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Overview of Bioactive Components in Milk and Dairy Products

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INTRODUCTION

Milk has been known as nature's most complete food. However, the traditional and contemporary view of the role of milk has been remarkably expanded beyond the horizon of nutritional subsistence of infants. Milk is more than a source of nutrients for any neonate of mammalian species, as well as for growth of children and nourishment of adult humans. Aside from nutritional values of milk, milkborne biologically active compounds such as casein and whey proteins have been found to be increasingly important for physiological and biochemical functions that have crucial impacts on human metabolism and health (Schanbacher et al. 1998; Korhonen and Pihlanto-Leppälä 2004; Gobbetti et al. 2007). Recent studies have shown that milk furnishes a broad range of biologically active compounds that guard neonates and adults against pathogens and illnesses, such as immunoglobulins, antibacterial peptides, antimicrobial proteins, oligosaccharides, and lipids, besides many other components at low concentrations (so-called "minor" components, but with considerable potential benefits). During the past decades, major progress has been made in the science, technology, and commercial applications of the multitude and complexity of bioactive components, particularly in bovine milk and colostrum. Cow milk has been the major source of milk and dairy products in developed countries, especially in the Western world, although more people drink the milk of goats than that of any other single species worldwide (Haenlein and Caccese 1984; Park 1990, 2006). Among the many valuable constituents in milk, the high levels of calcium play an important role in the development, strength, and density of bones in children and in the prevention of osteoporosis in elderly people. Calcium also has been shown to be beneficial in reducing cholesterol absorption, and in controlling body weight and blood pressure. Recent numerous research activities and advanced compositional identification of a large number of bioactive compounds in milk and dairy products have led to the discovery of specific biochemical, physiological, and nutritional functionalities and characteristics that have strong potential for beneficial effects on human health. Four major areas of bioactivity of milk components have been categorized: 1) gastrointestinal development, activity, and function; 2) infant development; 3) immunological development and function; and 4) microbial activity, including antibiotic and probiotic action (Gobbetti et al. 2007).

MILK AS A RICH SOURCE OF BIOACTIVE COMPONENTS

Milk contains a wide range of proteins that provide protection against enteropathogens or are essential for the manufacture and characteristic nature of certain dairy products (Korhonen and Pihlanto-Leppälä 2004). Milk has been shown to contain an array of bioactivities, which extend the range of influence of mother over young beyond nutrition (Gobbetti et al. 2007). Peptides are in a latent or inactive state within protein molecules but can be released during enzymatic digestion. Biologically active peptides released from caseins and whey proteins contain 3 to 20 amino acids per molecule (Korhonen and Pihlanto-Leppälä 2004). Researchers for the last decade have demonstrated that these bioactive peptides possess very important biological functionalities, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunomodulatory, opioid, and mineral-carrying activities. A simple schematic representation of major bioactive functional compounds derived from milk is presented in Figure 1.1.

Most of the bioactivities of milk proteins are latent, being absent or incomplete in the original native protein, but full activities are manifested upon proteolytic digestion to release and activate encrypted bioactive peptides from the original protein (Clare and Swaisgood 2000; Gobbetti et al. 2002). Bioactive peptides (BPs) have been identified within the amino acid sequences of native milk proteins. They may be released by proteolysis during gastrointestinal transit or during food processing. Enzymes such as digestive, naturally occurring in milk, coagulants and microbial enzymes, especially those from adventitious or lactic acid starter bacteria, usually generate these bioactive compounds. BPs are released from milk proteins during milk fermentation and cheese maturation, which enriches the dairy products (Gobbetti et al. 2002). The major biologically active milk components and functions in milk precursors and components are summarized in Table 1.1.

