Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
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Bioaktiva komponenter i mjölk och mejeriprodukter

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Abstract

Milk is a complex liquid that has expanded its more conventional role and is more than a source of nutrients for young mammals. In the last few decades, a great deal of research has shown that milk and dairy products provide a broad range of bioactive components. Bioactive components can be defined as components in foods that have a physiological function. The aim of this study was to give a general overview on bioactive components found in milk and dairy products and what valuable health effects they might have. Milk consists of a mixture of components but the main components are water, lactose, fat, proteins and minerals. The composition of milk can differ and are influenced by number of dissimilar factors. In this thesis calcium, specific bioactive proteins (lactorferrin, α-lactalalbumin, β-lactoglobulin, glycomacropeptide, and immunoglobulin) oligosaccharides and conjugated linoleic acid will be overviewed. However, these components are just a small fraction of all bioactive components found in milk and dairy products. Some of them are natural milk components, others emerge during digestive processes or fermentation processes in dairy products. Numerous of health effects of these bioactive components have been described in *in vitro* studies, animal trials, a small number of epidemiological studies and human clinical trials. The studies show that these bioactive components exhibit antimicrobial effect, anticarcinogenic properties, ACE-inhibitory activity, anti-hypertension properties, effects on the cholesterol levels etc. Although bioactive components in milk and dairy products is a well studied area there is a lack of human clinical trials and epidemiological studies to draw conclusions regarding health benefits in humans. However, the development of functional foods with health properties is a market that is expanding and will continue to do so in the coming future. Through the knowledge and understanding of how these components function, new health promoted products can be developed.

Keywords: bioactive components, milk, dairy products, health effects

Sammanfattning

Mjölk är en komplex vätska som är mycket mer än bara en källa av närings-ämnen för unga däggdjur. Under de senaste decennierna har en hel del forskning demonstrerat att mjölk och mejeriprodukter innehåller ett brett spektrum av olika bioaktiva komponenter. Syftet med denna studie var att ge en litteraturöversikt över bioaktiva komponenter som finns i mjölk och mejeriprodukter och vilka fördelaktiga hälsoeffekter som de kan ha. Mjölk består av en blandning av olika komponenter men de huvudkomponenterna är vatten, laktos, fett, proteiner och mineraler. Mjölkens sammansättning kan dock variera och påverkas av olika möjliga faktorer. I den här litteratur-studien kommer kalcium, specifika bioaktiva proteiner (laktoferrin, α-lactalalbumin, β-laktoglobulin, glykomakropeptid, immunglobulin), oligo-sackarider och konjugerad linolsyra att tas upp. Dessa komponenter är dock bara en liten del av alla bioaktiva komponenter som finns i mjölk och meje-jeriprodukter. En del av dessa är naturliga mjölkkomponenter medan andra uppkommer under matsmältningsprocessen eller genom fermentation i me-jeriprodukter. Många av hälsoeffekterna av dessa bioaktiva komponenter har beskrivits genomgående i *in vitro* studier, djurförsök och ett fåtal i epi-demiologiska studier och kliniska prövningar. Studierna visade att dessa bioaktiva komponenter uppvisar antimikrobiell effekt, anticarcinogena egenskaper, ACE-hämmande aktivitet, anti-hypertoniska egenskaper, på-verkan på kolesterolvärdet etc. Även om bioaktiva komponenter i mjölk och mejeriprodukter är ett välstuderat område är det brist på kliniska prövningar och epidemiologiska studier för att dra slutsatser angående hälsofördelar hos människan. Utvecklingen av funktionella livsmedel är en marknad som växer och kommer att fortsätta att göra det under den kommande framtiden. Genom kunskap och förståelse för hur dessa bioaktiva komponenter funge-rar kan nya hälsoförebyggande produkter utvecklas.

