Goat milk as close to perfect food as possible in nature

Milk is an excellent source of well balanced nutrients and also exhibits a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Goat milk is as close to perfect food as possible in nature. Its chemical structure is amazingly similar to mother’s milk. It is a complete protein that contains all the essential amino acids without the heavy fat content and catarrh (mucus) producing materials of cow milk.

Source of bioactive peptides

Milk protein is comprised of about 80% caseins and 20% whey proteins. These biological activities are mainly due to the peptides and proteins in milk. However, some of the biological activity of milk protein components is latent, and is released only upon proteolytic action.

Caseins are divided into α-, β- and κ-caseins (13). Principal CN in goat, sheep and cow milk are αs1-CN, αs2-CN, β-CN and κ-CN. The main forms of caprine and ovine CMP are the soluble C-terminal derivatives from the action of chimosin on κ-casein during the milk clotting process of cheesemaking. These main forms are identified and determined to be a good source of antithrombotic peptides.

Whey protein is composed of β-lactoglobulin, α-lactalbumin, immunoglobulins, glycomacropeptides, bovine serum albumin and minor proteins such as lactoperoxidase, lactoferin, lysozyme and lactoferrin.

Sheep and goat milk proteins are also important sources of bioactive ACE inhibitory and antihypertensive peptides. They can provide a non-immune disease defence and control of microbial infections. The activity of these biofunctional peptides is based on their inherent amino acid composition and sequence. The size of active sequences may vary from two to twenty amino acid residues. The total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin defence proteins such as LF, LP, lysozyme, and other peptides. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese. BP have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects.

Production of bioactive peptides

BP have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health. (12). BP are encrypted in milk proteins and are only released by enzymatic hydrolysis in vivo during gastrointestinal digestion, food processing or by microbial enzymes in fermented products. BP are formed from

ABSTRACT

Milk is an excellent source of well balanced nutrients and also exhibits a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Goat milk is as close to perfect food as possible in nature.

Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health. It can be generated during milk fermentation by the proteolytic activity of starter cultures. The beneficial health effects may be classified as antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory. Sheep and goat milk proteins are also important sources of bioactive ACE inhibitory peptides and antihypertensive peptides. They can provide a non-immune disease defence and control of microbial infections. The activity of these biofunctional peptides is based on their inherent amino acid composition and sequence. The size of active sequences may vary from two to twenty amino acid residues. The total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin defence proteins such as LF, LP, lysozyme, and other peptides. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese. BP have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects.
the precursor inactive protein (18). Due to their physiological and physico-chemical versatility, milk peptides are regarded as highly prominent components for health promoting foods or pharmaceutical applications. At present significant research is undertaken on the health effects of BP. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese.

Physiological effects of bioactive peptides
Effects of these peptides on the cardiovascular, nervous and immune systems are described, altogether with bioactive peptides that possess antimicrobial activity, effects on the nutritional status and dental health and other functional roles. The beneficial health effects may be classified as antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory (27, 34).

Major antimicrobial proteins in milk
The antimicrobial properties of milk have been widely acknowledged for many years. The antimicrobial activity of milk is mainly attributed to immunoglobulins, and to non-immune proteins, such as lactoferrin, lactoperoxidase and lysozyme. In some species minor proteins, including a folate binding protein, are also regarded.

Lysozyme is a relatively small basic protein and is classified as a 1,4-β- N-acetylmuramidase (6).

The function of Lf in vivo is not well understood. However, two main roles for Lf in vivo have been postulated: its antibacterial effect and role in the iron metabolism. The antibacterial effect of Lf in vitro is well established. The antibacterial mechanism has been assigned to the ability of capacity to chelate iron and thereby depriving bacteria of the iron that is essential for their growth. This is supported by the fact that Lf saturated with iron does not demonstrate an antibacterial effect (3).

LP is basic glycoprotein that has a molecular weight of 78 000 and contains one heme group. Buffalo’s milk, ewe’s milk and goat’s milk generally also contain high levels of LP (4).

Goat milk can have significant levels of folate binding protein and it may therefore be advisable to supplement infant formulas with folic acid in addition to the normal “humanizing” compositional adjustments when caprine milk is used in human neonate. One of the most potent antimicrobial peptides described so far corresponds to a fragment of the whey protein lactoferrin, named lactoferricin (LFcin). More recently, other whey proteins such as α-lactalbumin and β-lactoglobulin have also been considered as potential precursors of bactericidal fragments. Similarly, antibacterial fragments have also been derived from αs1-, αs2- and k-casein.

Occurrence of bioactive peptides in dairy products
It is now well documented that bioactive peptides can be generated during milk fermentation by the proteolytic activity of starter cultures. As a result, peptides with various bioactivities can be found in the end-products, such as various cheeses and fermented milks. These traditional dairy products may under certain conditions have specific health effects when ingested as part of the daily diet.

The activity of these biofunctional peptides is based on their inherent amino acid composition and sequence. The size of active sequences may vary from two to twenty amino acid residues, and many peptides are known to have multifunctional properties (27) e.g., peptides from the sequence 60-70 of β-casein show immunostimulatory, opioid and ACE-inhibitory activities. This sequence has been defined as a strategic zone (26, 28). The sequence is protected from proteolysis because of its high hydroscopicity and the presence of proline residues. Other examples of the multifunctionality of milk-derived peptides include the αs1-casein fraction 194-199 showing immunomodulatory and ACE-inhibitory activity, the opioid peptides α- and β-lactrophin also exhibiting ACE inhibitory activity and the CPPs, which possess immunomodulatory properties (34).

