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## Mediterranean milk and milk products

■ **Summary** Milk and dairy products are part of a healthy Mediterranean diet which, besides cow's milk, also consists of sheep's, goat's and buffalo's milk – alone or as a mixture – as raw material. The fat and protein composition of the milk of the various animal species differs only slightly, but in every case it has a high priority in human

nutrition. The milk proteins are characterized by a high content of essential amino acids. Beyond that macromolecules, which have various biological functions, are available or may be formed by proteolysis in milk. Taking this into consideration, the technology of different well-known Italian and German cheese types is presented and the differences as well as correspondences regarding nutrition are discussed.

Especially Ricotta and Mascarpone are discussed in detail. Ricotta represents a special feature as this cheese is traditionally made of whey and cream. Thus the highly

valuable whey proteins which contain a higher amount of the amino acids lysine, methionine and cysteic acid in comparison to casein and, additionally, to soy protein, are made usable for human nutrition. Finally, it is pointed out on the basis of individual examples that technologies to enrich whey proteins in cheese are already available and in use. Thus, the flavor of low fat cheese is improved and the nutritional value is increased.

■ **Key words** milk – cheese – technology – whey protein – fat replacement

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### Introduction

Health and the prevention of diseases are playing an increasingly meaningful role in nutrition. This paper aims to evaluate similarities and differences between German and Mediterranean milk products. However, it is worth noting that not all milk components can be discussed here for their relevance to this topic. Moreover, the function of special milk components has already been described in detail elsewhere [29]. The paper will focus on milk proteins in relation to traditional milk processing and will consider new technologies.

Milk and dairy products are part of a healthy Mediterranean diet which, besides cow's milk, also uses sheep's, goat's and buffalo's milk – alone or as a mixture – as raw material. Altogether, the proportion of the non-cow's milk dairy products remains small; however, a larger product variety results due to their availability.

The milk of the various species indicates only small differences in fat and protein composition, and they have a high priority in human nutrition. The high nutritional value of the milk protein fractions casein and whey protein both depend on the content of essential amino acids and the metabolic utilization. In addition, whey proteins present a high level of lysine, sulfur containing amino acids, methionine and cysteic acid, tryptophan and threonine, which are limited in different dietary protein sources.

Research for more than a century has demonstrated that milk contains protein with native or latent biological functionality. Activities of the native state are imputed to indigenous bioactive molecules including mediator and hormone-like substances, immunoglobulins and enzyme systems. Most of these components are contained in the whey protein fraction and exert a specific or non-specific activity against a great variety of pathogenic and non-pathogenic strains as well as food

spoilage microorganisms. In addition, they are known to stimulate the growth and differentiation of cells, and to enhance passive immunity and the regulation of immunity. The primary function of the components is related to the promotion of the cow's and the newborn calf's health. But due to their physiological function some of the components, notably immunoglobulins, lactoferrin and lactoperoxidase and lysozyme, have been recognized as potential for functional food, dietary supplements and pharmaceuticals.

Latent biological functionality only becomes active upon hydrolysis by certain proteolytic enzymes during processing, e. g. through the addition of chymosin in cheese-making, or during digestion, e. g. by pepsin and trypsin. Bioactive peptides have been described and tested for their physiological functionality derived from the hydrolysis of casein fractions,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. For example, the glyco-macropptide is released from  $\kappa$ -casein during cheese-making due to the addition of rennet (chymosin) and is lost in the whey. *In vitro* studies have shown that the glyco-macropptide binds bacterial toxins, inhibits the adhesion of bacteria and viruses and promotes the growth of bifido-bacteria [2]. Table 1 shows that many bioactive peptides are derived from the hydrolysis of milk proteins. They have gastrotestinal functions, a protective and defense function or a physiological function such as an ACE-(Angiotensin Converting Enzyme)inhibitory effect (see [3]). Additionally, there are bioactive peptides from  $\alpha$ - and  $\beta$ -casein that are able to bind minerals.

The above considerations demonstrate that milk represents not only a source for nitrogen and essential amino acids but also contains bioactive peptides which are released by digestion or processing. The latter may find use in the prevention and treatment of human dis-

eases in the future. Nevertheless, milk and milk products have played a key role in human nutrition for centuries because of their high nutritional value. To cover this protein source, the milk, which is perishable, has always been and still is converted into several cheese products with a longer shelf-life while preserving its nutrients. In order to preserve a product without thermal treatment and appropriate packaging, it is necessary to lower its water activity ( $a_w$ -value). One possibility is to add rennet to the milk in order to obtain gelling and dehydration after cutting, but valuable whey proteins are also separated at the same time as the water. Up to now a broad range of different available cheeses has been based mainly on regional conditions and the production technology, which has been repeatedly adapted and optimized.

