Chapter 6

Adjusting Bioactive Functions of Dairy Products via Processing

Katrin A. Kopf-Bolanz

Additional information is available at the end of the chapter

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Abstract

Milk is known for its high nutrient content that helps to maintain important body functions. In this regard, bioactive peptides that are encrypted in milk proteins and get released during processing and/or digestion might play a role. These peptides are able to inhibit enzymes, influence cell growth, or target specific receptors. The peptide profile that arises after protein digestion in the jejunum before the absorption into the blood takes place includes these bioactive peptides. The composition of the peptide profile is influenced strongly via processing and a modification in processing might target specific functionalities. Thermal, chemical, biochemical, and physical treatments affect protein digestion mainly by changing the protein structure for example via denaturation or protease actions. Parameters influencing this are external ones, like the matrix of the product, and internal ones, like specific enzyme deficiencies. However, considering all the important aspects that are involved, there might be the possibility in the future to adjust a bioactive function via processing.

Keywords: bioactive peptides, processing, bioavailability, bioactive function, dairy products

1. Introduction

Dairy products are appreciated for their high nutritional value [1]. Not only the high contents of protein, vitamins, and minerals determine the positive health effect of dairy products. Hidden components called bioactive peptides, encrypted in parent milk proteins, exhibit special functions that might influence our well-being. So far peptides with antihypertensive, anti-oxidative, anti-thrombotic, anticancer, immune-modulatory, antimicrobial, cholesterol-lowering, antidiabetic, mineral-binding, opioid and satiety properties were identified. These peptides occur directly in the dairy products after processing and are resistant to digestion enzymes or they are encrypted in dairy proteins and get released during digestion.

Interestingly, the processing method of the dairy products can influence the number and sequence of the resulting peptides after digestion and therefore also the content of bioactive peptides. Heat treatment, chemical and biochemical, and physical treatment can influence the bioactive functionality transmitted by the selected dairy product. In the future, it might be possible to target a wished bioactive function via processing. This book chapter just deals with the effects exhibited by bioactive peptides. However, also other milk components are affected by processing and can exhibit bioactive functions, e.g. effects on the lipids, minerals, and vitamins. Furthermore, the addition of certain bioactive ingredients to dairy products is also not discussed in this chapter. The focus is given to the possible effects transmitted via bioactive peptides and the effect of processing on the peptide profile.

2. Bioactive peptides and their functionalities

The health potential of dairy protein is not only originating from the unique amino acid composition and their great bioavailability (**Figure 1**). Especially the high content of essential amino acids and their fast release as free amino acids during digestion [2], plus the high content of certain vitamins and minerals is important for the high nutritional value of bovine milk. However, there is a more hidden health potential of dairy products that is displayed by bioactive peptides. Bioactive peptides contain usually 3–20 amino acid residues and their composition and sequence determine their activity. They are encrypted in the primary sequence of proteins and get released via three different ways [3]:

1. Hydrolysis by digestive enzymes
2. Hydrolysis by proteolytic microorganisms
3. Action of proteolytic enzymes derived from microorganisms or plants

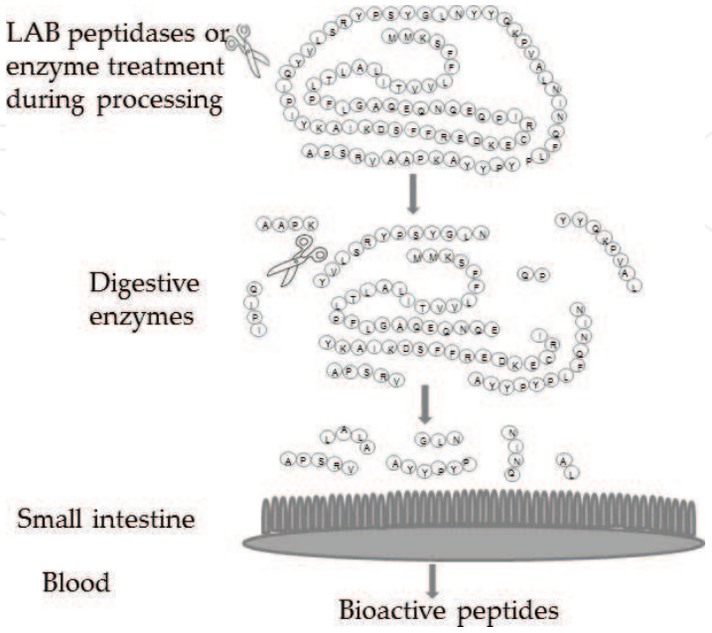

Figure 1. Illustration of milk protein degradation by lactic acid bacteria (LAB) and during digestion via digestive enzyme action resulting in bioactive peptides.