Antimicrobial peptides from goat and sheep milk proteins
Bioactive proteins and peptides derived from milk have been reported to provide a non-immune disease defence and control of microbial infections. It is generally accepted, that the total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and nonimmunoglobulin defence proteins such as LF, LP, lysozyme, and peptides. This may be due to the synergistic activity of naturally occurring proteins and peptides in addition to peptides generated from inactive protein precursors (9). It has been proved, that milk proteins can also act as antimicrobial peptide precursors, and in this way might enhance the organism’s natural defences against invading pathogens. Consequently food proteins can be considered as components of nutritional immunity (30).

Antimicrobial peptides derived from whey proteins
Peptides derived from LF are antibacterial peptides from proteins. Tomita et al. first reported the enzymatic release of antibacterial peptides with more potent activity than the precursor LF in 1991 (40).Shortly afterwards the antibacterial domains of bovine LF f(17-41) and human LF f(1-47), that are called bovine and human LFcin, were purified and identified (2). These peptides showed a potent antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria (44).

Concerning goat and sheep species, the chemical synthesis of fragment f(17-41) of caprine LF, consisted the first investigation, in which this fragment displayed antibacterial activity, although to a lesser extent than the bovine counterpart (42). Hydrolysis of caprine and ovine LF by pepsin resulted in antibacterial hydrolysates, and a homologous peptide to
LFcin, corresponding to fragment f(14-42) was identified in the caprine LF hydrolysate. Caprine LFcin showed lower antibacterial activity than bovine LFcin against \textit{Escherichia coli} but comparable activity against \textit{Micrococcus flavus} (Table 1).

Antibacterial peptides have been found to be active against a broad range of pathogenic organisms e.g. \textit{Escherichia}, \textit{Helicobacter}, \textit{Listeria}, \textit{Salmonella} and \textit{Staphylococcus}, yeasts and filamentous fungi. Depending on the target microorganism, inhibitory concentrations of peptides vary, e.g.: antimicrobial peptides $\alpha_s$-CN f183-207 and f164-179 exhibited inhibition against Gram-positive and -negative bacteria with MICs ranging from 8 to 95 μmol/l.

**Antimicrobial peptides derived from caseins**
In the same way as whey proteins, caseins are also a source of antimicrobial peptides (21, 22). In a preliminary study, an ovine β-casein hydrolysate with pepsine, trypsin and chymotrypsin has showed inhibition of bioluminescent production by \textit{Escherichia coli} JM103, but the peptides responsible for this activity have not been identified (10, 11).

Four antibacterial peptides were identified from a pepsin hydrolysate of ovine $\alpha_s$-casein (21). The peptides corresponded to $\alpha_s$-casein fragments f(165-170), f(165-181), f(184-208) and f(203-208), being the fragments f(165-181) and f(184-208) homologous to those previously identified in the bovine protein (36, 37) (Table 1).

These peptides showed a strong activity against Gram-negative bacteria. Of them, the fragment f(165-181) was the most active against all tested bacteria. The peptide corresponding to ovine $\alpha_s$-casein f(203-208) is a good example for a multifunctional peptide, because it exhibited not only antimicrobial activity, but also potent antihypertensive and antioxidant activity (35). These results can be extended to caprine proteins, because the amino acid sequence of these peptides is the same (Table 1).

**Future perspectives for bioactive peptides**
Milk provides the newborn (neonate) with nutrients and an array of antimicrobial factors. These are believed to help protect the neonates from infection until their own immune system has developed.

Developments in nano-encapsulation (39) and nano-emulsion technology, as well as research into delivery systems, may ultimately lead to the generation of specific formulations containing bioactive peptides with anti-bacterial activity. Antibacterial peptides from food protein deserve attention due to their mechanism of activity, which makes microbial resistance improbable (8), and for increasing the functional values of foods.

### TABLE 1

Some examples of bioactive peptides from goat and sheep milk with antibacterial activity

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide fragment</th>
<th>Biopeptide</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovine and caprine LF f/17-42/</td>
<td></td>
<td>Vorland L.H. et.al. (1998) (42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recio I. and Visser S. (2000) (38)</td>
</tr>
<tr>
<td>$\alpha_s$-casein</td>
<td>$\alpha$-La 1-23/</td>
<td>isracidin</td>
<td>Hill R.D. et.al. (1974) (15)</td>
</tr>
<tr>
<td>$\alpha_s$-casein</td>
<td>$\alpha$-La 165-170/</td>
<td>multifunctional</td>
<td>Lopez-Exposito I. et al. (2006) (21)</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-La 165-181/</td>
<td>peptides</td>
<td>Lopez-Exposito I. et al. (2006) (22)</td>
</tr>
<tr>
<td>$\alpha_s$-casein</td>
<td>$\alpha$-La 165-170/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\alpha$-La 184-208/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\alpha$-La 203-208/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin</td>
<td>$\beta$-Lg 15-20/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\beta$-Lg 18-24/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\beta$-Lg 30-32/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and other peptides</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**BIOTECHNOL. & BIOTECHNOL. EQ. 24/2010/2**
BP have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects (12).

REFERENCES