The introduction has shown that milk and milk products contain highly valuable nutrients as well as bioactive molecules. But this leads us to the question as to whether there are special technologies for Mediterranean milk products which may use the potential of the milk source better than others. In order to illuminate differences in processing and nutrition, Table 2 shows the composition of several cheese types which are well-known in northern Europe and Italy. The table is vertically arranged according to decreasing protein content, and horizontally according to decreasing duration of ripening.

First of all, there are cheeses belonging to the hard cheese group that are mostly produced from raw milk and have long maturing times. Interestingly, the Italian cheeses such as Parmesan, Pecorino and Grana Padano have long maturing periods and are mainly used for the seasoning of food, e. g. noodles. As mentioned previously, a high amount of bioactive peptides was probably formed in these products through ripening. But in Bergkäse as well as in Emmentaler, both made from raw milk, these bioactive peptides also appear during ripening. In all cases, most of the native bioactive molecules and the valuable whey proteins are lost in the whey.

Types of soft cheese are another interesting group. It has to be pointed out that beyond this group of cheese, the technology for processing some Italian products differs from typical German cheese-making, e. g. for Butterkäse (butter cheese). Mozzarella and Provolone are so-called "Pasta Filata" cheese. In this technology the curd grains are cut into small pieces and are then warmed up by applying hot water in order to melt the grains. The melted grains are intensively kneaded to form a continuous matrix. While warming up, the remaining microorganisms are inactivated to a large part. Maturation is reduced and only achieved by non-inactivated enzymes. Therefore, this cheese is mostly consumed unripened, e. g. au gratin or with vegetables. In Germany, these cheese types are already being produced and are available on the market. They mostly contain the

**Table 1** Bioactive peptides from milk proteins [1]

Bioactive peptide	Protein precursor	Bioactivity
Casomorphins	$\alpha_{51}$ , $\beta$ -Casein	Opiod, agonist
$\alpha$ -Lactorphin	$\alpha$ -Lactalbumin	Opiod, agonist
$\beta$ -Lactorphin	$\beta$ -Lactoglobulin	Opiod, agonist
Serum albumin	Serorphin	Opiod, agonist
Lactoferrroxin	Lactoferrin	Opiod, antagonist
Casoxins	$\kappa$ -Casein	Opiod, antagonist
Casokinins	$\alpha$ -, $\beta$ -Casein	ACE inhibitory
Lactokinins	$\beta$ -Lactoglobulin	ACE inhibitory
Casocidin	$\alpha_{52}$ -Casein	Antimicrobial
Lactoferricin	Lactoferrin	Antimicrobial
Isracidin	$\alpha_{51}$ -Casein	Immuno-modulating/ antimicrobial
Immuno peptides	$\alpha$ -, $\beta$ -Casein	Immuno-modulating
Casoplatelins	$\kappa$ -Casein, Transferrin	Antithrombotic
Phosphopeptides	$\alpha$ -, $\beta$ -Casein	Mineral binding

**Table 2** Composition of various cheese types (adapted from various sources)

Cheese type	Protein (g/100g)	Fat in TS (g/100g)	Fat (g/100g)	Ca (g/kg)	P (g/kg)	Milk source	Ripening
Parmesan	36.5	–	26.0	13.0	8.5	Cow	up to 2 (3) years
Pecorino		50	31.0			Sheep	8 months
Grana Padano		36	24.1	11.0	7.0	Cow	12–18 months
Gruyere	29.8	48	32.3	10.0	6.1	Cow	8 months
Bergkäse	28.0	50	31.0			Cow	3–12 months
Emmentaler	27.9	45	29.0	10.8	8.6	Cow	3–12 months
Edamer	25.5	45	26.0	7.5	4.5	Cow	6 weeks
Cheddar	25.4	50	32.2	8.0	5.0	Cow	up to 2 years
Gouda	25.4	45	29.0	8.2	4.4	Cow	6 weeks to 1 year
Bel Paese	25.4	49	30.2	6.0	4.8	Cow	ripened
Camembert	22.0	45	22.3	4.0	4.0	Cow	ripened
Butterkäse	21.1	50	28.8	6.9	4.2	Cow	ripened
Mozzarella	19.9	40	16.1	6.3	4.3	Buffalo, cow	unripened
Provolone	19.9	40	16.1	6.3	4.3	Cow	ripened
Gorgonzola	19.4	50	31.2	6.1	3.6	Cow	ripened
Feta	17.0	45	18.1	4.3	3.3	Sheep, goat, cow	10 to 15 days
Hüttenkäse	14.7	20	4.6	0.8	1.6	Cow	unripened
Quarg (skim)	12.5		0.25	0.9	1.6	Cow	unripened
Quarg	11.1	40	11.4	1.0	1.9	Cow	unripened
Zi(e)ger	9.5	60	15.0	2.7	2.7	Whey	unripened
Ricotta	10.0	60	15.0	2.7	2.7	Whey	unripened
Mascarpone	5.5	80	40	4.0	8.0	Cow	unripened

