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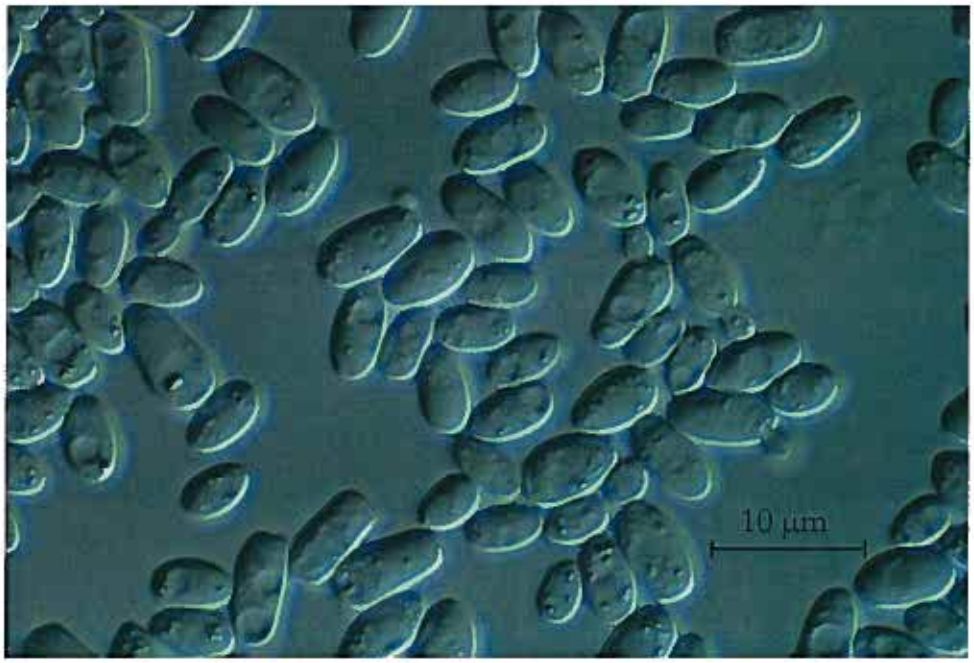

Yeasts in Dairy Products

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Publisher:
FAM
Swiss Federal Dairy Research Station
Liebefeld
CH-3003 Berne
Telefon +41 (0)31 323 84 18
Fax +41 (0)31 323 82 27
http://www.admin.ch/sar/fam
e-mail: info@fam.admin.ch

Author:
Marie-Therese Wyder

Address for queries:
M.-T. Wyder
e-mail marie-therese.wyder@fam.admin.ch
Tel +41 (0)31 323 82 23
Fax +41 (0)31 323 82 27

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Yeasts in Dairy Products

Marie-Therese Wyder
Swiss Federal Dairy Research Station
Liebefeld, CH-3003 Berne

1 Yeasts in general terms

Traditionally, and also from an economic point of view, yeasts are the most important microorganisms ever exploited by man. Yeasts have been used for several thousand years for the production of a wide range of food such as bread, wine, beer, kefyr, as well as for the production of ethanol for fuel, of biochemicals for the pharmaceutical industry and of many other substances. Originally, yeasts present on fruits, vegetables, all kinds of biological raw material, equipment and in homemade starters were responsible for spontaneous fermentation. It was only with the development of a technique to isolate pure cultures on solid media by Robert Koch that it became possible to select yeast strains on the basis of their fermentation characteristics [95]. However, yeasts also play an important role as spoilage organisms in foods and beverages because of their ability to grow at low temperatures and pH values, their resistance to physicochemical stresses and their metabolic activities [54]. Also, infections may arise from pathogenic yeasts, but they are usually not transmitted through food [35].
In 1680, the existence of yeasts was discovered by the Dutch scientist Antonie van Leeuwenhoek. During the second half of the nineteenth century, the French biochemist Louis Pasteur showed that yeasts were responsible for the conversion of sugar to mainly ethanol and carbon dioxide [95]. Yeasts may be defined as unicellular fungi reproducing by budding or fission [67]. Some authors regard yeasts merely as fungi that produce unicellular growth, but that are otherwise not different from filamentous fungi. Consequently, yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission and are capable of forming sexual states which are not enclosed in a fruiting body [8]. Organisms for which no sexual stage is known, are included into the deuteromycetes or fungi imperfecti [6]. At present, approximately 700 yeast species are recognised but only a few are commonly used.