These are the ways to produce bioactive peptides that can be afterwards purified and used as ingredients for manufacturers of functional foods. However, also the more natural way of processing via hydrolysis by proteolytic microorganisms can be an approach to enrich a specific bioactive function in a product. Bioactive peptides have been discovered not only in dairy products, but also in meat, eggs, fish, and other marine organisms and also in plant sources like certain grains, legumes, pulses, and oilseeds [4–6].

The production of bioactive peptides for use as additives can be done by enzymatic hydrolysis or microbial fermentation [7]. Enzymatic hydrolysis applies digestion enzymes. Mostly trypsin, a pancreatic proteinase is used, but also chymotrypsin, pepsin thermolysin, pancreatin, elastase, carboxypeptidase or a proline-specific endopeptidase can deliver bioactive peptides. Additionally, proteases from bacteria, fungi, and plants also showed interesting properties [7]. Microbial fermentation uses bacteria or yeast that exhibit proteolytic activity to generate peptides. They are grown and added in their exponential phase to the protein of interest. The degree of hydrolysis is then dependent on the strain and its proteolytic activity. In both ways, a purification of the peptides is necessary. This can be for example reached by centrifugation methods, freeze drying, desalting, and membrane filtration techniques [8]. Examples are the production of caseinophosphopeptides from α-s-casein with an immobilized trypsin in a fluidized bed bioreactor [9] and a combination of diafiltration and anion-exchange chromatography [10]. The peptide additives can be added to a product of interest to generate a functional food. For this purpose, also the stability of the peptides with regard to pH, temperature, and food matrix has to be considered. Furthermore, the more natural way to enhance dairy products with bioactive peptides is to directly add a bacterial culture to the dairy product and generate a fermented product containing bioactive peptides. This is the general processing method applied already for each fermented dairy product. If protein is not taken out, all dairy products result in a high quantity of bioactive peptides that might be absorbed in the small intestine. For the functionality of these peptides, the selection of bacteria strains is important to aim for a specific bioactive function via processing (see Section 3).

The possible, so far detected, functionalities of bioactive peptides are summarized in **Figure 2**.

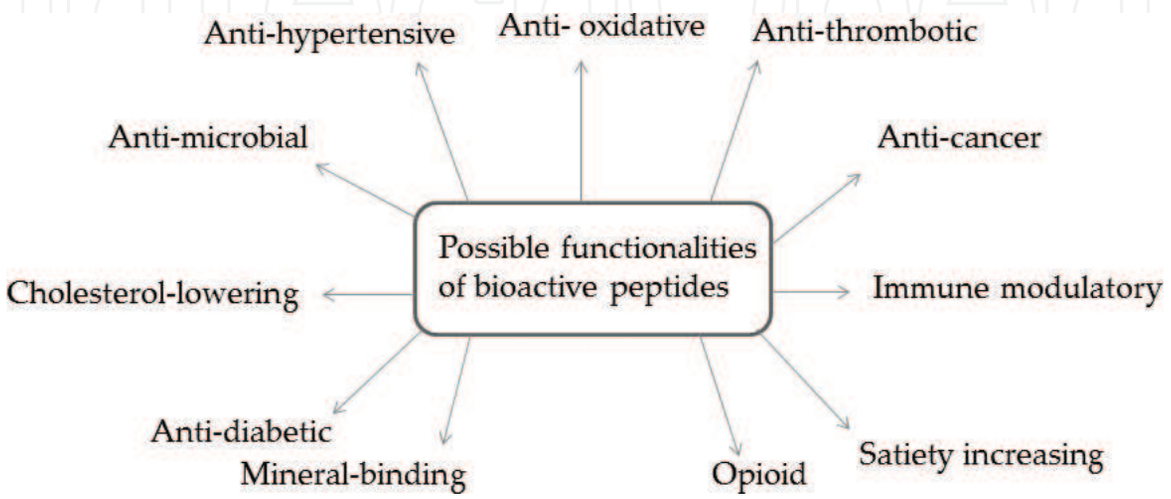

Figure 2. Possible functionalities of bioactive peptides.