Ca calcium; P phosphor; TS total solids

casein fractions, precursors and some bioactive peptides such as the glyco-macropeptide. The valuable whey proteins also become lost in the whey during cheese-making.

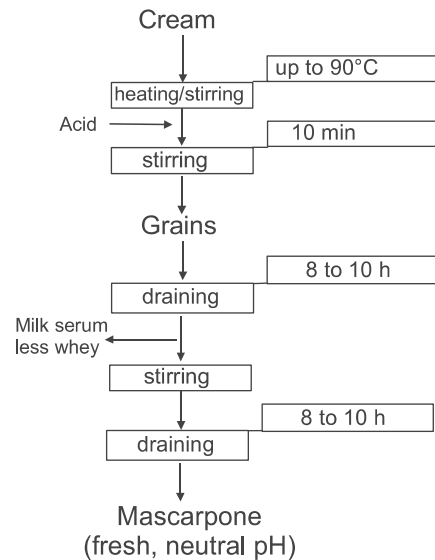
Up to this point only small differences have been found between traditional German and Italian technologies, although the cheese “Ricotta” has to be discussed in detail, as there is no comparable product. The cheese is made from cow’s milk whey. It is white, creamy and mild and is primarily used as an ingredient in lasagna. (Zieger is also made from whey but the flavor is different.) Some milk or cream is added to the whey in order to increase the fat content. The mixed whey is heated up to 70 °C, and citric acid is added to encourage destabilization of the whey proteins. Heating up to 90 °C and holding enhances the coagulation and creaming of the whey grains. The grains are skimmed off and put in baskets to drain for two days. The differences to traditional German processing have to be emphasized: 1) The valuable whey proteins are gained for food. 2) Whey proteins coagulate when whey is heated-up and the pH value is decreased by directly adding acid. Then the proteins can be separated from the watery milk phase (permeate). It has to be noted that besides fermentation usually applied in Germany, direct acidification is a useful and common process step in Italian milk processing. In

so doing whey proteins are transformed into a source of nutrition, although whey is used for feeding in traditional German cheese-making.

However, Mascarpone also represents an interesting cheese processing method in which direct acidification is applied (see Fig. 1). Cream is heated and, while stirring, acid is added in order to force the coagulation of the matrix. In addition, during the intensive heating the whey protein denatures and aggregates or sticks to the casein micelles and the fat globule membrane. As a result of this reaction whey proteins partly remain in the cheese matrix during the draining step. After a total of about 20 h of draining one obtains Mascarpone, which is often used in desserts because of its mild flavor and creamy consistency. The shelf life of the traditionally manufactured products is limited as re-contaminants may grow and hygienic problems may arise during the long draining period of 20 h.

### Integrating valuable whey proteins in cheese by ultrafiltration

In traditional rennet cheese manufacturing only 6 to 30 kg of the milk constituents in the form of curd is recovered from 100 kg milk depending upon the type of



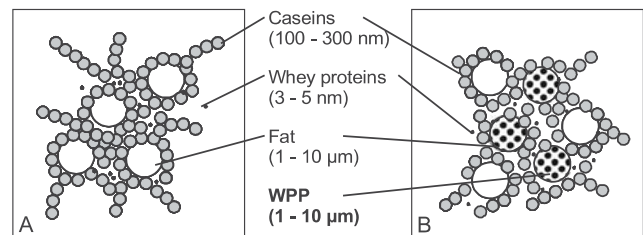
**Fig. 1** Scheme for the traditional processing of Mascarpone

