etiology of colorectal cancer. In a recent study, most of the fecal bile acids were DCA and lithocholic acid, which were generated by the bacterial 7α-dehydroxylation of primary bile acids. The absence of cholic acid 7α-dehydroxylase activity was demonstrated in 46 strains of the genera Lactobacillus, Bifidobacterium, Lactococcus, and Streptococcus which are widely used in fermented milk products and were originally isolated from human feces (Takahashi & Morotomi, 1994). These results suggest in vivo intake of lactic acid bacteria is safe in that it does not produce secondary bile acids that promote colon cancer. Lactobacillus GG in yoghurt was reported to increase the fecal numbers of lactobacilli and bifidobacteria with concomitant decrease in leucinase-negative clostridia in healthy Japanese volunteers (Hosoda et al., 1994).

IMMUNE RESPONSE
A cell wall preparation of Bifidobacterium infantis was evaluated for the antitumor effects on peritoneal and thoracic tumor-bearing animals, using mouse Ehrlich carcinoma and Meth A fibrosarcoma, as well as rat MADB 106 mammary tumor models. For peritoneal Meth A tumor-bearing mice, the i.p. injection of cell wall preparation (for example 100 mg of 10 times) did result in significant prolongation of survival time. Cell kinetic studies suggested that the bifidobacterial cell wall preparation induced and activated non-specific phagocytes, polymorphonuclear cells (PMNs) and macrophages in situ to reject growing tumor cells in animals (Sekine et al., 1994). The induction of several cytokines was demonstrated by i.p. injection of Bifidobacterium longum (B. longum) and B. animalis. In brief, both B. longum and B. animalis induced the expression of IL-1β, IL-6, IL-10 and TNF-α mRNA in mouse peritoneal cells. Although the physiological significance of the stimulation of the production of these inflammatory cytokines remains to be clarified, the induction of IL-6 and IL-10 might play a role in the promotion of antibody production by B lymphocytes. TNF might also play a role in the activation of the immune system, including tumor inhibition (Sekine et al., 1994).

The effects of oral administration of the preparation of L. casei (BLP, 1 x 10⁹/g) on the enhancement of the immune response were investigated in patients with Dukes A colorectal cancer (Sawamura et al., 1994). Briefly, 21 patients, with a good performance status and no prior chemotherapy or radiotherapy, participated in the study. They were divided into three groups according to the following dosage schedule: BLP 3 g/day for 7-10 days for group A, 15 g/day for group B and no treatment for group C. In patients of groups A and B, an increase of helper T cells and NK cells, and a decrease in suppressor T cells were found in peripheral blood. A decrease in suppressor T cells in group A and suppressor inducer T cells in group B was also found in regional lymph nodes. The results suggest that the immune response of lymphocytes from colorectal cancer patients was enhanced by oral administration of BLP.

CULTURED PRODUCTS, PROBIOTICS AND MINERAL ABSORPTION
The effects of Bifidobacterium lactis and lactulose on whey calcium (Ca) absorption and bone fracture properties were investigated with ovariectomized rats receiving a diet containing 0.01% Ca for 31 days. The femur fracture properties (breaking energy and breaking force) in group 2 fed whey calcium and Bifidobacterium culture in drinking water, and group 3 fed whey calcium, lactulose and Bifidobacterium culture were increased compared with the control group receiving only whey calcium. These results suggest that administration of Bifidobacterium and lactulose promotes whey calcium absorption and thereby increases the strength of bone (Igarashi et al., 1994). A similar effect of the administration of galactooligosaccharides (GOS) on calcium absorption and prevention of bone loss in ovariectomized rats was reported (Chhon et al., 1992). The administration of indigestible oligosaccharides resulted in an enhancement of volatile fatty acid production in the lower intestine, and led to an increase in soluble calcium ion concentration, thus stimulating calcium absorption and preventing bone loss. The effect of Lactobacillus acidophilus on iron bioavailability in rats was examined by the hemoglobin regeneration method (Oda et al., 1994). Although the final hemoglobin value or iron intake was not different between the skim milk group and the fermented product group, the hemoglobin regeneration efficiency was significantly higher in the rats given the fermented product, indicating that L. acidophilus SRT 2062 was effective in increasing iron bioavailability in rats. Recently, a selene-Lactobacillus strain has also been reported as a potential organic selenium source (Calonne et al., 1994).