2.1. Antihypertensive peptides

Antihypertensive peptides can inhibit the angiotensin I-converting enzyme (ACE) (EC 3.4.15.1;ACE) that is involved in blood pressure regulation. ACE increases the blood pressure by converting angiotensin I into the vasoconstrictor angiotensin II and additionally degrades vasodilative bradykinin. ACE-inhibitory peptides were detected in different food proteins like bovine casein and human casein, whey protein, zein, gelatin, yeast, and corn [11]. The most ACE-inhibitory peptide in studies of [28], had an IC_{50} value of 77 μM and was originating from α-lactalbumin with the peptide sequence α-lactalbumin f(104–108). Different studies showed the bioavailability of the ACE-inhibitory peptides Ile-Pro-Pro and Val-Pro-Pro in humans [12, 13]. These two tripeptides are the ones that are studied the most and show the highest evidence for their bioefficacy.

2.2. Anti-oxidative peptides

Anti-oxidative peptides help against the oxidative damage caused by reactive oxygen species. The amino acids cysteine, lysine, histidine, methionine, tryptophan, and tyrosine can act as radical scavengers [14]. Therefore, they act as potential antioxidants.

2.3. Antithrombotic peptides

The formation of blood clots can be reduced by antithrombotic peptides. Especially known is the glycomacropeptide (GMP) originating from kappa-casein after enzymatic milk coagulation. GMP can inhibit the aggregation of blood platelets and binding of the human fibrinogen gamma-chain to platelet surface fibrinogen receptors [15]. Also, the absorption into the plasma could be observed in humans for 2 anti-thrombotic peptides [16].

2.4. Anticancer peptides

Anticancer peptides can inhibit cancer cell growth. In vitro experiments with HL-60 human leukemia cells showed for example, that after skimmed milk digestion with a proteolytic enzyme from yeast *Saccharomyces cerevisiae* apoptosis can be induced [17].

2.5. Immune-modulatory peptides

Immune-modulatory peptides are mostly found in dairy products. Enzymatic hydrolysis resulted in a number of biologically active peptides that can influence immune cells and release specific signals [18]. Some peptides can stimulate or inhibit immune responses and their positive health effects have been investigated mostly in vitro. These assays are performed with immune cells and target for example proliferation, phagocytosis, differentiation, and cytokine production. A survey of these assays can be found in the review of Maestri et al. [6]. The immunomodulatory potential of peptides originating from whey protein is discussed in the study of Gauthier et al. [19]. Interestingly, there are some in vivo studies that have

demonstrated promising results. Otani et al. showed for example that feeding mice a dietary casein phosphopeptide influenced the level of serum IgA and intestinal antigen-specific IgA [20]. The exact mechanism of the action exhibited by immune-modulatory peptides still has to be determined.

2.6. Satiety peptides

Peptides that inhibit dipeptidyl peptidase 4 (DPP-IV, EC 3.4.14.5) are known as satiety increasing peptides. DPP-IV degrades the satiety regulating glucagon-like-peptide 1 [21]. Kopf-Bolanz et al, monitored the fates of specific peptides with known satiety increasing action. They compared different dairy products and found the relative abundance of three potent DPP-IV inhibitory peptides deriving from β-casein. The best source for these peptides was Gruyere cheese. Two other peptides deriving from α-S1-casein were not detectable anymore after the pancreatic phase of the digestion [22]. Tryptophan seems to be an important amino acid in peptides that exhibit a DPP-IV inhibitory potential [23]. Three dipeptides containing tryptophan Trp-Arg, Trp-Lys, and Trp-Leu with half maximum inhibitory concentrations (IC_{50}) < 45 μM could be detected in a study of Nongonierma and Fitzgerald that are potent inhibitors of DPP-IV [24].

Another interesting peptide that has an influence on satiety is the glycomacropeptide (GMP) resulting from cheese production. It has demonstrated in several animals and human studies that it can stimulate the release of cholecystokinin and promote satiety. However, further studies would be necessary to demonstrate a clear bioefficacy [25, 26]. Peptides that increase the satiety are also known as anti-obesity peptides [27].