cheese, while the rest is left over in the form of whey. The caseins form the essential structure of the cheese matrix (see Fig. 2A), which constitute about 80 % of the milk proteins. The remaining 20 %, the whey proteins, are lost to a large extent in the whey. In the meantime different technologies have been tried and tested, and some are now well established as integrating whey proteins into cheese and thus improve the recovery of the nutrients of the raw material milk. Whey proteins may be incorporated into cheese in principle both in a native form and in a denatured state [4–9]. Ultrafiltration technology has meanwhile been established on an industrial scale in modern dairies enabling valuable whey proteins and other milk constituents to be retained in the cheese. Examples of cheese made with this technology are fresh cheese (i. e. Quarg), fresh cream cheese, soft cheese (i. e. Camembert) and also Mediterranean cheese types such as Pasta Filata (i. e. Mozzarella), Feta cheese, Cheddar cheese, cheese base, cottage cheese and butter cheese [10–13].

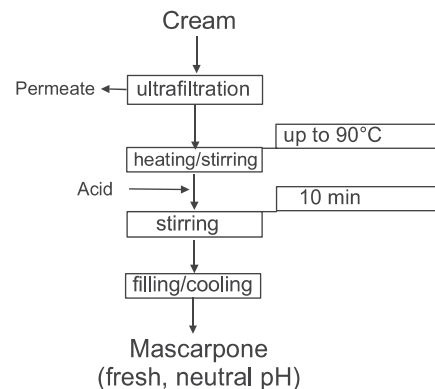
Ultrafiltration may be integrated into the cheese-making process either for “partial” or for “full” concentration. In the latter application curd cutting and whey drainage are entirely eliminated and 100 % of the whey proteins of milk are retained in the cheese matrix. When milk is ultrafiltered, the fat globules are also recovered in addition to the caseins and whey proteins. An example serves to demonstrate how ultrafiltration technology can be applied in order to improve the quality of milk products. Ultrafiltration enables the concentration of whole milk as well as cream and the standardization of the cheese milk prior to cheese-making [14]. The process for a modern Mascarpone production is schematically demonstrated in Fig.3 as an example.

Whey drainage is totally eliminated in comparison to the traditional process in Fig. 1 when ultrafiltration is integrated prior to heating and direct acidification. The separated permeate contains lactose and milk salts, which are further concentrated by nanofiltration and/or reverse osmosis, purified and applied for dietary supplements or pharmacy. Most of the milk constituents, especially the valuable whey proteins, are recovered in the cheese matrix. Besides the increase of the nutrient value the hygienic status of the Mascarpone production is enhanced by elimination of the drainage, which leads to a prolonged shelf-life.

The same procedure may be used for other cheese products. However, when applying membrane technology in order to recover most of the valuable constituents of raw milk also in other cheese types, the concentrated milk and the altered composition of the resulting cheese may influence ripening as well as the functional properties. Therefore, it is necessary to study traditional processes and, at the same time, the potential of new technologies. This enables the processing conditions to be adapted beyond the scope of high nutritional value and the flavor of cheese products depending on the technology applied.



**Fig. 2** Model for the casein network in cheese. **A** Rennet gel with immobilized fat globules. **B** Rennet gel with immobilized fat globules and whey protein particles (WPP)



**Fig. 3** Applying membrane technology for Mascarpone processing

## Cheese fortified with whey protein particles

When adding denatured whey proteins to cheese milk it was shown that these are mechanically retained in the rennet-induced gel network [15]. The addition of whey protein leads to an increased yield but may result in a slightly poorer quality in the cheese flavor and texture. An improved yield is attributed to both an increased retention of serum in the cheese matrix and the incorporation of whey proteins. In particular, denatured and highly hydrated whey proteins obstruct syneresis so that less water drains off during the cheese-making process [16]. By applying the usual technological countermeasures, such as intensified curd working, the water content may be reduced with beneficial influences on quality-relevant parameters.

Valuable whey proteins are retained preferentially in a denatured and more aggregated (particulated) state in a gel network. Particulation is a technology by means of which the whey proteins are denatured and aggregated by heating with simultaneous shearing into particles in a whey concentrate – the efficiency depends on the composition and process conditions, e.g. Simpless® [17, 18], Dairy-Lo™ [19] or is produced by other means [20–23]. Thus particulation technology takes up the proceedings during Ricotta processing because heat is also applied to coagulate the valuable whey proteins. Shearing only restricts the heat-induced growth into large whey grains.