Several milk-derived peptides have shown multifunctional properties, and specific peptide sequences have two or more distinct physiological activities. Certain regions in the primary structure of casein contain overlapping peptide sequences that exert different activities, as shown in Table 1.2. These fragments have been considered as strategic zones that are partially protected from further proteolytic

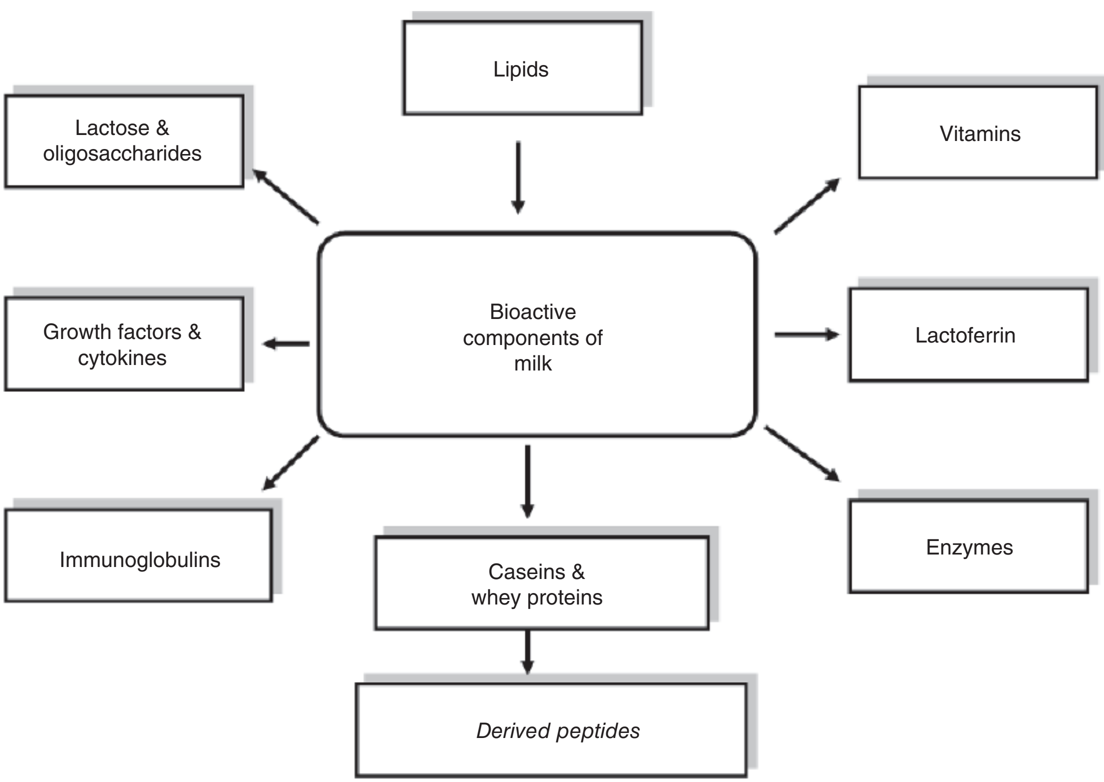

Figure 1.1. Schematic representation of major bioactive functional compounds derived from milk.

Table 1.1. Major biologically active milk components and their functions[1]

Milk Precursors or Components	Bioactive Compounds	Bioactivities Observed
α-, β-caseins	Casomorphins	Opioid agonist (Decrease gut mobility, gastric emptying rate; increase amino acids and electrolytes uptake)
α-, β-caseins	Casokinins	ACE inhibitory (Increase blood flow to intestinal epithelium)
α-, β-caseins	Phosphopeptides	Mineral binding (Ca binding; increase mineral absorption, i.e., Ca, P, Zn)
α-, β-caseins	Immunopeptides Casomorphins Casokinins	Immunomodulatory (Increase immune response and phagocytic activity)
α_{s1}-casein	Isracidin	Antimicrobial
α_{s2}-casein	Casocidin	Antimicrobial
κ-casein	Casoxins	Opioid antagonist
κ-casein	Casoplatelins	Antithrombotic
κ-casein	κ-casein glyco-macropeptide	Probiotic (Growth of bifidobacteria in GI tract)
α-lactalbumin (α-La) β-lactoglobulin(β-La)	Lactorphins	Opioid agonist
Serum albumin	Serorphin	Opioid agonist
α-La, β-La and Serum albumin	Lactokinins	ACE inhibitory
Immunoglobulins	IgG, IgA	Immunomodulatory (Passive immunity)
Lactoferrin	Lactoferrin	Immunomodulatory (Increase natural killer cell activity, humoral immune response, thymocyte trafficking immunological development, and interleukins-6; decrease tumor necrosis factor-α) Antimicrobial (Increase bacteriostatic inhibition of Fe-dependent bacteria; decrease viral attachment to and infections of cells) Probiotic activity (Increase growth of Bifidobacteria in GI tract)
Lactoferrin	Lactoferroxins	Opioid antagonist
Oligosaccharides	Oligosaccharides	Probiotic (Increase growth of bifidobacteria in GI tract)
Glycolipids Oligosaccharides	Glycolipids Oligosaccharides	Antimicrobial (Decrease bacterial and viral attachment to intestinal epithelial cells)
Prolactin	Prolactin	Immunomodulatory (Increase lymphocyte and thymocyte trafficking, and immune development)
Cytokines	Interleukins-1,2,6, & 10 Tumor necrosis factor-α Interferon-γ Transforming growth Factors-α, β; leukotriene B_4 Prostaglandin E_2, Fn	Immunomodulatory (Lympocyte trafficking, immune development)
Growth factors	IGF-1, TGF-α, EGF, TGF-β	Organ development and functions
Parathromone-P	PTHrP	Increase Ca^{+2} metabolism and uptake