2 Kefyr

2.1 The History of Kefyr

Kefyr is an acidic, mildly alcoholic and very ancient fermented milk beverage originating from the northern slopes of Caucasus, more specifically, from the village of Karatschajeff (2'500 m) at the foot of Elbrus (5'600 m) [22, 63, 66, 107]. The root of the name „Kefyr" can be referred to the Turkish word „kef" meaning pleasant, delightful, well-being, making drunken, fermenting, or to the word „kiaf" meaning froth, or to the Caucasian word „kefy" meaning best quality [48, 63, 116]. All these different meanings reveal a distinctive feature of kefyr i.e. it undergoes both a lactic acid and an alcoholic fermentation, latter due to yeasts. The altitude of the region of origin, and, therefore, the rather low temperatures, led to a selection of mesophilic microorganisms [66, 117].
For a long time, the manufacture of kefyr was known only to members of the

Ossete and Karabbiner tribes. They prepared kefyr from either cow, sheep or goat milk in bags of goat hides. In daytime, due to the rather cold climate, the sacks were subjected to sunlight and at night, they were taken into the house and hung at the door. Every person who passed by, had to kick the sack in order to mix the content. Fresh milk was added when some of the fermented milk was removed, providing a continuos natural fermentation [22, 23, 66, 85]. Depending on the outside temperature, the product could be quite different. Low temperature led to a rather high concentration of ethanol (up to 1 %) and carbon dioxide, whereas an elevated temperature to a more acidic product [117].
The actual starter culture of kefyr are the kefyr grains. But until this day, nobody really knows where and how these grains appeared. However, legends and presumptions are the only source for an explanation of their formation.
Kuntze [69] and Duitschaever et al. [23] refer to the above described manufacturing procedure of kefyr. During the ongoing spontaneous fermentation of kefyr, cauliflower-like aggregates were formed, consisting of a matrix of polysaccharides and coagulated proteins, in which a variety of microorganisms was embedded.
Skolotowski [110] reported on a saga of the Caucasian people. The grains were told to originate from another fermented milk, called „Ayran" which is similar to kefyr. Ayran is made by natural souring of the milk in oak vats, or sacks of goat hides, with pieces of either calf's or camel's stomach. The grains were collected from the walls of the vats and then added directly to fresh milk. The new sour milk, kefyr, was found to be much more pleasant than ayran. According to another story, the grains were found by shepherds in the bushes of the high mountains as a gift from heaven [69]. The most known but also most legendary explanation for the origin of the grains, also called „grains of the prophet", is reported by Podwyssozki [94] and Koroleva [66]. Allah himself gave the first grains to a chosen tribe, so to speak as a symbol of immortality. According to another version, Mohammed was the bearer of the grains who also told the people how the grains are to be used. He strictly forbade the secret of kefyr preparation or the grains to be given away. Otherwise, if unbelievers got hold of the grains, these would lose their magic and healing power. This legend explains why the method of kefyr preparation has been kept secret for so long. And still, until now nobody has ever been able to disclose the secret of the formation of kefyr grains.
The first one to report publicly on the beneficial effects of kefyr in the treatment of intestinal and stomach diseases was Dr. G. Dzhogan in 1867 [116]. This was the end of the secrecy of kefyr and the start of its spreading through Europe. The owner of the Moscow Dairy got the idea to produce kefyr on an industrial scale. To obtain the grains, he sent a beautiful woman, one of his workers, to the Caucasian tribes. She was kindly received by their prince but did not get the grains. On her way back, she was kidnapped by the mountain people to become the prince's wife. The woman was then set free by the gendarmes and as a compensation, the prince had to give her 10 pounds of „Mohammed grains" [66]. This is the story of how the grains started to move westwards. In the former USSR and in Bavaria, kefyr started to be produced on an industrial scale in the 1930's [130].