Nyckelord: Bioaktiva komponenter, mjölk, mejeriprodukter, hälsoeffekter

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Abbreviations

ACE	Angiotensin-converting-enzyme
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
GMP	Glycomacropeptide
Ig	Immunoglobulin
α-La	α-Lactalbumin
LF	Lactoferrin
β-Lg	β-Lactoglobulin

1 Introduction

Milk is a complex liquid and one of nature's most complete food (Park, 2009). The main role of milk is to fulfill the nutritional need for the neonate of the mammalian species during the first phase of its life (Raikos & Dassios, 2013). The conventional role of milk as nutrition for infants has expanded with time. Milk does not only provide growth to children but also nourishment for adults. However, aside from the nutritional values of milk, milk and dairy product have also become known to provide bioactive components. Many valuable constituents in milk have diverse but yet significant roles. Bioactive components could be defined as compounds in foods that may exert physiological activity. Bioactive components of food origin could be provided to humans by intake of conventional food, functional foods or dietary supplement (Gobbetti & Minervini, 2007). Extensive research reports different advantageous health effects from bioactive components in milk and dairy products. The interest of bioactive components in milk and dairy products is greater than ever in today's society. As research into the health profits of food components have expanded, both food manufacturers and consumers have developed a greater understanding of the relationship between foodstuff and human health. With this knowledge of how bioactive components function, new treatments, drugs or food can be developed in the near future (Park, 2009).

1.1 Aim

The aim of this thesis was to provide a literature overview on bioactive components in milk and dairy products and their beneficial effects on health.

1.2 Demarcation

This thesis will include studies made on milk from different species (human, bovine, ovine, caprine, and goat). In addition, the studies in this literature study will include various types of study designs e.g. animal studies, *in vitro* studies, epidemiological studies and clinical studies.

2 The composition of milk

Milk is the primary source of nutrients for infants of mammals and is defined as the secretion from mammary glands of mammals. Milk consists of a complex mixture of components. The major components are lactose, lipids, protein, minerals and water. The composition of milk varies and is reliant on which mammal, breed and even the individual animal. Moreover the stage of lactation and the feed could have a significant impact of the milk composition (Walstra *et al.*, 2005).

2.1 Lactose

The major carbohydrate in milk is lactose or β-D-galactopyranosyl-(1,4)-D-glucopyranose. Lactose is a disaccharide composed of D-galactose and D-glucose. Lactose mainly provides the infant mammal energy but also have other functions such as giving milk the sweet flavor. For the blood to be able to take up lactose it must first be hydrolyzed by the enzyme *lactase* to glucose and galactose. Individuals who metabolize lactose poorly due to a significant reduction in lactase activity are called lactose mal-absorbers. This condition can eventually lead to lactose intolerance (Walstra *et al.*, 2005).

2.2 Lipids

The term lipid refers to all components soluble in non-polar organic solvents - phytosterols, tocopherols, free fatty acid etc. The term fat can sometimes be mixed up with lipids though fat refers to the mixture of triglycerides that are solidified at room temperature. The word fat will sometimes be used in this context since lipids is such an extensive term (Walstra *et al.*, 2005). The majority of the lipids in milk occur as fat globules. Milk contains approximately 3- 5 % lipids, a value that varies, and milk fat is emulsified in the aqueous water phase (Jensen, 2002). Milk fat mainly consists of triglycerides (TAG) which make up approximately 98 % of the milk fat. The rest is free fatty acid, cholesterol, diglycerides, monoglycerides and phospholipids. An exceedingly vital factor on determining the lipid properties is the pattern for the fatty acid, for instance the chemical reactivity and melting range. The fatty acid composition varies depending on the degree of unsaturation, chain length and position of the double bond (Walstra *et al.*, 2005).

2.3 Proteins

The primary function of protein in milk is to provide amino acids and nitrogen to young infants but milk proteins are also an essential part of the dietary proteins in adult humans (Nagpal *et al.*, 2011). The two major fractions of proteins in milk are casein and whey protein.

Casein is the primary protein of these two and compromising ca 80 % of the milk protein. Casein micelles are formed through interaction between various casein proteins and calcium phosphate. This large colloidal complex particle has a spherical structure. Casein can further be divided into α_{s1}-, α_{s2},-, β-, and -κ-fractions (Chandan, 2007). Caseins are phosphoproteins that enclose phosphate groups esterified to the amino acid serine. The α_s- and β-casein are more hydrophobic and are largely present in the interior of the micelles while κ-casein is primarily found on the surface of the micelles. κ-Casein provides stability for the micelles in milk during the standard handling conditions. This is caused by the hydration of the κ-casein and the negative charge together with the fact that the hydrophilic fragment has a tendency to stick out from the micelle surface. It gives a hair-like structure and grants a spatial stability for the micelles. When manufacturing cheese, casein micelles coagulate by adding the enzyme complex rennet to the milk. A protease cleaves a definite bond in κ-casein and will make the hair-like structure detach from the micelle, making the surface uncharged and unstable. The micelles therefore aggregate and form a gel and can further be separated from the whey in the process of making cheese (Christian, 2008).