2.7. Opioid peptides

Peptides that have an affinity for the opioid receptor are categorized in this group. There are receptors that are responsible for specific physiological effects like emotional behavior and food intake. Opioid peptides have the same N-terminal sequence Tyr-Gly-Gly-Phe. There are also atypical opioid peptides with the ending of Tyr-X-Phe or Tyr-X1-X2-Phe. A tyrosine residue at the N-terminal and another aromatic amino acid at the third or fourth position are specific binding motifs of the opioid receptor. The first food-derived opioid peptides were β-casomorphins. Also, casoxins, lactorphins, and exorphins can bind to opioid receptors [28]. So far, a weak opioid activity for α-lactorphin (α-lactalbumin f(50–53)) and β-lactorphin (β-lactoglobulin f(102–105)) was detected in guinea pigs [11], but human data are still missing. Concentrations released from in vivo digestion of milk are quite low. The total amount of α-lactorphin and β-lactorphin in 1 L of bovine milk would be 32 mg (64 μmol), respectively 90 mg (162 μmol), but it might be difficult to obtain a full release of the possible peptide during in vivo digestion. It is so far not clear whether they can get liberated by in vivo digestion at all, but it was demonstrated that casomorphins are liberated in vivo [11].

2.8. Antidiabetic peptides

Diabetes is treated by synthetic antidiabetic drugs that can result in side effects like hypoglycemia or weight gain [8]. To overcome this issue, the application of antidiabetic peptides originating from food sources might be a solution. Antidiabetic peptides could be for example detected in sheep milk [29].

2.9. Mineral-binding peptides

Mineral-binding phosphopeptides can carry different minerals by forming soluble organophosphate salts [30]. Caseinophosphopeptides (CPP) can increase calcium absorption by limiting calcium precipitation in the ileum. Caseins are phosphorylated in the mammary gland at primary sequences rich in serine and glutamic acid forming triplet regions SerP-SerP-SerP-Glu-Glu that occur in α-S1-casein (66–70), α-S2-casein (8–12), (56–60), (129–133) and β-casein (15–19). The presence of CPPs has been shown in vivo. Several animal studies have demonstrated the effect of CPP to enhance calcium bioavailability. In contrast, convincing results from human are still missing [31]. A human study with CPP-enriched preparations (containing candidate functional food ingredients) on calcium absorption from a calcium lactate drink showed no significant results [32]. Another interesting peptide is lactoferricin consisting of 25 amino acid residues. The molecule is folded into two globular units, each capable of binding one ferric (Fe^{3+}) ion [33].

2.10. Cholesterol-lowering peptides

So far mainly peptides derived from soy proteins have been shown to suppress cholesterol in the blood. Some can for example target the cholesterol receptor or suppress the presence of LDL. Important for the functions are mainly the hydrophobic residues [34]. A novel peptide (Ile-Ile-Ala-Glu-Lys) from a trypsin-treated hydrolysate of β-lactoglobulin showed a hypocholesterolemic effect in an animal study [35].

2.11. Antimicrobial peptides

Peptides that induce for example the lysis of bacterial membranes are antimicrobial peptides. They could be detected in α-lactalbumin, β-lactoglobulin, all casein fractions, and lactoferrin [36].

2.12. Safety issues

The safety and toxicity of bioactive peptides has to be considered. Different studies demonstrated that casein hydrolysates and Val-Pro-Pro from powdered fermented milk did not show any toxicological potential [37–39]. Processing can lead to Maillard reaction and result in the production of allergenic compounds [40]. Processing changes the protein structure and might influence the protein degradation and therefore also the response of the immune system. Therefore, it is important to determine the allergic potential that can arise from bioactive peptides. If fermentation takes place, for example, de novo peptides might originate and their allergenic potential has to be determined. First, a comparison with already known allergenic sequences can be done, followed by laboratory tests. The problem is that allergenic sequences

can occur over the whole dairy protein sequences, and there can be rare cases that people are allergic to a new peptide sequence arising from fermentation. However so far, mostly positive reports about the effect of fermentation are published [41, 42]. It is also important to mention that these functionalities were observed to a great extent with in vitro methods. Only very few human studies have demonstrated an effect of bioactive peptides in vivo.