[1]Adapted from Schanbacher et al. (1998), Meisel (1998), and Clare and Swaisgood (2000).

Table 1.2. Examples of physiologically active milk peptides derived from milk[1]

Peptide Sequence[2]	Name	AA[3] Segment	Physiological Classification	Release Protease	Reference
FFVAP	α_{s1}-Casokinin-5	α_{s1}-CN (f23–27)	ACE inhibitor	Proline endopeptidase	Maruyama et al. (1985)
AVPYPQR	β-Casokinin-7	β-CN (f177–183)	ACE inhibitor	Trypsin	Maruyama et al. (1985)
YGLF	α-Lactorphin	α-LA (f50–53)	ACE inhibitor and opioid agonist	Synthetic peptide	Mullally et al. (1996)
ALPMHIR	β-Lactorphin	β-LG (f142–148)	ACE inhibitor	Trypsin	Mullally et al. (1997)
KVLPVPQ	Antihypertensive Peptide	β-CN (f169–174)	Antihypertensive peptide	Lactobacillus CP790 protease, and synthetic peptide	Maeno et al. (1996)
MAIPPKKNQDK	Casoplatelin	κ-CN (f106–116)	Antithrombotic	Trypsin and synthetic peptide	Jollès et al. (1986)
KDQDK	Thrombin	κ-CN glycol-inhibitory peptide macropeptide (f112–116)	Antithrombotic	Trypsin	Qian et al. (1995b)
KRDS	Thrombin inhibitory peptide	Lactotransferrin (f39–42)	Antithrombotic	Pepsin	Qian et al. (1995a)

Peptide Sequence[2]	Name	AA[3] Segment	Physiological Classification	Release Protease	Reference
QMEAES*IS*S*S*EEIVPNS*VEQK	Caseinophospho-peptide	α_{s1}-CN (f59–79)	Calcium binding and transport	Trypsin	Schlimme and Meisel (1995)
LLY	Immunopeptide	β-CN (f191–193)	Immunostimulatory (+)	Synthetic	Migliore-Samour et al. (1989)
FKCRRWQWRMKKLGAPSITCVRRAF	Lactoferricin B	Lactoferrin (f17–41)	Immunomodulatory (+) and antimicrobial	Pepsin	Bellamy et al. (1992)& Miyauchi et al. (1998)
YQQPVLGPVR	β-Casokinin-10	β-CN (f193–202)	Immunomodulatory (+/–) and ACE inhibitor	Synthetic	Meisel and Schlimme (1994)
RYLGYLE	α-Casein exorphin	α_{s1}-CN (f90–96)	Opioid agonist	Pepsin	Loukas et al. (1983)
YGFQNA	Serorphin	BSA (f399–404)	Opioid agonist	Pepsin	Tani et al. (1994)
YLLF.NH_2	β-Lactorphin amide	β-LG (f102–105)	Opioid agonist = ACE inhibitor	Synthetic or trypsin	Mullally et al. (1996)
YIPIQYVLSR	Casoxin C	κ-CN (f25–34)	Opioid antagonist	Trypsin	Chiba et al. (1989)
[YVPF PPF]	Casoxin D	α_{s1}-CN (f158–164)	Opioid antagonist	Pepsin-chymotrypsin	Yoshikawa et al. (1994)
YLGSGY-OCH_3	Lactoferroxin A	Lactoferrin (f318–323)	Opioid antagonist	Pepsin	Yamamoto et al. (1994)

[1]Adapted from Clare and Swaisgood (2000).
[2]The one-letter amino acid codes were used; S* = Phosphoserine.
[3]Amino acid.

breakdown (Fiat et al. 1993). Various peptide fragments have different physiological activities. Peptides containing different amino acid sequences can exhibit the same or different bioactive functionalities. The specific bioreactions associated with each physiological class have been characterized, and recent research data have been classified according to their physiological functionality. Some examples of BPs are compiled as shown in Table 1.2 (Clare and Swaisgood 2000).