The other major protein fraction is whey, also called milk serum representing 20 % of the milk proteins (Ko, 2009). This fraction includes β-lactoglobulin, α-lactalbumin, lactoferrin, immunoglobulines, bovine serum albumin and various of enzymes. The spherical structures of whey proteins are much more in an ordered form than casein proteins. Whey proteins have disulfide linkages and are precipitated under heat circumstances rather under acid conditions (Chandan, 2007).

Varieties of proteins and peptides derived from milk proteins have bioactive function and growing evidence on beneficial health effects has been put forward.

2.4 Vitamins and minerals

Milk contains both fat-soluble and some water soluble vitamins. Vitamin A can naturally be found in the fat component of whole milk (Chandan, 2007). It is not unusual to fortify milk with vitamin A prior to sale since the separation process of milk leads to small amounts of vitamin A in the low fat milk fraction (Christian, 2008). Vitamin D is also naturally present in whole milk though in small amounts and therefore it is commonly fortified as well (Chandan, 2007).

Calcium and phosphorus are the main minerals present in milk. Calcium can be found combined with the protein casein as calcium caseinate, with phosphorus as calcium phosphate and as calcium citrate. Furthermore, magnesium, potassium, sodium and sulfur is other minerals present in milk (Christian, 2008).

3 Twenty first century diseases

3.1 Cardiovascular disease

Cardiovascular disease (CVD) is the foremost cause of mortality globally, today. More people die annually from CVD than from any other diseases or causes. CVDs include a group of disorders of heart and blood vessels such as, coronary heart disease, cerebrovascular disease etc. CVD can be developed from the presence of one or more risk factors such as diabetes, hypertension and hyperlipidemia. These factors can be prevented by addressing a healthy diet, physical activity and nonusage of tobacco.

3.1.1 Hypertension and antihypertensive activity

Hypertension, also known as high blood pressure, is defined as systolic blood pressure above 140 mm Hg and/or a diastolic blood pressure over 90 mg Hg. The development of hypertension is a serious health condition and associated with CVD. Furthermore, it could influence other outcomes e.g. heart attack, stroke or heart failure (WHO, 2015).

Angiotensin- converting enzyme (ACE) is an enzyme with multiple functions and is an essential component of the renin-angiostin-system. ACE can act as one of the major regulators of blood pressure by converting the enzyme angiotensin I to angiotensin II. Angiotensin II is thereby the hormone that increase the blood pressure by contracting the blood vessels (Nagpal *et al.*, 2011). To prevent this, patients suffering from hypertension use ACE-inhibitors. This could be in form of pharmaceutical drugs. However, over the past decades numerous studies have demonstrated that bioactive peptides derived from milk protein have ACE-inhibitory activity.

3.2 Osteoporosis

Osteoporosis is a well-defined disease and is a condition of skeletal fragility. The condition is characterized by low bone mass and micro-architectural weakening of bone tissue which consequently increase the risk of fractures (Heaney, 2000). Calcium has a significant role in a wide range of biological functions. One of the most important roles is to mineralize the skeleton. Lack of calcium can eventually contribute to osteoporosis. Since calcium cannot be produced of the body itself, it needs to be supplied through dietary intake (Peacock, 2010). Milk and dairy products are rich in calcium and hence major contributors of the mineral. Nutrition plays an essential role to maintain and develop the bone mass, although there are other aspects that could influence the development of osteoporosis additionally (Heaney, 2000).

3.3 Cancer

The second leading cause of mortality worldwide is cancer. The term cancer is somewhat broad and includes a large group of diseases that could affect any part of the body. A general defining aspect of cancer is the rapid formation of abnormal cells that grow beyond their common limitations. These abnormal cells can invade and spread to other organs, so called metastases. The metastases are the foremost cause of death from cancer. In spite the major advances that have been achieved in this field, the incidence of cancer is expected to continue rising the coming years. It is estimated to rise up to 70 % over the next 2 decades (WHO, 2015).

Considerable amounts of studies have investigated the relation between diet and cancer prevention of different food constituents. During the past years, peptides derived from food components have proven to lower the cancer risk in number of investigations (Hsieh *et al.*, 2015). Particularly peptides derived from milk have been well studied which will be brought up in this thesis.