2.13. Physiology of digestion

To exhibit really a bioactive function in vivo, the peptides must be released during digestion from their originating protein or if they are already in the product as such, they have to be resistant to digestion enzymes. During digestion, the proteins get denatured by gastric acidification and subsequently degraded by pepsin and pancreatic peptidases like trypsin into peptides and amino acids. Furthermore, the final actions of the enzymes at the brush-border membrane in the small intestine have to be taken into account. There are peptidases that cleave amino acids or dipeptides from the N- or C-terminal of the interior bond of the oligopeptides. The mean size of the peptides in the jejunum considering the action of aminopeptidases and dipeptidases from the enterocytes is 3–6 amino acids. Di- and tripeptides can be transported actively by the peptide transporter PEPT1 [43]. Longer peptides can probably get absorbed ether via paracellular or transcellular pathways. The possible transport of a heptapeptide was shown using a cell culture model [44]. In the blood, the peptides must be able to reach their target site in the peripheral organs. In a human study of van Platerink et al., 17 ACE-inhibiting peptides with 5–6 amino acids length could be detected in the blood after consumption of drinks enriched with those peptides [13]. The first proof that the tripeptide Ile-Pro-Pro does not undergo intestinal degradation and can reach the circulation intact was shown from Foltz et al. [12]. Another human study showed the presence of a longer peptide after soybean consumption in the blood [45]. At the target cells, it is assumed that peptides can internalize via endocytosis and get digested in the lysosome. Peptides that do not enter target cells can accumulate in the liver and kidney and can be detected in urine or bile [6]. There is still the need to demonstrate a clear bioefficacy of the peptides and confirm the positive health effects in human studies. In the future possibly health claims for certain bioactive peptides could be developed. So far Japan declared certain antihypertensive peptides such as Val-Pro-Pro, Ile-Pro-Pro, Val-Tyr, and Cys-Pro-Pro as Food of Specific Health Use (FOSHU). In contrast, the European Food and Drug Association (EFSA) did not authorize any claims regarding the effect of bioactive peptides in foods yet [46].

2.14. Detection of bioactive peptides

Experiments concerning bioactive peptides are mainly done in vitro. Most of the time, a dairy product is inserted into an in vitro digestion model that mimics human digestion.

There are numerous in vitro digestion models that can be applied. It is important that a model close to human physiology and validated is applied. Recently, a harmonized digestion model was established during the COST digestion action. This model is very physiological and might be used for mimicking digestion [47]. The resulting peptides generated during the digestion process can be detected by peptidomic methods. Analytics of bioactive peptides aim toward three main directions [48]:

1. Tracing the pathways of formation of bioactive peptides from the parent proteins
2. Identifying the biological properties
3. Improving the "positive" properties discovered in natural peptides by design of synthetic structural analogues or peptide mimetics

Peptidomics is the comprehensive qualitative and quantitative analysis of all peptides in a biological sample. In earlier days, protein digestion could be followed by HPLC or Edman sequencing [49]. Nowadays, MS-based techniques such as Liquid chromatography coupled to mass spectrometry (LC-MS) can be applied [22, 50]. Peptidomics of food hydrolysates, for example, led to the discovery of the exact sites of rennet cleavage on kappa-casein or the cleavage sites produced by bacteria during cheese ripening [49]. The detailed human study of Boutrou et al. was identified in the jejuna effluents of healthy adults, after consumption of 30 g milk casein and whey proteins, 356 and 146 peptides [50]. The in vitro model developed by Minekus et al., almost resulted in similar peptides [47]. The different analytical approaches that can be applied are summarized in the review of Dallas et al. [49]. Technology allows the prediction of the peptide sequence and can generate a peptide fingerprint. The peptides can be then compared to the known bioactive peptides from the literature in various databases. An example is the milk bioactive peptide database by Nielsen et al. [51]. This database comprises information on bioactive peptides from across hundreds of original research articles and is available to the public. Furthermore, whole in silico strategies for bioactive function generation including computational modeling might be applied, that still have limitations, but might be used in the future for the design of new products.