REQUIREMENTS OF PROBIOTIC BACTERIA FOR THE DAIRY INDUSTRY
The basic requirements for strain selection have been described in a number of studies and are summarized in Table 1.

SAFETY OF LACTIC ACID BACTERIA
The use of lactic acid bacteria in foods has a long history and lactobacilli have a "generally recognized as safe" status. The safety of lactic acid bacteria has recently been reviewed (Gasser, 1994) and also discussed by Harty et al. (1994). It was concluded that as opportunistic bacteria the frequency of occurrence of lactic acid bacteria is extremely rare. Also, the occurrence of some virulence factors remains to be shown. The International Union of Microbiological Societies (IUMS) reviewed the literature and concluded that the risks represented by lactobacilli and related organisms is not substantiated by clinical data. However, since new probiotic preparations include also bifidobacteria, enterococci, propionibacteria and
### Table 1: Desirable properties of probiotic bacteria

<table>
<thead>
<tr>
<th>Health and clinical properties</th>
<th>Stability and technological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human origin</td>
<td>Ability to maintain verified viability</td>
</tr>
<tr>
<td>Acid and bile stability</td>
<td>Maintenance of good flavor and aroma profiles after</td>
</tr>
<tr>
<td></td>
<td>fermentation</td>
</tr>
<tr>
<td>Adherence to human intestinal cells</td>
<td>Maintenance of mild acidity throughout storage time, good</td>
</tr>
<tr>
<td></td>
<td>acidity profile</td>
</tr>
<tr>
<td>Colonization of the human gut</td>
<td>Maintenance of colonizing properties throughout processing</td>
</tr>
<tr>
<td></td>
<td>and storage</td>
</tr>
<tr>
<td>Production of antimicrobial substances</td>
<td>Development of good storage stability in fermented products</td>
</tr>
<tr>
<td>Antagonism against carcinogenic and pathogenic bacteria</td>
<td>Stability after freeze-drying and other drying methods</td>
</tr>
<tr>
<td>Safety in human use</td>
<td>Accurate strain identification (genus, species)</td>
</tr>
<tr>
<td>Clinically validated health effects</td>
<td>Dose-response data for required effects</td>
</tr>
</tbody>
</table>

Even *Saccharomyces* strains, it is of importance to verify the safety of new probiotic strains. No firm guidelines exist for safety testing. An example of safety assessment is given in the testing of one strain (Salminen & von Wright, 1993). Safety assessment should include studies on the risk of microbial invasion (infectivity), basic toxicology including the development of deleterious intestinal metabolism, degradation of intestinal mucus, and also epidemiological data and evidence on safety. Further guidance may be found in European novel food guidelines and Japanese standards for functional foods. Further evidence for safety is acquired by selecting strains of human origin and strains that are relatively common in the human intestinal tract (Johansson et al., 1993). Such strains have been isolated and administered to humans and they include *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus casei* subsp. *rhamnosus* and others. Common yoghurt bacteria are rarely isolated in the lower parts of the intestinal tract, but they have a long history of safe use.

A number of studies on the survival and colonizing abilities of different culture strains were reported. *Lactobacillus gasseri* was shown to colonize elderly subjects (Pedrosa et al., 1995). *Lactobacillus acidophilus* LC1 was shown to be adherent in in vitro test systems (Bernet et al., 1994) and *Lactobacillus GG* was shown to colonize temporarily human subjects in addition to dairy products also in the form of gelatin capsules (Saxelin et al., 1995). Most recently, *Lactobacillus reuteri* was also shown to colonize human subjects (Wolf et al., 1995).

### NEW STUDIES ON HEALTH EFFECTS

The most important new developments are listed in Table 2. Additionally, there are some probiotic strains such as *Saccharomyces boulardii*, which are not used in dairy products or other foods at present. New documented published effects of cultured milks and probiotics include, for example, treatment of various types of diarrhea, alleviation of gastrointestinal side effects of antibiotic treatment, lactose intolerance, adjuvant effects on cancer treatment, and the general balancing and stabilization of intestinal integrity.