4 Bioactive components in milk

Bioactive components are referred to as specific constituents found in foods in small quantities and that beyond the nutritional function comprises beneficial physiological function. However, the term is somewhat extensive and could be interpreted differently. These components are being intensely studied because they may promote health and prevent diseases. All the studies of the different bioactive components that will be mentioned in this section are summarized in Table 1.

There is a large volume of published studies describing the role of bioactive components in milk. Many of them have been studied in milk from different species. However, to date milk proteins are currently the foremost source of bioactive components and therefore the most well studied constituent in milk. Even though there is a large amount of studies related to other components in milk besides from milk proteins, no other constituents have been intensively studied and described as well like milk proteins. The majority of the bioactivity of the milk protein is dormant, incomplete or even absent in the original protein. However the liberation of the bioactivity could be achieved via proteolytic digestion to release the bioactive peptide from the native protein (Park, 2009). There are different ways peptides with biological activity can be produced. During food processing, such as milk fermentation using proteolytic starter cultures and in cheese maturation, bioactive components are generated (Clare & Swaisgood, 2000). Bioactive components could also be released during enzymatic hydrolysis through digestive enzymes in the gastrointestinal transportation. Digestive enzymes can naturally occur in milk and therefore supplement those products with bioactivity (Park, 2009). Lastly, bioactive peptides could be released by enzymes derived from microorganisms or plants (Korhonen & Pihlanto, 2007).

4.1 Calcium

Calcium is an essential mineral and one of the most abundant mineral in the human body. It is a crucial nutrient required in numerous biological functions in the body such as skeletal mineralization. Therefore, the loss of calcium through nail, skin, hair as well as urine and feces must be replaced from the daily diet. The calcium requirement is however reliant on the state of the calcium metabolism which is individual (Peacock, 2010). Today the predominant source of calcium in the human diet is milk and dairy products. The majority of the calcium in milk is bound to casein, and to minor extent to other milk proteins, phosphorus and citrate. There is also a small fraction of the calcium that exist in an unbound form (Park, 2009). During the past twenty years, research has proven that calcium in dairy products has a beneficial role and the level of consumption of milk and dairy product is correlated to many of the 21st century diseases (Ibeagha-Awemu *et al.*, 2009).

A study made on adolescent girls showed that an increase in milk consumption augments the bone mineral acquisition and could change attainment of peak bone mass (Cadogan *et al.*, 1997). It is also reported in a two year prospective analysis that higher intake of calcium is associated with reduced fat mass in young women (Lin *et al.*, 2000).

4.2 Conjugated linoleic acid (CLA)

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acids with 18 carbons and two conjugated double bonds (C18:2) in cis or trans position (Aro *et al.*, 2000). The most biologically active isomers of CLA is reported to be *cis*-9, *trans*-11 which can be found in milk and dairy products. *Cis*-9, *trans*-11 CLA is also referred to as rumenic acid. In milk fat, approximately 75-90 % of the total CLA is in the *cis*-9, *trans*-11 form (Collomb *et al.*, 2006).

During the past years, numerous of animal trials have proven various health profits of CLA in milk (Dhiman *et al.*, 1999). In rats, the isomer *cis*-9, *trans*-11 CLA has been reported to have an anticarcinonegic effect (Corl *et al.*, 2003). Furthermore, studies in human have also proven a number of positive effects (Roche *et al.*, 2001). A clinical study by Jiang *et al*. (1999) investigated the relation between the bovine milk fat and occurrence of positive effects in the human adipose tissue. The investigation showed that there was a significant correlation between intake of milk fat and the amount of CLA found in the serum of the participants. It was concluded in the study that the negative health aspects of milk fat should be re-evaluated since CLA have demonstrated to have positive health effect according to various amount of literature. Moreover additional scientific data are required to complement the result of this study.

4.3 Glycomacropeptide (GMP)

GMP is a fragment of the C-terminal f(106- 169) from κ-casein which is released by action of the protease chymosin (Keogh & Clifton, 2008). It is an hydrophilic peptide and has a molecular weight of roughly 8000 Daltons which makes it a rather small molecule (Park, 2009). GMP is released during cheese making upon adding rennet that contains the key component, chymosin. GMP remains in the whey fraction through this process and will not be included in the cheese curd (Brody, 2000).