Whey protein particles are inserted inertly into the pores of the casein network like fat globules (Fig. 2B) while added undenatured whey proteins as well as native whey proteins of milk become lost in the whey (Fig. 2A). The pore size of the network is indicated as approx. 10 µm [24, 25], which means that this is the critical diameter for added particles. Whey protein particles between 1 and 10 µm are integrated inertly into the structure; larger particles, however, disturb the homogeneity of the network and result in a reduced firmness [26].

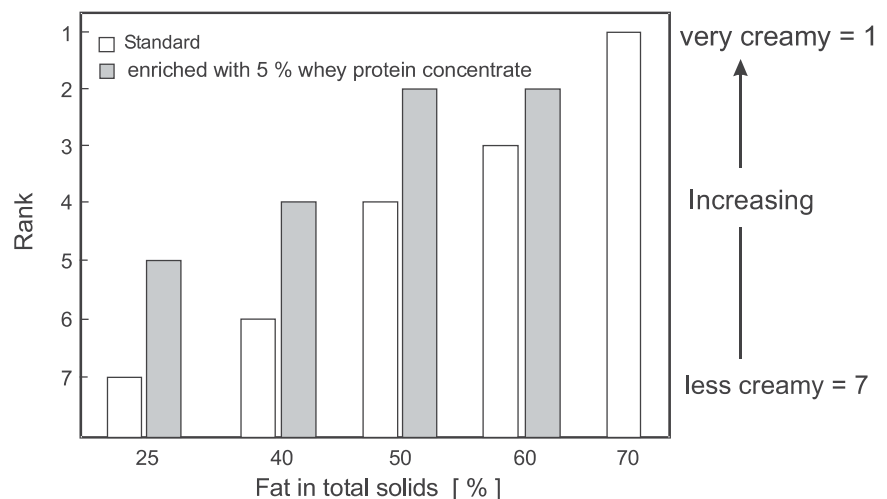
To conclude, whey protein particles are integrated into the cheese network just like fat globules. Now the question arises whether whey protein particles also influence the flavor in the same manner. Fig. 4 shows a sensory test on the creamy texture. The white columns represent standard soft cheese with varying fat contents in comparison with the gray columns for soft cheeses fortified with whey protein particles. The texture of the cheeses fortified with whey proteins was creamier than the control cheese with the same fat content. As a result, whey proteins act as efficient fat replacements in the cheese matrix, so that the quality of cheese, particularly with a low fat content, is improved [25] (Fig. 4, see 25 %). In addition, the nutritional value of the cheese is enhanced due to the integration of whey proteins, which are usually left over in the whey.

However, adjustments in cheese processing must be made when recycling whey proteins in the form of particles in order to lower the water content, on the one hand, and to remove milk constituents, which are additionally recycled with the whey protein particles such as lactose, from the cheese, on the other hand [27, 28]. Therefore, the extent of the recycling of whey protein particles is restricted depending on e.g. the moisture specification of individual cheese types and the texture. In this manner, high quality cheeses may be produced with fortified whey proteins.

## Conclusion

Mediterranean milk products are quite comparable to German ones regarding their composition and manufacturing technology. In addition there are some special products, the technology of which is already known, which are produced and marketed in Germany. The new approach to supply whey proteins originating in milk to cheese by applying suitable technology makes German

**Fig. 4** Ranking of soft cheese samples with varying fat contents fortified with whey protein particles in comparison to standard cheese [25]





milk products again more valuable for human nutrition.

In traditional cheese-making, casein forms the curd structure while the whey proteins are lost in the whey. In order to use the nutritionally valuable whey proteins, several attempts have been made to recover or to re-integrate them into the cheese matrix. Whey proteins are retained by ultrafiltration in order to reduce the aqueous phase before cheese-manufacturing. Alternatively, whey proteins may be restrained and recovered from drained whey by ultrafiltration so that after special treatment (i. e. particulation) they are added to the curd or recycled into the cheese milk. Meanwhile, several advanced processes, depending on the type of cheese, are being established in dairies.

The advantages of incorporating whey protein into cheese are a higher nutritional value and, especially in the case of low fat cheese, sensory improvement. Both result in a higher rate of transformation of the valuable components of milk into cheese compared to traditional cheese-making. But in order to benefit from the incorporation of the whey protein, cheese-making conditions require adaptations to suit the composition of the cheese milk and the requirements of the product in order to produce a high quality cheese. It seems likely that the nutritional value of milk products will be further improved by connecting traditional knowledge to new technologies.

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