BIOACTIVE COMPOUNDS IN FOODS AND FUNCTIONAL FOODS

In recent years, functional foods and bioactive components in foods have drawn a lot of attention and interest of food scientists, nutritionists, health professionals, and general consumers. A functional food may be similar in appearance to a conventional food, is consumed as a part of normal diet but has various physiological benefits, and can reduce the risk of chronic diseases beyond basic nutritional functions. The volume of market sales for functional foods has grown steadily. The global functional foods market continues to be a dynamic and growing segment of the food industry (Marketresearch.com 2008). Rapid growth is predicted to continue for the next year, but taper off by 2010, when functional foods are expected to represent 5% of the total global food market. The current global functional foods market is estimated to be US$7–63 billion, depending on sources and definitions (Marketresearch.com 2008), and is expected to grow to US$167 billion by 2010. The global growth rate for functional foods will likely achieve an average of 14% annually through 2010. After 2010, the functional foods market size is expected to comprise approximately 5% of total food expenditures in the developed world (Marketresearch.com 2008). Mintel International Group Ltd. (2008) reported that U.S. sales in the dairy segment of functional (bioactive) foods increased by more than 33% over the review period of 2005–07, and its share of the total market grew from 71–74% with a value of nearly US$2 billion, to nearly 75% of the total market (Table 1.3). In the bars and snacks segment, sales of functional foods more than doubled from 2005 to 2007 to a value of US$197 million. However, the segment still remains quite small, accounting for just 7% of the market in 2007. Functional cereal sales gains were modest as 6% from 2005 to 2007 with a value of US$434 million, making up 16% of market share. Bakery's share of the functional food sales stood at just 2%, which was a 29% decrease for the same period, suggesting that functional foods for the bakery segment may be the one that represents the most untapped potential (Table 1.3).

The consumption volumes of functional foods, especially for those manufactured using dairy bioactive compounds, are likely to increase in the developed countries. The U.S. sales of functional foods were forecasted to increase by 46% from 2007 to 2012 at current prices and by 28% at inflation-adjusted prices, following strong performance from 2002 to 2007 (Table 1.4). The number of new functional products drastically increased between 2002 and 2007. Product proliferation is a boon to this market because it promotes familiarity with the functional concept. However, the Mintel reports (2008) predicted that proliferation would lead to market saturation and that future growth in the spe-

Table 1.3. Sale volumes of functional foods in the U.S. during 2005 and 2007

	2005		2007		Change 2005–07
	$million	%	$million	%	%
Dairy and margarine	1,459	71	1,959	74	34
Cereal	410	20	434	16	6
Bars and snacks	92	5	197	7	113
Bakery	79	4	56	2	–29
Total	**2,041**	**100**	**2,646**	**100**	**30**

Data may not equal totals due to rounding.
Source: Mintel/based on Information Resources, Inc. InfoScan® Reviews Information.

Table 1.4. Total U.S. sales and forecast of functional foods at current prices, 2002–2012

Year	$million	% Change	Index (2002 = 100)	Index (2007 = 100)
2002	1,620	—	100	61
2003	1,666	2.8	103	63
2004	1,776	6.6	110	67
2005	2,041	14.9	126	77
2006	2,385	16.9	147	90
2007	2,646	10.9	163	100
2008 (fore)	2,879	8.8	178	109
2009 (fore)	3,117	8.3	192	118
2010 (fore)	3,359	7.8	207	127
2011 (fore)	3,611	7.5	223	136
2012 (fore)	3,874	7.3	239	146

Source: Mintel/based on Information Resources, Inc. InfoScan® Reviews Information; Mintel forecasts inflation-adjusted growth of 28% during 2007–12.

cifically defined market may slow down as manufacturers and marketers favor more general promotional methods (Table 1.4).