There have been several studies on GMP and its acquired bioactivity. Research has shown that GMP has antimicrobial activity *in vitro* by inhibiting production of several toxins produced by bacteria. Kawasaki *et al*. (1992) concluded that GMP from bovine milk inhibits cholera toxin from binding to its receptor. The study was made on Chinese hamster ovary (CHO)-Kl cells. Azuma *et al.* (1984) has shown that GMP from human milk promotes the growth of bifida bacteria and therefore suggested to be an important bifida factor. This study also reported that GMP from human milk had four times higher growth-promoting activity for bifidobacteria than GMP from bovine milk. In this case, GMP from bovine milk was used as a reference. The outcome of this is not clear, but the study suggests that when incubated with pepsin, GMP from human milk has the ability to be hydrolyzed by further steps. Furthermore, intact κ-casein showed growth-promoting activity as well though the activity was enhanced when hydrolysis of either chymosin or pepsin was used to release GMP.

4.4 Immunoglobulin

Immunoglobulins (Ig) are antibodies present in the milk and colostrums of lactacting mammals produced as a response to stimulation by specific antigens (Korhonen, 2009; Mehra *et al.*, 2006). The biological function and also the main role of Ig is to give the offspring protection from pathogens and infection. The young mammals are therefore depending on colostrum as a passive immune system throughout the development of their own immune system (Mehra *et al.*, 2006). Ig:s are divided into different classes depending on the structure, properties and biological function. The five main classes are IgA, IgD, IgE, IgG, and IgM (Chandan, 2007).

A randomized, double-blind, parallel-group, placebo controlled study reported that consumption of Ig from bovine milk emerged to have a positive effect on patients suffering from mild hypercholesterolemia. The result showed that both total cholesterol measured from baseline and LDL-cholesterol decreased (Earnest *et al.*, 2005).

Over the past decades, a vast amount of studies have demonstrated that milk with Ig can be effective in avoidance of diseases due to pathogenic microbes. In a study by Tacket *et al.* (1992) it was demonstrated that an intake of oral bovine Ig concentrate derived from colostrum had a protective efficiency against the pathogen *Shigella*. The study was performed on healthy young adults in a randomized, double blinded study.

4.5 α - Lactalbumin

α-La is a predominant protein in milk, mostly in human and bovine milk. The protein contains the amino acid cysteine, methionine and tryptophan in high quantities. The sulfur amino acids give rise to the disulfides in the molecule. α-La has revealed to obtain a vital physiological role in biosynthesis of lactose in the mammary gland where α-La is produced. Alongside the enzyme uridine-diphosphate galactosyl transferase, α-La is a part of the lactose synthesis through catalyzing the conveyance of galactose to glucose to lastly form lactose (Chandan, 2007).

α-La has also revealed to be a metal-binding protein, specifically for calcium. This may further have an important biological function according to Hiraoka *et al.* (1980). Another study has suggested that the metal-binding property could be significant for the lactose synthase complex and hence have a physiological meaning (Lönnerdal & Glazier, 1985). Animal studies have also been conducted to investigate the bioactivity of α-La. A study by Matsumoto *et al.* (2001) reported that α-La had an antiulcer function. The study investigated the effect of bovine α-La on gastric mucosal injury in rats. Before the gastric mucosal injury was induced by ethanol and stress, the test proteins were given orally. Matsumoto *et al*. (2001) concluded that α-La had an protective effect against gastric mucosal injury. It has been known that milk is effective in preventing ulcers that had been induced for experimental reasons (Kivinen *et al.*, 1992; Dial & Lichtenberger, 1987). However, the study conducted by Matsumoto *et al.* (2001) is the first of its kind. Due to α-La being rich in the amino acid cysteine, tryptophan, and methionine, studies investigating if these amino acids have a function have been performed. One such study looked into if α-La derived from bovine milk could be involved in increasing the brain tryptophan and serotonin activity. It is known that high serotonin activity may be related to the ability to cope with stress and mood. Serotonin is synthesized from the amino acid tryptophan. The brain serotonin concentration increases with intake of food with high tryptophan availability. For this reason, a diet with high concentration with tryptophan was given to the participants. α-La, with high concentration of tryptophan, was therefore used in this double-blind, placebo-controlled study. The study did indeed show that an enriched α-La diet significantly increased the plasma ratio of tryptophan to other large amino acid (Trp-LNAA ratio) (Markus *et al.*, 2000).

4.6 Lactoferrin

Lactoferrin (LF) is an iron-binding glycoprotein found in milk and in other exocrine secretion. The structure of LF consists of a single chain polypeptide with two globular lobes (Korhonen, 2009). Due to its iron-binding property, LF has shown to provide several physiological purposes in the biological system. The LF concentration differ significantly among the milk type as other bioactive components do (Lönnerdal & Iyer, 1995).

