THERAPEUTIC PROPERTIES OF FERMENTED MILKS

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ELSEVIER APPLIED SCIENCE
LONDON and NEW YORK

ELSEVIER SCIENCE PUBLISHERS LTD
Crown House, Linton Road, Barking, Essex IG11 8JU, England

Sole Distributor in the USA and Canada
ELSEVIER SCIENCE PUBLISHING CO., INC.
655 Avenue of the Americas, New York, NY 10010, USA

WITH 34 TABLES AND 5 ILLUSTRATIONS

British Library Cataloguing in Publication Data

Therapeutic properties of fermented milks.
1. Food. Health aspects
I. Robinson, R. K. (Richard Kenneth)
613.2

ISBN 1-85166-552-8

Library of Congress Cataloging-in-Publication Data

Therapeutic properties of fermented milks/edited by R. K. Robinson.
p. cm.—(Elsevier applied food science series)
Includes bibliographical references (p.) and index.
ISBN 1-85166-552-8
1. Fermented milk—Therapeutic use. 2. Fermented milk—Microbiology. I. Robinson, R. K. (Richard Kenneth) II. Series.
RM234.T48 1991
615'.36—dc20

Photoset by Enset (Photosetting), Midsomer Norton, Bath, Avon
Printed in Great Britain at The University Press, Cambridge.

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PREFACE

Nutrition, probably more than any other aspect of food science, thrives on a mixture of fact, speculation and myth, and the main reasons for this situation are not difficult to determine. Thus, nutritional studies on humans are notoriously difficult to perform, partly because of the problem of obtaining large test populations, and partly because any individual who does agree to participate will, to some degree, be a unique being. Certain basic metabolic pathways are obviously universal, but differences both qualitative and quantitative abound. The result of this diversity is that the same foodstuff may well affect different individuals in different ways, and perhaps in a manner that is too subtle to be recognised by standard clinical diagnoses.

It is this inherent variability between individuals that feeds the plethora of myths and 'old wives tales' that surround food habits. In reality, many of these 'much ridiculed' tales contain more than a 'grain of truth', particularly when assessed in a well-defined context. The early work of Metchnikoff, for example, suggested that the health and longevity of the Balkan peoples was founded on their regular consumption of yoghurt, while the literature of Russia contains similar accounts of illnesses avoided or cured by the inclusion of kefir or kumiss in the diet. Applied to different populations and/or products, such ideas may become untenable, and it is for this reason that, for the last fifty years or so, controversy has raged over the alleged health benefits of fermented milks.

Nevertheless, the basic hypothesis has never been disproved, and numerous authorities have claimed that fermented milks can be effective in combating a range of clinical or subclinical conditions. Indeed, evidence in favour of the view that certain 'beneficial' bacteria can have a health-promoting role has been steadily accumulating, and today few clinicians would feel disposed to state categorically that fermented milks cannot offer a health benefit over and above that indicated by a crude analysis for protein, vitamins or minerals. Obviously the type of bacterial culture is important, and clearly not every consumer will react in the same manner—even given the same pattern of consumption/complementary diet—but even so, genuine interest has replaced wholesale scepticism.

It is, therefore, a most opportune time to offer this critical examination of the dietary roles of fermented milks, and to this end, specialists from around the world have been invited to comment on the possible therapeutic properties of the major types found in the market-place. It must be admitted, of course, that definitive proof of health-promoting activity based on large-scale clinical trials is still lacking, and that the reaction of different test groups is often contradictory. Nevertheless, sufficient fragments of data have been accumulated to make the basic hypothesis look entirely plausible; so plausible in fact that it can no longer be simply dismissed.

It is a pleasure, therefore, to acknowledge with gratitude the efforts of the contributors that have made this book possible, and if it serves to keep alive the interest in the possible health-promoting properties of fermented milks, it will prove to have been a most worthwhile project.

R. K. Robinson

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Chapter 1

MILK AS A FOOD

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Milk is a complete food for newborn mammals. It is the sole food during the early stage of rapid development and as such contains those constituents, particularly proteins, fats and minerals, required for mammalian growth and development. As well as its nutritional properties, milk also affords protection against infection in the neonate by virtue of the protective factors it contains.