Globally, digestive health claims are leading functional claims for foods. A recent report also showed that functional foods and beverages providing digestive health benefits are growing, both in the traditional categories where the claim emerged for "yogurt and dairy-based beverages" and in unique and new categories, such as prepared meals and snack mixes (Prepared Foods 2008). In coming years, functional foods and beverages are expected to grow continuously. This trend stems from an ever-growing number of products capitalizing on natural ingredients that provide digestive health benefits. When it comes to digestive health, the key to business successes for manufacturers is to identify creative positioning strategies and launch new product introductions that would make a clear difference (Prepared Foods 2008). Table 1.5 shows the top 10 U.S. digestive health subcategories by number of new product introductions up to May 2008.

Table 1.5. Top U.S. digestive health subcategories (by number of new product introductions)

Category	2008*	2007	2006	2005
Spoonable yogurt	42	28	24	0
Cheese	7	15	1	0
Dairy-based frozen products	7	1	0	0
Snack/Cereal/ Energy bars	5	9	2	0
Soy yogurt	5	0	0	0
Juice	4	2	0	1
Prepared meals	4	0	0	0
Hot cereals	3	2	0	0
Drinking yogurts/ liquid cultured milk	2	6	1	1
Cold cereals	2	3	1	0
Snack mixes	2	0	0	0

Source: Mintel GNPD (*through May 5, 2008); Prepared Foods (2008).

The term *bioactive components* refers to compounds either naturally existing in food or ones formed and/or formulated during food processing that may have physiological and biochemical functions when consumed by humans. Food scientists have been exploiting bioactive components of milk and dairy products for application in functional foods and for potential pharmaceutical use. Milk is often considered as a functional food since it contains varieties of different bioactive components. Because of its chemical composition and structural properties, milk is also a good vehicle to formulate functional foods. Although bioactive compounds in milk and dairy products have been extensively studied during the last couple of decades, especially in human and bovine milk and some dairy products, there are very few publications on this topic available for other dairy animal species that provide valuable nourishment, especially in developing countries in Asia and Africa.

WHY IS THIS BOOK UNIQUE?

So far only a limited number of publications have been available about the biochemistry and technology of bioactive and nutraceutical compounds in bovine and human milk, and the milk of other mammalian species has not received much research attention in this regard. This book is therefore unique in also covering extensively bioactive components in milk and dairy products of goats, sheep, buffalo, camels, and horses by internationally renowned scientists who are in the forefront of research in functional components of milk and dairy products and their chemistry and technology. The bovine milk chapter starts the discussion in order to present the updated reference scientific information and research data in the field of bioactive components and functional food ingredients with respect to those in other dairy species. This book benefits readers around the world, including students, scientists, and health-conscious consumers who are looking for scientific information on bioactive compounds and therapeutic substances in milk and dairy products from different dairy animal species as referenced to cow milk. This book presents the best available current knowledge and reports on the up-to-date information written and forwarded by world authorities and experts in bioactive and nutraceutical components in milk and processed milk products of different dairy species.

This volume is uniquely different from other published works because it not only contains rich compilations of a variety of research data and literature on milk of different mammalian species, but because it also extensively delineates bioactive compounds in the various important manufactured dairy products. Other integral aspects of functionality of bioactive compounds are also included in this book, such as potential for improving human health with these components in milk and dairy products. The introductory section describes the overview of bioactive components in milk and dairy products and the general concept of bioactive compounds and functional foods derived from milk and dairy products. Section I covers the bioactive components in milk of the different major dairy species, which makes this book a special reference source of detailed information not otherwise available. This work therefore would be valuable to readers who seek special scientific information and data for their unique locations, environments, traditions, and availabilities of their own dairy species. Considering this rapidly emerging and fascinating scientific area in human health and nutrition, this work is a special reference source because of its unique and significant contributions to the field. Section II looks closely at the bioactive components in manufactured dairy products, such as caseins, caseinates, cheeses, yogurt products, koumiss and kefir, whey products, probiotics, and prebiotics. Section III touches on other related issues in bioactive compounds in dairy products, such as regulatory issues and functional health claims on bioactive compounds, new technologies for isolation, and analysis of bioactive compounds. This section also delineates several aspects of potential for improving human health, including immunomodulation by dairy ingredients, calcium bioavailability of milk and dairy products, and iron fortification of dairy products.

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