To date, several studies have investigated the antimicrobial activity of LF. Many of these studies have explored the correlation between the antimicrobial property of LF and its ability to bind iron. In a study by Ellison *et.al* (1998) it was demonstrated that LF from human milk increased the antibacterial effect by damaging the outer membrane of gram-negative bacteria. A different study also investigated the correlation between LF and the iron-binding property and stated a similar conclusion (Masson *et al.*, 1966). However this study was conducted several years prior Ellison *et al.* (1998) and only concluded that the antimicrobial feature could be due the iron-binding properties of LF (Masson *et al.*, 1966). It has moreover been reported that orally administered LF could be a potential natural antibacterial agent. This particular study showed that LF had protective effects regarding bacterial infection by *Staphylococcus aureus* (Bhimani *et al.*, 1999). Bovine LF has also shown to have antimicrobial property against infection by *Escherichia coli* (Teraguchi *et al.*, 1995) and *Heliobacter pylori* (T. Wada, 1999).

LF has furthermore revealed to have anticancer effect in animal studies. In a study by Sekine *et al.* (1997), it was implied that bovine LF, which was orally administered, could operate as a chemopreventer for colon cancer in male rats.

4.7 β - Lactoglobulin

β-Lactoglobulin (β-Lg) is one of the main whey proteins in milk of some mammalian genera. It has been reported that β-Lg has four genetic variants, A, B, C and D. The protein contains a large amount of sulfur due to its cystein residues (Chandan, 2007). β-Lg has proven to have a wide range of bioactivities and numerous studies have investigated the ACE-inhibitory activity and effect on blood cholesterol.

A study investigating ovine and caprine whey reported ACE - inhibitory activity of β-Lg. It was observed that higher activity was obtained with peptides from β-Lg after action of microbial enzymes rather than digestive enzymes. Hernández-Ledasma *et al.* (2002) suggested that the cleavage was more adequate for the creation of ACE - inhibitory peptides with enzymes from bacterial origin . Hernández-Ledasma *et al.* (2002) also demonstrated potent ACE - inhibitory activity *in vitro* when β-Lg was administered orally in rats. Two peptides were evaluated and both indicated decrease in blood pressure after administration. However, when the peptide was subjected to a simulated gastrointestinal digestion in the same study there was no indication of ACE - inhibitory activity. Therefore, it may be crucial to validate and perform further studies.

In a model study conducted by Nagaoka *et al.* (2001) peptide derivates from β-Lg effectively influenced on serum cholesterol levels and also had a great hypocholesterolemic activity.

4.8 Oligosaccharides

Other than lactose, oligosaccharides are also important carbohydrates in milk. These sugars make a substantial contribution to the carbohydrate content, in human milk for example up to 1.4 % of the entire carbohydrate content (Korhonen & Pihlanto, 2007). Oligosaccharides contain between 3 - 10 monosaccharide units that are linked through glycosidic bonds (Mehra & Kelly, 2006). Great deals of previous research on oligosaccharides are mostly focused on human milk since they are highly abundant in human milk. That the amount of oligosaccharides in milk from other animals is low compared to human milk is the reason for lack of studies on milk from other species (Kunz & Rudloff, 2006). There is an increasing evidence that the principal role of oligosaccharides seems to be to administer the protection against pathogens (Kunz *et al.*, 2000). By functioning as competitive inhibitors on the binding site of the intestinal epithelial surface may give physiological protection (Gopal & Gill, 2000).

A study on oligosaccharides from goat milk in rats suggested that they play an important role in protecting the intestine and repair the damage of induced colitis (Lara-Villoslada *et al.*, 2006).