2.2 The Kefyr Grain

Kefyr grains are characterised by an irregular form, a white or slightly yellow colour and by its elastic consistency. The various types of grains can range from flat sheets to scrolls and rolls, to the cauliflower floret forms and finally to millet-like grains [79]. They are the result of a strong and specific symbiotic relation of microorganisms and grow as biologically 'independent units'. In spite of much effort, all attempts to obtain new kefyr grains by various combinations of microorganisms isolated from them have failed so far [9, 65].
The following microorganisms may be

part of the basic symbiotic microflora: mesophilic homofermentative lactic acid streptococci (*Streptococcus lactis, Str. cremoris, Str. diacetylactis*), lactobacilli (*Lactobacillus brevis, L. casei, L. delbrueckii, L. helveticus, L. acidophilus, L. kefir*), mesophilic heterofermentative LAB (*Leuconostoc mesenteroides*), yeasts (*Kluyveromyces marxianus, Torulaspora delbrueckii, Saccharomyces cerevisiae, Candida kefir*) and acetic acid bacteria (*Acetobacter aceti, A. pasteurianus*) [65, 116]. Lactobacilli were found in the grain in a concentration of $10^9 - 10^{10}$ CFU/g, leuconostoc in a concentration of 10^7 CFU/g. For yeasts the detected counts were $10^6 - 10^8$ CFU/g and for acetic acid bacteria $10^2 - 10^8$ CFU/g [49, 60, 88, 105]. Only streptococci were not always detectable in the grain [25, 51, 60, 85]. The microorganisms are embedded in a fibrous grain matrix consisting of coagulated casein, polysaccharides, fat and lysed cells (Fig. 1). Investigations using scanning electron as well as optical microscopy revealed a specific distribution of the microorganisms in the grain which, however, can differ strongly. Rosi [105], Bottazi and Bianchi [9] and Bottazi et al. [10] observed the presence of yeasts particularly in the centre but also along the peripheral channels. This could not be confirmed by Molska et al. [85] who found that yeasts in the inside were not as common as in the periphery. Mann [77] and Koroleva [65] reported on the dominance of non lactose fermenting yeasts in the centre of the grains, whereas the lactose positive species were located mostly on the surface, together with bacteria. Quite often lactobacilli were found associated with yeasts mostly on the surface, suggesting that they develop in micro-colonies building the grain mass [9, 71, 77, 85, 112]. Only Toba et al. [112] could not find any particular arrangement of microorganisms in the grain.
The total dry matter of the grain is about 10 % having the following composition: protein 30 – 34 %, fat 3 – 4 %, ash 7 – 12 % and polysaccharides 45 – 60 % [10]. La Rivière and Koolman [71] were the first ones who examined the composition,

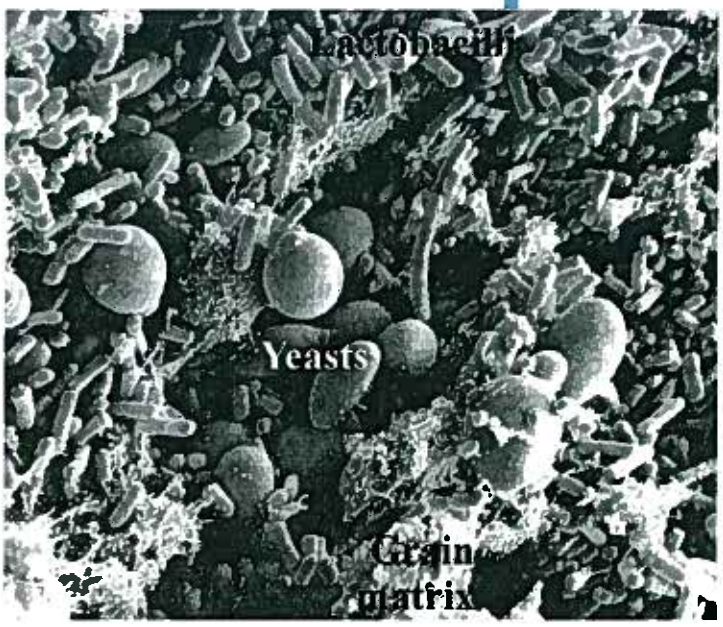

Fig. 1:
Scanning electron micrograph of a kefyr grain (intermediate zone)