New and more recent reports with some probiotic strains include the immune enhancing effects, vaccine adjuvant effects and also some effects on lowering serum cholesterol levels as well as effects on colon cancer related parameters. The immune enhancing effects have been reported in several studies for two strains — *Lactobacillus acidophilus* LC1 and *Lactobacillus GG*. *Lactobacillus strain GG* (ATCC 53103) has been further defined as *Lactobacillus rhamnosus*, but most published studies use the name *Lactobacillus GG*. Both strains appear to have properties that may enable them to enhance natural immunity and act as immunoadjuvants. *Lactobacillus acidophilus* LC1 was reported to enhance the effect of oral *Salmomella* vaccination in human volunteers (Link-Amster et al., 1994) and *Lactobacillus GG* was shown to enhance the vaccine take in oral rotavirus vaccination (Isolauri et al., 1995).

Studies on the treatment of acute infant diarrhea have been successfully verified with *Lactobacillus GG* in a study by Raza et al. (1995). Similarly, a clinical study on the treatment of rotavirus diarrhea using *Bifidobacterium bifidum* preparations was published recently (Saaedra et al., 1994).

A recent review on travellers’ diarrhea covers most probiotic as well as other studies (Scarpignato & Rampal, 1995).

In a study on yoghurt strains, Pedrosa et al. (1995) found a significant reduction in faecal β-glucuronidase, nitroreductase and azoreductase activities in elderly subjects after a 12-day *Lactobacillus gasseri* supplementation, but not after *Streptococcus thermophilus* and *Lactobacillus bulgaricus* supplementation which confirms the findings reported earlier for yoghurt strains. Thus, some strains appear to be able to alter the metabolic activity of the colon, but further studies are needed to verify the practical health effects of this altered metabolism.

In an epidemiological case control study in Holland, 232 cases and 259 controls were studied with respective response rates of 60 and 57%. No significant associations were observed between the intake of fermented dairy products and colon cancer (Kampman et al., 1994).

The anti-ulcer effects of bifidobacteria, lactobacilli and streptococci were examined using the acetic acid induced gastric ulcer and ethanol induced erosion models in rats (Nagaoka et al., 1994). Anti-ulcer effects were confirmed by oral administration of these cultures of *Bifidobacterium breve* YIT 4007 and 4043, and *B. bifidum* YIT 4007 and their cell wall polysaccharide fractions (PSFs). The major effective component of anti-ulcer polysaccharides was rhamnose. In addition, the administration of the PSP from *B. bifidum* YIT 4007 resulted in the increase of epidermal growth factor and basic fibroblast growth factor in gastric tissues. Furthermore, the production of 6-ketoprostaglandin 1 α by macrophages.
Table 2: Successful probiotic bacteria and their reported effects

<table>
<thead>
<tr>
<th>Strain</th>
<th>Reported effects in clinical studies</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>Immune enhancer, adjuvant to an oral vaccine, adherent to human intestinal cells, balances intestinal</td>
<td>Link-Amster et al., 1994</td>
</tr>
<tr>
<td>LC1</td>
<td>microflora</td>
<td>Bernet et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Prevention of radiotherapy related diarrhea and side-effects, antibiotic diarrhea</td>
<td>Bernet et al., 1994</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td></td>
<td>Schiffirin et al., 1995</td>
</tr>
<tr>
<td>NCFO 1748</td>
<td></td>
<td>Salmiinen et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Treatment and prevention of rotavirus diarrhea, treatment of relapsing <em>Clostridium difficile</em> diarrhea,</td>
<td>Majamaa et al., 1995</td>
</tr>
<tr>
<td>Lactobacillus GG</td>
<td>Crohn's disease, antagonistic against carcinogenic bacteria</td>
<td>Raza et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kalla et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meurman et al., 1994</td>
</tr>
<tr>
<td>Lactococcus casei Shirotii</td>
<td>Positive effects on the treatment of superficial bladder and colon cancer</td>
<td>Asa et al., 1995</td>
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<tr>
<td></td>
<td></td>
<td>Sawamura et al., 1994</td>
</tr>
<tr>
<td>Streptococcus thermophilus;</td>
<td>No effect on rotavirus diarrhea, no immune enhancing effect during rotavirus diarrhea, no effect on fecal enzymes</td>
<td>Majamaa et al., 1995</td>
</tr>
<tr>
<td>Lactobacillus bulgaricus</td>
<td></td>
<td>Pedrosa et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saavedra et al., 1994</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em></td>
<td>Treatment of rotavirus diarrhea, balancing intestinal microflora, treatment of viral diarrhea, anti-ulcer properties, eradication of Helicobacter</td>
<td>Saavedra et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nagaoka et al., 1994</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em></td>
<td>Anti-ulcer properties</td>
<td>Yamamoto et al., 1994</td>
</tr>
<tr>
<td><em>Lactobacillus gasseri</em> (ADH)</td>
<td>Fecal enzyme reduction, survival in the intestinal tract</td>
<td>Nagaoka et al., 1994</td>
</tr>
<tr>
<td><em>Lactobacillus reuteri</em></td>
<td>Colonizing the intestinal tract, mainly animal studies so far, possibly an emerging human probiotic</td>
<td>Pedrosa et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wolf et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Molin et al., 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Johansson et al., 1993</td>
</tr>
</tbody>
</table>