For young humans after weaning, milk, especially cows' milk, still plays an important dietary role providing fat for energy, proteins for muscle development and minerals for bone development. As part of a mixed diet milk has a proven record as a healthy, nutritious food. For the adult, products derived from milk become more important as constituents of a nutritionally balanced diet.

The history of milk as a food for man most likely extends back to prehistoric times. Cave drawings estimated to be 15 000 years old show that cattle were known in prehistoric times and there is evidence that domestication of cattle occurred before 4000 BC. Illustrations found on artifacts discovered in the Middle East, in Egypt and Mesopotamia, show that different types of cattle and various aspects of dairy farming existed about 3000 BC.

Dairy farming became well established in Ancient Egypt, as records have been found listing a cattle census in 2800 BC and paintings, dated by egyptologists as being done about 2500 BC, show cows being milked.

In Europe, domestication and the development of dairy farming occurred much later. Cattle were kept on common land, and a number were culled in the winter because of the shortage of grazing and the need for meat. Milk was consumed warm directly after milking. Surplus milk was converted into products, such as butter and cheese, as a means of preservation and sold locally. It was not until the 18th and 19th centuries that development of a more widespread distribution of milk as a food for the population occurred.

As the population of towns increased and the Industrial Revolution spread, so the need to supply the urban population with milk increased. It was not, however, until the beginning of the 20th century with the acceptance and widespread development of commercial pasteurisation of milk, together with improvements in milk production practices, that milk became established as a wholesome and safe food.

CONSTITUENTS OF MILK

Milk as a complete food for the newborn contains all the main nutrients required of a food. The amounts of the various constituents of milk are species dependent, and cows' milk differs in content from human milk in several important areas. For example, although the total fat content is similar, human milk fat contains a higher proportion of the long-chain unsaturated fatty acids. The protein fraction of human milk also contains a higher proportion of whey proteins and the lactose concentration is about twice that of cows' milk.

Cows' milk contains 3–4% protein, 3–6% fat, about 5% lactose and about 0·7% ash giving a total solids content of 11·5–15·5%. Many factors, such as breed characteristics, lactational, seasonal and dietary effects influence milk composition giving rise to variations in content.

In terms of dietary energy supply, a litre of milk of average composition, i.e. 3·5% protein, 3·8% fat and 4·7% lactose, would contribute about 660 kcal or 2750 kJ. Detailed nutritional composition data for milk and milk products is given by Paul and Southgate (1978) and Souci *et al.* (1981).

MILK PROTEIN

The importance of milk as a source of high quality protein for the growth and development of the young has already been mentioned. The nutritional importance is not only related to the amino acid composition of the proteins, but also to physico-chemical properties that enhance their value as a food. The proteins of cows' milk together with some of their properties are listed in Table I. The average protein content of milk is about 3·5%, but will vary slightly according to breed, stage of lactation, season of the year and the health of the animal.

TABLE I
PRINCIPAL PROTEINS IN COWS' MILK

Protein	*Composition in skim-milk (g/litre)*	*Number of genetic variants*	*Molecular weight*
α_{s1}-Casein	12–15	5	22 068–23 724
α_{s2}-Casein	3–4	4	25 230
β-Casein	9–11	7	23 944–24 092
κ-Casein	2–4	2	19 007 and 19 039
β-Lactoglobulin	2–4	8	18 205–18 363
α-Lactalbumin	0·6–1·7	2	14 147 and 14 175
Serum Albumin	0·4	1	66 267
Immunoglobulin G_1	0·3–0·6	—	153 000–163 000
Immunoglobulin G_2	0·05–0·1	—	146 000–154 000
Immunoglobulin A	0·05–0·15	—	385 000–417 000
Immunoglobulin M	0·05–0·1	—	1 000 000
Secretory Comp.	0·03–0·1	—	79 000

Source: Eigel *et al.*, 1984.