properties and the origin of the polysaccharides. Acid hydrolysis of the polysaccharides yielded only D-glucose and D-galactose in approximately equal portions. The specific optical rotation was + 65° ± 4°. No other polysaccharide was known to have the same characteristics, therefore, the new polysaccharide was designated as „kefiran". Also, La Rivière and Koolman [71] were able to isolate *L. brevis* as the responsible strain for the production of kefiran. *L. brevis* produced kefiran as capsular material only in presence of the lactose negative yeast species *S. delbrueckii*. Kefiran is soluble in hot but insoluble in cold water, has constant viscosity over a wide pH range and cannot be hydrolysed by enzymes [51]. All these properties are essential for kefyr grains to maintain their particular form through repetitive fermentation cycles. Further examinations of the grain by Hirota [51] and Kandler and Kunath [51, 60] revealed that the predominant lactobacillus was *L. kefir* (formerly *L. brevis*), but it was not assumed to be responsible for kefiran production, this in contrast to Hosono et al. [52] and Pintado et al. [93] who claimed that *L. kefir* is the responsible strain for capsular kefiran production.

Pidoux et al. [92] reidentified *L. kefir* as *L. hilgardii* which produces kefiran, a gelling dextran. Finally, Fujisawa et al. [37] and Toba et al. [112] isolated also an encapsulated lactobacillus and proposed to name it *L. kefiranofaciens*. Thus, it still remains undecided which microorganism is responsible for kefiran production.

2.3 The Kefyr

In the preparation of kefyr the two phases, fermentation and ripening, can be distinguished. Fermentation is generally done at 18 – 22 °C during 18 – 20 hours, lower temperatures favour yeasts and higher temperatures LAB and, thus, the acidification process [10, 62, 82, 130]. The quantity of inoculated kefyr grains (2 – 5 %) has also an effect on the fermentation. Large inoculum shortens the fermentation pro-cess due to a rapid accumulation of lactic acid and results in low content of streptococci and yeasts at the end of fermentation. Low quantity of grains leads to an increasing number of the major groups of microorganisms. Stirring the inoculated milk during fermentation results in increasing numbers of streptococci, yeasts and of acetic acid bacteria if present. And finally, washing of the grains prior to inoculation results in a decrease of the main microorganisms groups and, consequently, to a longer fermentation time [65]. Even though streptococci cannot be detected microscopically in the milk after inoculation with kefyr grains, they provide rapid acidity development during the first hours of fermentation and are found to dominate in the end. After subsequent subculturing of kefyr starter without grains, lactobacilli and yeasts tend to disappear and streptococci become dominant [25, 49].
After separating the grains from the kefyr, ripening is performed at a temperature of 8 – 10 °C for 1 – 3 days. During this phase, the concentration of ethanol and other flavour components increase due to the fermenting activity of yeasts [29, 39, 62, 65].
According to Koroleva [65], a properly prepared kefyr should have the following composition of microorganisms:

- homofermentative mesophilic lactic acidstreptococci 10^8 – 10^9 CFU/mL
- thermophilic lactobacilli 10^5 CFU/mL
- heterofermentative lactic acid strepto cocci 10^7 – 10^8 CFU/mL
- yeasts 10^5 – 10^6 CFU/mL
- acetic acid bacteria 10^5 – 10^6 CFU/mL

The pH of the fermented milk prepared with kefyr grains is around 4.6 – 4.3, the lactic acid content can vary between 8 – 11 g/L and the ethanol content between 0.1 – 5 g/L [10, 49]. The carbon dioxide content which is formed during alcoholic fermentation and which is responsible for the prickly taste of kefyr, was reported to be around 1.33 g/L [16].
For the production of kefyr on an industrial scale, grains are applied, if ever, only to prepare the starter culture which is then used for the inoculation of milk for kefyr production. This method has a few disadvantages such as a large amount of grains is needed, the fermentation procedure becomes time consuming, and the composition of the microflora in the product is varying [22]. Therefore, kefyr grains are usually replaced by starters composed of pure microorganisms isolated from grains. Such cultures can be prepared as freeze-dried starters. The quality and the taste of the resulting kefyr product are found to be uniform [78, 90, 130]. Other procedures are based on two fermentation stages. The lactic acid fermentation with lactobacilli, leuconostoc and streptococci is performed at 25 – 32 °C until the pH is lowered to 4.7 – 4.4. The yeasts are incubated either separately in milk and then added to the sour milk, or they are added directly to the fermented milk and then incubated at 10 °C for 24 hours [10, 63] . This method results in a product of good quality and flavour [23, 24].