was also enhanced by PSFs. These results indicated that these bacteria and their polysaccharides induced host repair and protective systems in the gastric ulcer model. The clinical effects of the *B. bifidum* YIT 4007 preparation (BBI 4007) on the 10 patients with gastric ulcers was investigated using endoscopy (Yamamoto et al., 1994). The administration of BBI 4007 at the dose of 1.5 g/day for 30 days resulted in healing of the ulcer in 50% of the patients, with eradication of *Helicobacter pylori* from the gastric mucous membrane in 30% of the patients.

Effects of skim milk and products fermented by *L. acidophilus* SBT 2062 on plasma and liver lipid levels were investigated in diet-induced hypertriglyceridemic rats (Oda & Hashiba, 1994). Skim milk prevented the elevation of triglyceride level in plasma and liver, while its fermented milk product had a significant effect on only liver triglyceride. The effects of milk whey and its fermented products with *B. longum*, *L. acidophilus* and *S. thermophilus*, on amelioration of the peroxidation of hepatic mitochondrial lipids and liver injury were investigated in bile duct-ligated rats (Zommarra et al., 1994). The rats fed on diets containing milk whey fermented with *B. longum* ameliorated the elevation of organ weights (liver and spleen), serum alkaline phosphatase activity, bilirubin concentration, and content of mitochondrial lipid hydroperoxide, while milk whey and milk whey fermented with *L. acidophilus* and *S. thermophilus* suppressed the elevation of mitochondrial lipid hydroperoxide only. The suppression of the elevation of serum lipid hydroperoxide was also observed in rats fed on diets containing milk whey and milk fermented with *B. longum* and *S. thermophilus*. These results suggest that a milk whey fermented with lactic acid bacteria exerts a beneficial effect on free radical mediated hepatic injury due to, in part, amelioration of the reduction of plasma α-tocopherol.

**STABILITY OF PROBIOTIC BACTERIA DURING STORAGE**

Only decades ago that cultured milk was consumed mainly for its taste, texture and improved shelf life whilst today, probiotic properties are also desired. Since the probiotic properties of desirable bacteria are largely dependent on their ability to remain viable and to colonize the surface of human intestinal cells it is necessary that sufficient numbers of viable bacteria should be present at the time of consumption. The number of 1 x 10^8 CFU/g has been suggested as the "therapeutic minimum". The stability of lactic acid bacteria and probiotic preparations has recently been reviewed by Lee & Salminen (1995). Most probiotic bacteria, for example *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, show a short stationary growth phase which is followed by rapid losses of cell viability. Their short shelf life represents a logistic problem for the manufacturers and retailers, and a technical challenge for researchers.

**LITERATURE**


(Spanish with English summary.)
BIOAVAILABILITY OF CALCIUM

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1 INTRODUCTION
This paper is aimed to provide an overview of the factors which influence the bioavailability of dietary calcium. An extensive review of the relevant literature is beyond the scope of this paper. Knowledge on the bioavailability of calcium is considered to be important with respect to the prevention of chronic diseases related to calcium, for example osteoporosis and hypertension [1].