Table 1. Bioactive components in milk and the resulting bioactivity as reported in various studies

Bioactive component	Bioactivity and suggested health effect	Study design	Reference
Calcium	Reducing fat mass Increased bonemass	Prospective analysis Open randomised intervention study	Lin *et al.*, 2000 Cadogan *et al.*, 1997
Conjugated linoleic acid (CLA)	Anticancerogenic Significant higher amount of CLA in blood serum	Animal study Observational study	Corl *et al.*, 2003 Jiang *et al.*, 1999
Glycomacropeptide (GMP)	Promotes growth of bifida bacteria (bifidus factor) Inhibition of binding cholera toxin	In vitro (using bifidobacterium infantis) In vitro (using Chinese hamster ovary (CHO)-Kl cells)	Azuma *et al.,* 1984 Kawasaki *et al*., 1992
Immunoglobulin	Cholesterol-lowering effects Protective efficiency against *Shigella*	Randomized, double-blind, parallel-group, placebo-controlled study In vitro	Earnest *et al*., 2005 Tacket *et al*., 1992
α - Lactalbumin	Protection against gastric mucosal injury Calcium-binding Increased the plasma ratio of tryptophan	Animal study (Rats) In vitro Double-blind, placebo-controlled study	Matsumato *et al*., 2001 Hiraoka *et al*., 1980 Markus *et al*., 2000
Lactoferrin	Antimicrobial Antimicrobial Anticancer	In vitro Animal study (mice) Animal study (rats)	Ellison *et al*., 1988 Bhimani *et al*., 1999 Sekine et al., 1997
β - Lactoglobulin	Influence serum cholesterol levels and exhibit a greater hypocholesterolemic activity ACE - inhibitory activity ACE - inhibitory activity	Animal study (mice) In vitro Animal study	Nagaoka *et al*., 2001 Hernández-Ledesma et al., 2002 Hernández-Ledesma et al., 2007
Oligosaccharides	Reduce intestinal inflammation (Antiinflammatory)	Animal study (Rat)	Lara-Villoslada *et al*., 2007

5. Bioactive components in dairy products

5.1 Cheese products

Making cheese has distinctive food processing procedures that make cheese unique. These processes include fermentation and ripening and it is also these processes that give rise to important bioactive components. Cheese products have been manufactured through a wide range of process techniques, to meet requirements that consumers are demanding. By using a diverse range of lactic acid bacteria as a starter culture, different tastes of cheese and specific health requirements can be made. All the studies on the cheese products that will be mentioned in this section below are summarized in Table 2.

Higurashi *et al*. (2007) studied the effect of Gouda-type cheese consumption on the accumulation of abdominal adipose. The experiment was performed on rats to examine the effects of Gouda-type cheese on some biological markers of the metabolic syndrome. It was discovered that the cheese fed rats had an extensively lower weight of the adipose tissues. These results were compared to rats fed with a diet comparable with respect to fat content but instead contained butterfat and casein. Furthermore, the cheese-fed rats revealed to have a decreased level of cholesterol in very low density lipoprotein as well of the level of serum in low density lipoproteins. Higurashi *et al*. (2007) suggests that cheese consumption has a suppressive effect on abdominal adipose accumulation and may prevent the metabolic syndrome.

Up to now, a number of studies have showed that ripened cheese contains peptides with antihypertensive effects. Meisel *et al.* (1997) have reported ACE-inhibitory peptides in ripened cheese. The ACE-inhibitory activity increased during cheese maturation. However, the ACE-inhibitory activity decreased when a certain level of proteolysis of the peptides was exceeded. Ryhänen *et al*. (2001) produced a new type of cheese with a mix of starter cultures from *Lactobacillus acidophilus* and *Bifidobacterium* spp. The "Festivo" cheese was ripened for 13 weeks and stored for 4 months before test was performed. The analysis detected that the "Festivo" cheese had ACE-inhibitory activity. Several peptides isolated from Italian cheese (Mozarella, Italico, Crescenza and Gorgonzola) have reported to have inhibitory activity against proteolytic enzymes from lactic acid bacteria used as starter cultures. Moreover, most of these cheese peptides showed ACE-inhibitory activity (Smacchi & Gobbetti, 1998). Antihypertensive peptides have also been found naturally in gouda cheese (Saito *et al.*, 2000).

5.2 Yoghurt and other fermented dairy products

Yoghurt can be referred to as fermented milk and is produced through lactic acid fermentation with the presence of *Lactobacillus delbreuckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Nonetheless, it is very common to add other lactic acid bacteria species to give the product characteristic features. Bovine milk is the most common milk for producing yoghurt in the Western world however yoghurt from ovine, caprine and buffalo milk are used as well (Korhonen & Pihlanto, 2007). The studies brought up in this section are summarized in Table 2. Numerous studies have been published claiming that fermented dairy products, including yoghurt, have beneficial health effects. For instance, Donkor *et al.* (2007) in an *in vitro* study identified ACE-inhibitory peptides in probiotic yoghurt. These peptides were most likely degraded from caseins.