Investigations have shown that the composition of commercial kefyr can vary to a great extent. Lactobacilli were found either in counts of up to 10^5 CFU/g, or else they were absent. Yeasts were detected in a range of 0 – 10^8 CFU/g

and ethanol is usually found in a concentration of 0 – 0.4 %. Thus, it is evident that the flavour strongly depends on the manufacturing procedure [29, 31, 41, 63, 118].

Many attempts have already been done to make kefyr on an industrial scale using pure cultures of microorganisms [10, 24, 65]. However, other starters than grain itself always resulted in a completely diffe-rent final product. Lactobacilli and yeasts tend to disappear whereas streptococci become dominant [29].

2.4 The Yeast Flora of Kefyr

The sharp acid and yeasty flavour together with the prickling sensation contributed by the carbon dioxide can be considered as the typical kefyr flavour [23]. The yeasts play a leading role in the development of the characteristic taste and aroma because of their ability to ferment carbon sources releasing ethanol and carbon dioxide [65]. However, to obtain best flavour, the count of yeasts should reach at least $10^3 – 10^5$ CFU/mL kefyr [21, 40]. Also, the flavour characteristics are very much determined by the yeast species present in kefyr [29, 30, 78, 101].
Several working groups reported on the yeast count in grains and in kefyr obtained with them, as well as in commercial kefyr products. The microbial counts in grain depended strongly on the applied method of determination. By direct microscopic counting, 10^8 yeasts/g grain were detected, whereas by plate count only $10^6 – 10^7$ CFU/g [16, 60, 105]. After adding the grains to milk and stirring, a number of 10^5 CFU/mL milk was found [60]. In kefyr obtained with grains, the amount of yeasts was very similar to that in the grain itself i.e. $10^5 – 10^7$ CFU/mL [16, 21, 29, 60, 68, 105]. In the fermented milk made with kefyr i.e. without grains, the yeast count was 10^5 CFU/mL [68]. Commercial kefyr samples differed strongly from traditionally prepared kefyr. Many samples contained no yeasts at all, in others again the count reached up to 10^6 CFU/mL [21]. Manufacturers usually try to keep the yeast number as low as possible to avoid blowing of the packages [38]. In addition, there are no compelling regulations on the composition of the kefyr microflora except for the IDF Standard which proposes a minimal yeast count of 10^4 CFU/g in kefyr [53].
An often discussed question is whether all yeasts found belong to the specific kefyr yeast flora and if not, which yeasts must be considered as contaminants. Quite often, yeasts found in kefyr are the same as those species causing spoilage in other milk products [39]. Some authors claim that only lactose fermenting yeasts should be considered as specific for the kefyr flora because of their leading role in the alcoholic fermentation [41, 118]. Nevertheless, a high percentage of the yeasts found in kefyr are lactose negative [21, 31, 53, 87, 97, 98].
The first one to examine the microbial flora of kefyr grains was Kern [61] who showed that a symbiosis between a yeast and a bacterium existed. The yeast was *Saccharomyces cerevisiae*, a non lactose fermenting species. After him, other investigations followed. Tab. 1 shows the yeasts which since have been isolated from kefyr grains, and Tab. 2 the frequency in which yeasts were isolated from kefyr grains or kefyr products.
The role of yeasts is not only limited to their contribution to kefyr flavour. For example La Rivière [70] reports that appreciable growth of *L. brevis* occurred only in presence of a yeast. Therefore, yeasts also promote symbiosis among microorganisms by providing LAB with growth stimulants. On the other hand, LAB produce β-galactosidase which splits lactose into glucose and galactose. Nearly all the yeasts are able to utilise either glucose or galactose or both [21, 29, 64, 65, 93, 105].