The principal proteins in milk are the caseins, comprising about 85% of the total protein. They are a group of phosphoproteins which, by virtue of their physico-chemical properties, form stable complexes with calcium phosphate to produce protein mineral aggregates ranging in size from about 100 to 250 nm in diameter, the casein micelles. It is the micelles by reason of light scatter which give milk its white colour.

The phosphorylation of the caseins during synthesis is an essential biological process, as these groups are required to form stable casein micelles—necessary in the subsequent behaviour of the caseins as nutrients. The presence of phosphoserine residues provides a mechanism by which the mineral concentration of milk can be increased without causing instability, and also allows enzymes to preferentially act on the 'Ca-sensitive' caseins as principal substrates (West, 1986). Although nutritionally important, the caseins may have other specific functions. One suggestion is a relationship to the riboflavin-binding proteins.

A riboflavin-binding protein in chicken eggs, required for the transport of riboflavin across the yolk sac membrane, has eight serine phosphates arranged in sequence similar to those in casein. The protein is not involved with the vitamin binding site, but is presumed to be involved in binding to a recognition site on the membrane. No transfer of vitamins takes place if the protein is dephosphorylated. The similarity of the arrangement of the serine phosphates between the riboflavin-binding proteins and the caseins suggests the possibility that, although the caseins do not function as riboflavin binding proteins, they might act as a membrane recognition signal in the vitamin transfer process (West, 1986).

Fragments of β-casein have been isolated which have opiate-like activity in animal and isolated organ experiments. Some caseolytic strains of bacteria were found to produce these peptides, termed β-casomorphins, when incubated in cows' milk (Hamel *et al.*, 1985). The principal member of the group β-casomorphin (1–7) has an amino acid sequence. TRY-PRO-PHE-PRO-GLY-PRO-ILE. However, no evidence has been found of the presence of β-casomorphins in human plasma after consuming cows' milk or milk products. Nevertheless, it has been speculated that these peptides may act as local regulators of intestinal function, in other words as 'food hormones' (Morley, 1982; Teschemacher *et al.*, 1986).

Organisation of the micelle structure gives a highly concentrated arrangement of proteins, calcium and phosphate having an open, sponge-like structure which permits access of digestive enzymes. The *k*-casein linkage between residues 105 and 106 is uniquely susceptible to proteolytic attack. The hydrophilic portion of the molecule is released and the hydrophobic portion remains in the micelle. This change leads to micelle destabilisation and to the formation of a coagulum. The curd formed in the stomach of the young by the action of chymosin and pepsin is, however, less accessible to subsequent enzymic digestion than if the components were all soluble. The curd formation allows for greater retention in the stomach and a slower and prolonged digestion and release of nutrients (Poiffait & Adrian, 1986).

The curd formed from bovine milk is harder than that formed from human milk, and its digestion and release of nutrients is slower. Ca^{2+} absorption in the gut has been shown to be influenced by the presence of the casein phosphate groups, because if the casein is dephosphorylated, the absorption of Ca^{2+} is less. There is a high calcium content in bovine milk, about 32 mMoles, but little is present as the free ion Ca^{2+}. In the soluble phase it is complexed with citrate, and in the micellar phase as colloidal calcium phosphate.

The bioavailability of divalent metal ions such as Ca^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+} is influenced by their strong binding to the caseins. Although Zn^{2+} is released under acid conditions, it is retained in a chymosin formed coagulum.

Whey proteins, which are globular proteins, comprise the rest of the protein fraction. Most of the whey proteins, such as β-lactoglobulin and α-lactalbumin are synthesised in the mammary gland, whilst some of the rest, such as serum albumin and immunoglobulin, are derived from the blood.

Whey proteins constitute about 20% of the total milk protein, but contribute proportionally greater amounts of essential amino acids. There are 511 mg of essential amino acids/g of casein, but whey proteins contain 609 mg/g of protein. Whey proteins per gram contain 60% more of the sulphur-containing amino acids, methionine plus cysteine, than the caseins.