3. Influence of processing on protein digestion and peptide profile

Dairy products are processed by the application of different physical and chemical methods. These methods change the protein structure irreversible or reversible depending on the impact of the treatment. The protein can be mainly denatured, hydrolysed, or glycosylated. This structural change can influence the access of the digestion enzymes to the protein and therefore changes the action of the digestion enzymes. An impact on the peptide profile that is generated before absorption into the blood takes place is the result. It is necessary to determine which processing methods and which processing variables are necessary to be able to reach or maintain a specific bioactivity.

3.1. Thermal treatment

Thermal processing is an important step to improve the microbial quality of milk. Additionally, enzyme activities are inactivated and some physicochemical changes can occur that might support processing. The nutritional value is greatly affected by thermal processing. Denaturation, β-elimination, racemization, or iso-peptide bond formation can occur that influence the nutritional value [52]. Denaturation is influenced by pH, protein concentration, ionic environment, genetic variant, and presence of ligands [53]. Heating might even particularly destroy tryptophan, can convert Arginine into citrulline and ornithine, can deamidate glutamine and

asparagine, and desulphur cysteine and cysteine. Resulting end products might be lanthionine, lysine-alanine, iso-peptides and ornitho-alanine [52]. The digestibility of whey proteins increases after thermal treatment because the sites for enzymatic hydrolysis are easier to reach for the digestive enzymes. However, strong denaturation reduces digestibility [54]. Kopf-Bolanz et al. showed that heat treatment of dairy products led to an increased number of β-lactoglobulin peptides after in vitro digestion [22]. There is a greater susceptibility to hydrolysis following heat treatment [55]. Regarding the antidiabetic action of casein, there was a significant reduction observed after boiling compared to the raw casein [29]. The denaturation of whey protein via thermal processing led to an increase in the antibacterial activity of α-lactalbumin [56] and lysozyme [57]. The antioxidant action of whey proteins can be maintained by low-temperature processing. This results in high levels of specific dipeptides that can promote the synthesis of the antioxidant glutathione [58]. Extrusion cooking might also affect protein digestibility shown for example in a study of Onwulata et al. [59]. Data on the effect of ohmic heating are rare. Depending on the used temperatures, similar effects like with application of other heating methods might be expected [52]. It was also shown that spray drying or freeze drying did not exhibit negative effects on the immunomodulatory activity of a whey protein hydrolysate. The study also used whey protein concentrate (WPC) and sodium alginate as carriers for encapsulation to reduce bitter taste and resistance to hygroscopicity. They showed that spray drying of whey protein concentrate hydrolysate with the proper carriers did not affect the immunomodulatory activity and might therefore widen its application in food systems [60].

3.2. Chemical treatment

Hydrolysis by acid is applied which is known to improve their protein digestibility. It is used for example for enteral and hypoallergenic infant nutrition. For Mozzarella, the type of acid used is important for the protein yield obtained in the pre-cheeses [61] and might therefore also affect the profile of bioactive peptides. Treatment with alkali for hydrolysis is rarely applied in the food industry. It would result in racemization and loss of protein digestibility [62].

3.3. Biochemical treatment

Fermented dairy products like yoghurt and cheese result in a high number of bioactive peptides produced by the lactic acid bacteria. Especially the type of the starter culture, type of probiotic bacteria, and the fermentation parameters play an important role for the bioactive effect that the product might have. Furthermore, only via this way de novo peptides can be generated that do not occur after digestion of milk as such. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* possess bacterial activity against Streptococci in vivo that probably derives from the antimicrobial peptides that they produce during fermentation [63]. It is very promising to test different lactic acid bacteria strains for their effect on a bioactive function. One study of Gobbetti et al. showed that a fermentation with *L. delbrueckii* ssp. *Bulgaricus SS1* versus a fermentation with *Lactobacillus lactis* subspecies *cremoris FT4* resulted in a higher ACE-inhibitory activity [64]. The most investigated ACE-inhibitory peptides were obtained after fermentations with *L. helveticus* and *L. helveticus CP790*. Also, the Finnish milk product Evolus contained *L. helveticus LBK-16H* strain as a starter and