A placebo-controlled study by Hata *et al. (*1996) studied the effect of sour milk on elderly hypertensive patients. The sour milk contained starter culture from *Lactobacillus helveticus* and *Saccharomyces cerevisiae*. The patients ingested 95 mL of the sour milk for 8 weeks. It was found that both diastolic and systolic blood pressure of the hypertensive patients significantly decreased. Furthermore, blood pressure remained decreased for 4 weeks after the study ended. It was suggested that these effects could be due to two types of tripeptides in the sour milk . The tripeptides were previously reported to be ACE-inhibitors in studies on rats (Nakamura *et al.*, 1995).

In a population-based cohort study conducted by Larsson *et al*. (2005) the relation between high-fat dairy product (whole milk, full-fat cultured milk, cheese, cream, sour cream and butter) consumption and colorectal cancer was investigated. The participants were women aged 40-76 and were participants of the Swedish Mammography Cohort. The participant reported their daily food intake by answering questionnaires. Larsson *et al*. (2005) suggested that the prospective data indicated that a high consumption of dairy foods might lower the risk of colorectal cancer, and that this observation could be related to CLA intake. CLA is a bioactive component that has been shown to possess anti-carcinogenic effects, however Larsson *et al*. (2005) point out that other potentially anti-carcinogenic components cannot be excluded.

Table 2. Bioactive components in dairy products and the resulting bioactivity as reported in various studies

Dairy product	Bioactivity and suggested health effect	Study design	Reference
Gouda cheese	Beneficial effect on abdominal adi-pose	Animal study (rats)	Higurashi *et al.*, 2007
Cheese	ACE-inhibitory	-	Meisel *et al.*, 1997
''Festivo'' cheese	ACE-inhibitory	In vitro	Ryhänen *et al.*, 2001
Italian cheese(Mozzarella, Italico, Crescenza, and Gorgonzola)	ACE-inhibitory	In vitro	Smacchi & Gobetti, 1998
Yoghurt	ACE-inhibitory	In vitro	Donkor *et al.*, 2007
Sour milk	Reduce hypertension	Placebo controlled study	Hata *et al.*, 1996
High fat dairy products(whole-milk, full-fat cultured milk, cheese, cream, sour cream and butter)	Lower risk of colorectal cancer	Prospective cohort study	Larsson *et al.*, 2005

6. Discussion

The occurrence of bioactive components in milk and dairy products is well documented and investigated. The research into the health benefits has expanded the past decades and the potential health benefits from the various components have gained commercial interest globally in the perspective of producing health promoting functional foods (Park, 2009). Milk and dairy products are one of the few foods that have bioactive components present naturally and studies have reported various type of health benefits from their consumption. The studies summarized in Table 1 and 2 together with other extensive research have revealed milk and dairy products to be significant sources of bioactive components. However, the design and the model of the studies can affect the outcome. This need to be taken into consideration when stating health claims. There are several aspects to be taken in account when evaluating the bioactivity of the substances in milk and dairy products.

Milk proteins are the most studied constituents of milk. There are great amount of studies and evidence on stability, bioavailability, and impact on health that these components provide. Not only do proteins contribute the nutritional value but also extensive research has proven that peptides originating from milk proteins possess health affect such as ACE-inhibitory activity. Many studies performed *in vitro* have investigated peptide derivates from milk proteins by absorbing the peptide *in vivo* and see the biological effect of the intact molecule. However, there are restrictions in conducting human clinical investigations or epidemiological studies designed to investigate the bioactivity of milk proteins and other components in milk and dairy products. Therefore, many existing studies are made *in vitro* which can more or less be used as an approximation and as an alternative to investigate the properties of milk components. Performing a study *in vitro* has its advantages and disadvantages. *In vitro* studies gives an insight into how the mechanism of an bioactive components can operate (Chatterton *et al.*, 2004). Consequently, there is lack of information available in terms of how it would function and affect the human health. To confirm beneficial health effects of bioactive components it is required to design human clinical trials or epidemiological studies based on information that could explore new biomarkers associated to the different observed health benefits (Hsieh *et al.*, 2015). Further research is necessary, particularly in humans to completely understand the role of the bioactive components. The expansion of the functional food or foods with health properties beyond the generally accepted nutritional benefits is quickly evolving in the food industry and even beyond. The evolving research in this area will in the future be vital for development of new functional foods, but also for understanding the diverse roles components in foods could have beyond the nutritional aspects.

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