Although the principal whey proteins, α-lactalbumin and β-lactoglobulin, have an important nutritional role, other functions for these proteins have been postulated. It was shown over 20 years ago that α-lactalbumin could be substituted for one of the subunits of the enzyme lactose synthetase and enzyme functionality maintained, suggesting an enzymic role for this protein (Ebner *et al.*, 1966).

More recently crystallographic analysis of baboon α-lactalbumin has shown that there is similarity with egg-white lysozyme. The amino acid sequences are similar, and it has been suggested that the class C lysozymes and α-lactalbumin are homologous proteins and that α-lactalbumin evolved from a lysozyme precursor (Smith *et al.*, 1987).

The structure of the crystal form of β-lactoglobulin shows remarkable similarity to a retinol binding protein found in blood plasma. Such homology may suggest that β-lactoglobulin could have a role in vitamin A transfer (Papiz *et al.*, 1986). However, any metabolic function of these two whey proteins remains as yet unproven.

Milk contains a number of protective proteins whose function is to protect against infection of the mammary gland and/or protect the neonate (Reiter, 1985). Lysozyme, a basic protein of about 15 000 daltons is present in cows' milk in very small quantities compared with human milk, where concentrations of about 40 mg/100 ml are found. This protein can attach to bacterial surfaces and, in the presence of anions, especially thiocyanate SCN^- and bicarbonate HCO_3^-, has lytic activity. Lactoperoxidase, a glycoprotein of about 77 500 daltons, occurs at about 3 mg/100 ml, and for its inhibitory activity requires the presence of peroxide, e.g. H_2O_2, and thiocyanate. Oxidation products of SCN^- are formed of which hypothiocyanate $OSCN^-$ is probably the most important. This anion attaches to the inner membrane of bacteria and inhibits membrane transfer of nutrients.

Lactoferrin, a glycoprotein of about 76 500 daltons, with two metal binding sites, each able to bind Fe^{3+}, occurs in cows' milk at concentrations between 2 and 35 mg/100 ml. It can only inhibit bacteria that have high iron requirements, such as the coliforms, and its iron binding capacity depends upon the presence of bicarbonate HCO_3^-. Citrate counteracts the bacteriostatic activity unless the concentration of bicarbonate is high.

Xanthine oxidase, a protein found associated with the milk fat globule membrane, may have some protective contribution. It is involved in the catabolism of purines and, in this reaction, superoxide (O_2) and H_2O_2 are generated. The synergistic action of the protective proteins with the immunoglobulins gives, overall, greater protection than does the sum of the individual components.

Immunoglobulin of the types IgA, IgG and IgM, together with free secretory component (FSC), are found in milk. Colostrum contains high concentrations of these proteins, while in milk, concentrations of about 14 mg of IgA, 60 mg of IgG_1, 2 mg of IgG_2, 5 mg of IgM and 5 mg of FSC per 100 ml of milk have been reported (Eigel *et al.*, 1984). The immunoglobulins enter the milk through the mammary secretory cells, but they are synthesised by plasma cells derived from lymphocytes. These plasma cells are located in various sites, but those synthesising IgA in particular appear to be situated in the mammary tissue.

A large adhesive glycoprotein, fibronectin, molecular size 233 000 daltons, has been isolated from milk where it occurs at a concentration of 2 μg/ml, and from colostrum where it is present at 30 μg/ml (Sato & Hayashi, 1985). It appears to be similar to blood plasma fibronectin which plays a role in the non-immune, self-defence system.

The protective factors being proteins are denatured by heat treatment, and under the conditions used in the preparation of a fermented milk, i.e. 85°C for 30 min or 95°C for 10 min, little could be expected to survive.

Genetic variants of all the principal proteins have been found. These protein variants usually differ in composition by substitution of one or more amino acids; up to nine for example in β-lactoglobulin B, or in the case of α_{s1}-casein A by the deletion of the 13 amino acids in positions 14 to 26 in the α_{s1}-casein molecule. The occurrence of some variants, e.g. α_{s1}-casein D is quite rare, and the effect of variants on the physical and nutritional properties of milk, particularly in bulk milk supplies, is virtually negligible (Eigel *et al.*, 1984).