they all contained the tripeptide IPP and exerted a hypertensive effect [65–67]. Another study demonstrated the effect of the time of cheese ripening on the ACE-inhibitory activity. Cheese was produced with a mixture of 12 different strains and showed an increase of the inhibitory effect during ripening as long as a certain level of proteolysis was not exceeded [68]. In 10 Swiss cheese types, the ACE-inhibiting peptides V-P-P and I-P-P were quantified. They detected contents of 19.1 mg/kg to 182.2 mg/kg depending on the cheese type that shows the huge effect of different processing ways probably via different lactic acid bacteria [69]. Also, the application of new techniques like next-generation sequencing that reveals the whole genome of bacteria strains might help to select promising strains with specific protease expressions. It was also demonstrated that fermentation reduced the allergenic potential of α-lactalbumin and β-lactoglobulin [41, 42]. The peptides that result after fermentation and enzyme hydrolysis might remain susceptible to further hydrolysis as long as the process goes on. This might lead to a decrease of bioactive function of these peptides. More important is also the stability of the generated peptides. They might be degraded by the digestive enzymes and result in zero activity in the body. The stability versus the action of gastric and pancreatic enzymes has to be tested beforehand. Another problematic point is that the microbial fermentations have to be reproducible [8]. Fermentation with known and established lactic acid bacteria cultures is a great strategy to enrich certain bioactive peptides with a special functionality. This would be a possibility to enhance a bioactive function in a natural way with a minimal processing approach that meets the interests of the consumer. The functionality and bioavailability of bioactive peptides generated via fermentation has to be more clarified.

The use of milk-clotting enzymes and digestive enzymes to produce bioactive peptides is another processing approach. However, most of the resulting peptides had a bitter taste [7]. Membrane-separation technique is applied to enrich peptides with a specific molecular weight [3]. It was also shown that hydrolysed infant formulas show a different peptide profile compared to the standard formulas assuming that infants fed hydrolysed formulas might obtain bioactive peptides that promote other bioactive functions than the ones provided by the standard formulas [70].

3.4. Physical treatment

Homogenization applies pressure (14–18 MPa) and shear stress that alter the protein structure and improve the digestibility [52]. Use of ultra-high pressure homogenization with pressure around 400 MPa results in more severe protein denaturation [71]. Application of high hydrostatic pressure processing increased digestibility of β-lactoglobulin with pepsin with increasing pressures (400–800 MPa) [72]. Penas et al. also combined high hydrostatic pressure processing with selected food-grade proteases and demonstrated a reduction in antigenicity of the whey protein hydrolysates that can be used as ingredients of hypoallergenic infant formulae [73]. Ultrasound treatment is a non-conventional processing technique that can denature α-lactalbumin and β-lactoglobulin. In whole milk compared to skim milk, the denaturation was stronger and heat addition even increased this effect [74]. A very soft technology is membrane filtration that enables to separate proteins in their native state. This technology only enables a fractionation of different milk components and does not alter the protein structure as such, and it only influences the milk composition.

4. Influence of other factors on bioavailability of bioactive peptides

Not only processing can influence the profile of bioactive peptides. Also, other external factors can influence protein digestion and therefore the bioavailability and generation of bioactive peptides. It is important to consider the effect of the food matrix and meal composition on digestion. For example, the addition of inulin to the dairy product can influence digestion and peptide bioavailability [75]. Also, proteins can form complexes with polyphenols, etc. that could lower protein bioavailability [76]. Furthermore, internal factors can influence the peptide profile. Children and the elderly have different enzyme activities and therefore the digestion enzymes will act slightly different and change the peptide profile [77, 78]. Genetic variations in people for example enzyme deficiencies or changes in the composition of the digestion juices due to different transporter expression can have an impact. The action of digestion enzymes depends on daytime, age and on *Helicobacter pylori* infection [79]. Also for a lot of other special physiological states, certain diseases and so on, the enzyme activity is affected and might therefore result in a different bioactive peptide bioavailability. It is very important to consider all the factors that can affect peptide bioavailability in the target group of the product.