According to O’Dell [2], bioavailability can be defined as the proportion of a nutrient in food which is absorbed and utilized; utilization is the process of transport, cellular assimilation and conversion to biologically active form(s). Absorption should be differentiated into True absorption and Apparent absorption. True absorption is the proportion of a nutrient in the food which moves from the intestinal lumen, across the mucosa into the body; Apparent absorption is defined as the difference between nutrient intake and faecal excretion. So Apparent absorption is lower than True absorption, because in the case of True absorption no correction is made for the endogenous excretion of the nutrient into the faeces. Both True- and Apparent absorption can be expressed in absolute terms or as a fraction of the intake. It should be stressed that dietary factors increasing the endogenous losses of a particular nutrient via urine or faeces have to be addressed when it comes to their effect on bioavailability of that nutrient. Bioavailability of a nutrient is not exclusively dependent on endogenous factors, like characteristics of foods or food components. It may also be dependent on endogenous factors, like sex, age, pregnancy and lactation.

The application of radio- and stable isotopes in nutrition research [3, 4] has provided a lot of knowledge on dietary factors affecting bioavailability and this is particularly the case for calcium. True absorption is an important component of bioavailability, whereas, particularly for calcium, factors which enhance its urinary loss (protein, sodium and caffeine) attracted a lot of attention during the last 15 years.

After a brief discussion of the mechanisms of intestinal absorption and renal excretion of calcium, the effects of the various endo- and exogenous factors affecting calcium bioavailability will be reviewed briefly.

2 BIOAVAILABILITY OF CALCIUM

2.1 Intestinal calcium absorption and endogenous calcium excretion
The intestinal absorption of calcium occurs via two processes [5]: active transcellular calcium transport, mainly in the duodenum and jejunum, mediated by calcitriol, the active component of vitamin D, and passive paracellular transport, mainly in the jejunum and ileum. Most of the calcium absorbed from foods is passively moved across the gastrointestinal barrier. It is clear that availability for absorption requires calcium to be solubilized, either in a free ionic or complexed form. In adults true fractional calcium absorption at medium intakes is in the order of 25-35% [5]. The endogenous excretion of calcium into the intestinal lumen is about 150 mg/d, part of which will be reabsorbed, leaving an endogenous faecal excretion of about 100 mg at medium intakes [6]. It is likely that the reabsorption is largely dependent on the same factors that influence calcium absorption. The urinary excretion of calcium equals the filtered calcium load in the kidneys (glomerular filtration rate times the ultrafiltrable plasma calcium) minus the amount of calcium reabsorbed in the tubuli. Reabsorption is stimulated by parathyroid hormone and calcitriol. Because of the glomerular filtration and the maintenance of the plasma calcium concentration within narrow limits, calcium losses in urine are inevitable and amount to at least about 100-120 mg/d [6]. Calcium losses through skin are small and probably not much higher than 25 mg/d [6]. There is evidence that the loss is higher under conditions of extreme sweating.

2.2 Effects of endogenous factors
Endogenous factors affecting calcium absorption are: age, sex, pregnancy and lactation.

Age and sex
Calcium absorption (and retention) is increased when high demands for calcium exist. This situation occurs during periods of rapid growth, such as early infancy and puberty [6]. This adaptive response is mediated by an increased synthesis of calcitriol in the kidneys and an enhanced active calcium absorption in the intestine. During the early postmenopausal years estrogen deficiency is associated with an increased net calcium resorption from bone, leading to a decreased intestinal calcium absorption [1, 8]. Evidence exists that at old age active calcium absorption and the adaptive response to low intakes are decreased probably as a result of a lower synthesis of calcitriol in the kidneys or a lower calcitriol receptor activity [1, 8]. It is also possible that a decreased secretion of gastric acid in elderly people is the cause of a reduced calcium solubilization in the intestinal lumen [5, 9].