Non-protein nitrogen constituents in milk comprise about 5% of the total nitrogen. The principal component of this fraction is urea, forming about half of the NPN. Other components include free amino acids, creatine and nitrate. Feed high in nitrogen will increase the levels of nitrate and urea in milk. The levels of urea in milk are thought to be important in the stability of heat-treated and concentrated milks; higher levels yielding more stable products, possibly through increased conversion of urea to cyanate and the subsequent reaction of cyanate with protein-bound lysine. The resultant change in charge, it is suggested, improves colloidal stability of the casein micelles (Muir & Sweetsur, 1978).

Milk proteins are relatively rich in essential amino acids. Individual protein fractions may vary and thus give rise to differences in amino acid content, but on average, milk protein contains about 46 g of essential amino acids per 100 g protein: 1·4 g tryptophan, 5·2 g phenylalanine 10·4 g leucine, 6·4 g isoleucine, 5·1 g threonine, 2·7 g methionine, 8·3 g lysine and 6·8 g valine. A seasonal variation has been reported in which the proportion of essential amino acids increases during the warmer months of the year, although total protein production during this period is less (Kirchmeier, 1973).

NUTRITIONAL VALUE OF MILK PROTEINS

There are a number of indices that can be used to compare nutritional properties of proteins. The most commonly used are: (a) biological value, where 100 g of dietary protein can replace the equivalent amount of adult body protein. A replacement value of 100 is given to whole egg protein, and higher or lower values reflect the quality of a protein; (b) net protein utilisation (NPU) determined from animal feeding experiments and is similar to biological value; and (c) protein efficiency ratio (PER) which relates to the weight gain produced by 1 g of dietary protein in a growing animal.

These indices give similar but not identical results, and a comparison of the values obtained for milk protein with some other food proteins is given in Table II.

In the case of the individual milk proteins, the PER value for casein is considered to be 2·9, whilst α-lactalbumin which is richer in essential amino acids has a value of 4 and β-lactoglobulin a value of 3·5.

Good quality protein, particularly animal protein, is especially important in the diet of the young and the elderly. Consumption of half a litre of milk a day will provide the recommended intake of essential amino acids for children and adolescents with the exception of methionine and cystine. In the case of these two sulphur-containing amino acids, milk

TABLE II
NUTRITIONAL VALUE INDICES

Protein	*Biological value*	*NPU value*	*PER value*
Egg	100	94	3·8
Milk	91	82	3·1
Beef	80	73	2·9
Soya	74	61	2·1
Wheat	54	41	1·5
Beans	49	39	1·4

provides 60% of the requirement for adolescents and 85% of the requirement for children. A daily intake of say 200–250 ml of milk in a mixed diet would ensure an adequate supply of essential amino acids for the young.

The requirement for protein increases in the elderly because the rate of protein metabolism is reduced, and there is a corresponding loss of muscle tissue. There is also an increased requirement for some essential amino acids, especially lysine and methionine, and these metabolic changes which take place after the age of 50 emphasise the need for a mainly animal protein diet. As there is also a reduction in energy requirement for the elderly, their diet should be balanced so that protein provides about 15% of the energy supplied. A daily intake of say 300 ml of milk and 50 g of cheese would, in a mixed diet, provide a significant part of the protein requirement; the use of low fat milk being advantageous.

MILK LIPIDS

Milk fat is secreted in the mammary gland as globules surrounded by a membrane derived from the secretory cell. The integrity of the membrane is effected by the cooling and processing of milk; part is released and also exchange takes place between the globule surface and other milk constituents. Fat globules have an average size of 2–6 μm, although there is a considerable distribution in size. The fat content of milk varies according to breed, season and to some degree in relation to the cows' diet (Faulkner *et al.*, 1986). An average value of 3·8 g milk fat per 100 g of milk is a figure generally quoted. Milk fat consumption has tended to decrease in recent years partly due to health and diet implications, but