5. Possible approach for design of a dairy product with a satiety increasing effect

An enrichment of peptides that can inhibit DPP-IV could result in increasing satiety after consumption of a dairy product. The most promising approach to steer the peptide profile of the

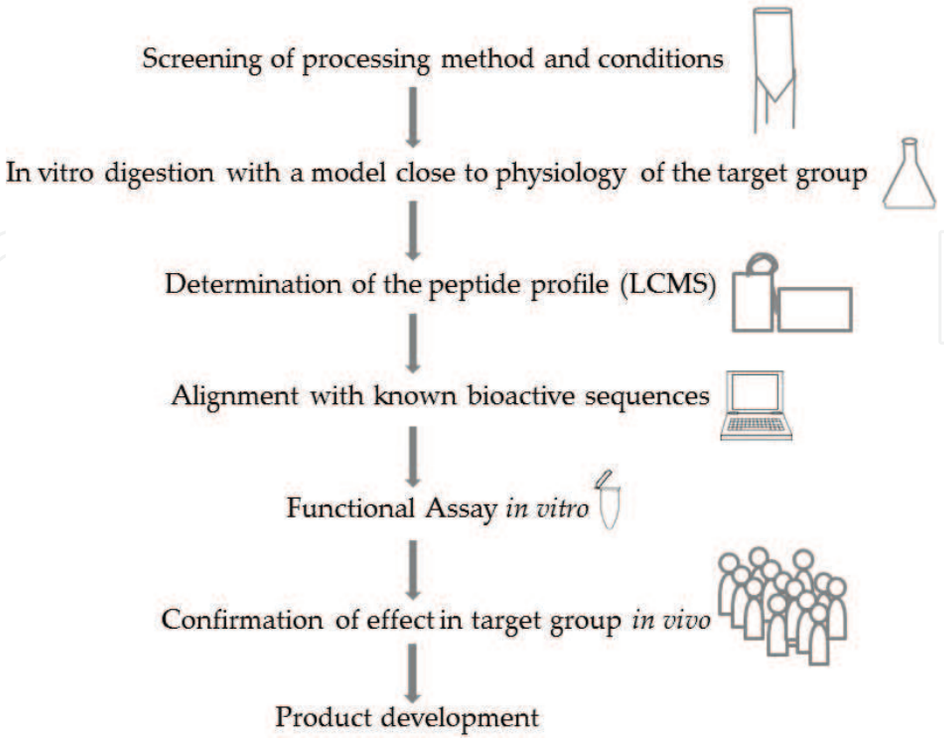

Figure 3. Targeting bioactive function in product development.

product is via fermentation with lactic acid bacteria. It would be promising to test different starter bacteria with different protease activities. Differences in their proteolytic action result in different peptide profiles. Then, an in vitro digestion model might be applied that is mimicking the digestion physiology of the target group. Next step would be peptide profiling and alignment of the results with already known sequences of DPP-IV inhibitory peptides. Furthermore, the inhibitory effect could be also tested using directly an enzyme assay. It is important to consider also all the other processing parameters that can affect protein digestion. Finally, the bioactive effect has to be confirmed in humans that represent the target group of the product (**Figure 3**).

6. Conclusion

After ingestion of food containing protein, a peptide pattern is generated that probably contains bioactive sequences and is present in the jejunum before absorption takes place. Especially for dairy products, many different bioactive peptides could be identified that might be very interesting for the development of products with a specific functionality. The peptide patterns are strongly influenced by processing. Thermal treatments are used in general to ensure the microbial quality of dairy proteins. They exhibit a great influence on the protein structure for example by protein unfolding. Easier access is then given to the digestion enzymes and the resulting peptide profile is changed. Highly interesting is the fermentation of dairy products with different lactic acid bacteria strains. Certain strains have different protease activities and increase for example the number of antihypertensive peptides resulting after digestion. The angiotensin-converting enzyme inhibition is the most studied functionality and there are reports that could detect bioavailable peptides in the blood. The peptide concentrations reached for example in cheeses are promising to exhibit a bioactive function. Chemical and physical approaches can also influence the protein structure and therefore the protein digestion. The impact of new processing techniques on protein digestion should be always monitored. For the adjustment of a specific bioactive function in a product, an example approach is mentioned. However, it will always also depend on other factors whether the wished functionality is really reached or not. External factors like the meal composition and internal factors like age or genetic preconditions can also have an impact and have to be considered. Furthermore, for safety reasons, there is the small chance that generated de-novo peptides might act as epitopes for rare cases of cow milk allergy. However so far, fermentation with established lactic acid bacteria strains seems to reduce the allergenic potential of dairy products in general. In the future, it is necessary to perform well designed human studies that ensure a bioactive effect and allow the admission of health claims.

Author details

Katrin A. Kopf-Bolanz

Address all correspondence to: katrin.kopf@bfh.ch

Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences HAFL, Zollikofen, Switzerland

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