Pregnancy and lactation
At birth the human baby contains about 25 g of calcium, most of which is delivered to the foetus during the last trimester of pregnancy. During a 6-month lactation period a total of about 50 g of calcium is delivered to the baby. These calcium fluxes are associated with an increased intestinal calcium absorption, mediated by increased circulating levels of PTH and calcitriol [10] and most probably prolactin [11].

2.3 Effects of exogenous factors
Exogenous factors reported to affect calcium bioavailability are calcium intake level, vitamin D, phytate, oxalate, dietary fibre, phosphorus, phosphopeptides, fat, protein, sodium, ammonium chloride, alcohol, caffeine, lactose, and congestion of a meal.

Calcium intake level
The fractional intestinal calcium absorption shows an inverse relationship to calcium intake, whereas urinary calcium increases with calcium intake. The adaptive responses to low intakes are mediated by increased circulating levels of PTH and calcitriol, promoting the active component of the intestinal calcium absorption and renal tubular calcium reabsorption [12–14]. Moreover, a low calcium diet in the rat results in a significant upregulation of the concentration of the vitamin D receptor in the intestine [15], whereas in the same animal calcium deprivation enhanced the inactivation of vitamin D in the liver [16].
Vitamin D
Vitamin D, obtained from synthesis in the skin under the influence of sunlight and/or from dietary sources, is required for the formation of calcitriol in the kidneys. Deficiency of vitamin D leads to a decrease in the active transcellular calcium transport in the intestine, hypocalcaemia, hyperparathyroidism and bone resorption [14].

Phytate and oxalate
Phytate (in cereals, beans and pulses) can form insoluble complexes with calcium, magnesium and zinc, leading to a reduced availability for absorption [17, 18]. There is no evidence that in balanced mixed diets this is an important anti-nutritional effect. In macrobiotic and vegan diets, however, the intake of phytate can be substantial and reduce significantly the bioavailability of calcium. Moreover evidence exists that high phytate intake levels may adversely influence the vitamin D status by reducing the plasma half life of calcidiol [19]. Oxalate forms insoluble complexes with calcium and has a strong negative influence on calcium absorption [20].

Dietary fibre
Dietary fibre (cellulose, hemicellulose, lignin, non-cellulose polysaccharides) and resistant carbohydrates, like amylose fractions, sugar alcohols, and undigestible oligosaccharides, could influence calcium absorption, either negatively or positively, by the formation of insoluble or soluble calcium complexes [3]. Resistant carbohydrates could increase calcium absorption in the distal part of the intestine (ileum and colon) by stimulating fermentation and reducing the pH of the intestinal lumen. Bacterial digestion of plant constituents in the lower intestine may be an important factor in calcium bioavailability, since calcium absorption in the colon is possible [21, 22]. Such a process is probably very important for persons on macrobiotic or vegan diets. Although several dietary fibre components have been demonstrated to inhibit calcium absorption or to decrease calcium balance [3], there is no evidence for long-term adverse effects in persons on balanced mixed diets. Excessive intakes of fibre-rich foods, particularly those which also include phytate, should not be recommended in view of the well-established adverse effects on mineral nutrition [17].

Phosphorus
There is no convincing evidence that dietary phosphorus (not polyphosphate or phytate phosphorus) reduces calcium bioavailability. Although calcium can form insoluble complexes with phosphorus in the intestine and reduce phosphorus absorption, this appeared to have no consequence for the amount of calcium absorbed [23, 24]. Dietary phosphorus reduces the urinary calcium excretion [23] and counterbalances, at least in part, the calciferic effect of dietary proteins from natural protein-rich foods, like meat, cheese and milk, which are also rich in phosphorus.

Phosphopeptides
Casein phosphopeptides, released during the digestion of milk protein, can form soluble complexes with calcium in the intestinal lumen and have been reported to increase passive calcium absorption in vitro or in situ [25]. Whether phosphopeptides exert such an effect in vivo remains controversial [26].

Fat
Fatty acids, particularly long chain saturated fatty acids, can form insoluble complexes with calcium and render calcium unavailable for absorption [3]. Fat intake, however, is not a significant factor for calcium bioavailability in healthy individuals. It becomes important in conditions of fat malabsorption, where calcium absorption is reduced by calcium soap formation. In human milk 85% of the palmitate is at the 2-position of the triglyceride molecule and is absorbed as the monoglyceride, since pancreatic lipase preferentially hydrolyses fatty acids from 1- and 3-positions. This certainly contributes to the high bioavailability of calcium from breast milk as compared to that from formulas with palmitate merely at 1- and 3-positions.

Protein
Dietary protein increases urinary calcium loss by enhancing the glomerular filtration rate and by inhibiting tubular calcium reabsorption [27]. The latter effect is caused by the increased excretion of sulfate originating from sulfur containing amino acids [28]. The calciferic effect of dietary protein is counterbalanced by dietary phosphorus [23, 24]. One might expect a decreased calcium bioavailability as a consequence of the excess urinary loss, but there is no evidence that protein intake is a significant factor in the loss of bone mass and the etiology of osteoporosis. This would mean that compensatory intestinal calcium absorption takes place. However, there is no evidence for any effects of dietary protein on circulating levels of calcitriol or active intestinal transport of calcium.

Sodium
Like protein, sodium intake raises the urinary excretion of calcium [29-31]. This will increase the amount of calcium that has to be absorbed from the diet to maintain calcium balance, otherwise calcium would be resorbed from the skeleton to compensate the reduced bioavailability. The possible long term effects of sodium intake on calcium retention in man have not yet been evaluated, but it has been demonstrated that dietary sodium increases the obligatory urinary calcium excretion in postmenopausal women, suggesting an increase in bone resorption [32].

Ammonium chloride
This salt is applied in liquorice. A high intake of double-salted liquorice could cause a mild metabolic acidosis with an increased urinary calcium excretion [33]. At present it is not clear whether in the long term regular consumption of liquorice reduces calcium bioavailability or has any effect on the bone mass.

Alcohol
Alcohol consumption has an acute positive effect on urinary calcium excretion [34], but moderate alcohol consumption seems to be associated with a positive effect on bone in postmenopausal women [35]. So alcohol in moderation does not seem to influence adversely calcium bioavailability.

Caffeine
Caffeine increases urinary calcium excretion [36]. Whether regular consumption of several cups of coffee per day adversely influences calcium bioavailability remains controversial. A moderate dose of 400 mg caffeine did not shift the calcium balance significantly into a negative direction [37]. On the other hand, a calcium kinetic study in postmenopausal osteoporotic women indicated that a high intake of coffee (in excess of 1 lid) would induce an extra calcium loss of 1.6 mmol/d [38]. A recent epidemiological study reported an increased hip fracture risk with increasing intakes of caffeine [39]. So the caffeine dose may be a significant factor for calcium bioavailability.

Lactose
Lactose has long been known to stimulate passive intestinal calcium transport in rodents and humans [14, 40]), probably because a part of the sugar reaches the distal intestine, where it stimulates fermentation, reduces the luminal pH and increases calcium solubility. In humans the effect of lactose
on calcium absorption has not always been found [1]. One
explanation could be that Caucasians generally maintain a
high intestinal lactase activity, so that only small amounts of
lactose reach the distal intestine. Another explanation could
be that an increased passive calcium absorption in humans
leads to a feedback on the active intestinal calcium transport
component, so that the net effect of lactose is zero.

Meal effects on calcium absorption
Several recent studies have indicated that calcium
absorption from different sources, including calcium salts,
vegetables and milk, is comparable, with the exception of
spinach [20, 41–44]. Calcium from calcium citrate/malate
showed a slightly higher availability than calcium from cal-
cium carbonate. An important observation is that the conges-
tion of a meal with a calcium salt increases the intestinal
absorption of calcium, probably as a consequence of stimula-
tion of gastric acid secretion, delayed stomach emptying and
better calcium solubilization [45,46].

3 CONCLUSION
The available evidence indicates that many factors influ-
ence calcium absorption and urinary calcium excretion. The
implications of these effects, however, with respect to human
health and particularly with respect to prevention of osteo-
porosis is not always clear. This is particularly the case for
those dietary factors that increase the urinary loss of calcium,
like high intake levels of protein, sodium, caffeine, ammonium
chloride and alcohol. It is likely that with western diets, being rich
in these dietary components, increase calcium requirements.

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