Tab. 1:
Yeast species isolated from kefyr and kefyr grains

Yeast species	New nomenclature [67]	Reference
K. bulgaricus	*K. marxianus* var. *bulgaricus*	[98]
K. fragilis	*K. marxianus* var. *marxianus*	[16, 21]
K. lactis	*K. marxianus* var. *lactis*	[97, 101]
K. marxianus	*K. marxianus* var. *marxianus*	[21, 31, 53, 87, 88, 97, 98, 101]
S. carlsbergensis	*S. cerevisiae*	[51, 82, 97]
S. cerevisiae	*S. cerevisiae*	[21, 23, 31, 51, 53, 61, 64, 87, 98, 105]
S. delbrueckii	*T. delbrueckii*	[21, 93, 98]
S. exiguus	*S. exiguus*	[53, 79]
S. fragilis	*K. marxianus var. bulgaricus*	[51, 70, 98]
S. florentinus	*Zygos. florentinus*	[82, 117]
S. italicus	*S. cerevisiae*	[51]
S. kefir	*K. marxianus var. marxianus*	[98]
S. lactis	*K. marxianus var. lactis*	[51, 88]
S. unisporus	*S. unisporus*	[21, 31, 53, 93, 97, 101]
C. holmii	*C. holmii*	[31]
C. kefir	*C. kefir*	[21, 31, 63, 79, 87, 97, 98, 117, 129]
C. lambica	*C. lambica*	[21]
C. lipolytica	*C. lipolytica*	[21]
C. pseudotropicalis	*C. kefir*	[21, 51, 70, 79, 88, 98, 117]
C. tenuis	*C. tenuis*	[30, 88, 97]
C. valida	*C. valida*	[97]
T. delbrueckii	*T. delbrueckii*	[21, 70, 71, 87, 93, 98, 105]
Tor. holmii	*C. holmii*	[70, 129]
Tor. kefir	*C. kefir*	[82, 98]
B. anomalus	*B. anomalus*	[97]
G. candidum	*G. candidum*	[66, 71, 98, 117]
I. occidentalis	*I. occidentalis*	[31]
P. fermentans	*P. fermentans*	[54, 101]
Y. lipolytica	*Y. lipolytica*	[101]

B = Brettanomyces; C = Candida; D = Debaryomyces; G = Geotrichum; I = Issatchenkia; K = Kluyveromyces; P = Pichia; S = Saccharomyces; T = Torulaspora; Tor = Torulopsis; Y = Yarrowia; Zygos = Zygosaccharomyces

3 Cheese

To make cheese, milk from domestic animals is transformed into a coagulum by the action of rennet and of LAB. Then, water is expelled by physical and microbial interactions in order to concentrate selectively casein and fat. During the ripening period, casein, fat and carbon sources are metabolised in a complex process by enzymes of the starter culture microorganisms. The endproduct is a cheese with characteristic flavour, taste, consistency and shape.
According to their consistency, cheeses have been classified into extra-hard, hard, semi-hard, semi-soft, soft and fresh cheeses [12]. Cheeses may also be grouped by the raw material, fat content, the exterior and so on.

3.1 Brief History

The rich and fertile agricultural area situated between the rivers Euphrates and Tigris in Iraq is known to be the cradle of civilisation. The staple foods were mainly bread and cheese. In an archaeological survey, remnants of material found were proved to have been cheese made either from the milk of cows or goats. From carvings and other findings it is also assumed that milk was stored in skin bags where a fermentation process took place. Most probably either yoghurt, laban, koumiss or kefyr was produced, or the whey was drained off through a cloth or a perforated bowl and the solid curd then salted. The whey was usually used as a refreshing drink. The early coagulants for milk which were applied in addi-

Yeast species*	Utilisation of carbon sources					Frequency of mentioning in literature **
	Lactose		Galactose		Lactic acid	
	A	F	A	F	A	
C. kefir [1)]	+	+,–	+	+	+	28
S. cerevisiae	–	–	+,–	+,–	+,–	27
K. marxianus var. *marxianus* [1)]	+	+,–	+	+	+	21
T. delbrueckii	–	–	+,–	+,–	+,–	14
S. unisporus	–	–	+	+	–	11
K. marxianus var. *lactis*	+	+	+	+	+	9
K. marxianus var. *bulgaricus*	+	+,–	+	+		7
C. holmii [2)]	–	–	+	+	+,–	6
G. candidum	–	–	+	–	+,–	6
C. tenuis	+,–	–	+	+	+,–	4
C. valida [5)]	–	–	–	–	+,–	4
P. fermentans [3)]	–	–	–	–	+	4
P. membranaefaciens [5)]	–	–	–	–	+,–	4
B. anomalus	+	+	+	+	+,–	3
S. exiguus [2)]	–	–	+	+	–,d	3
Zygos. florentinus	–	–	+(–)	+,–	–	3
C. lambica [3)]	–	–	–	–	+	2
C. lipolytica [4)]	–	–	–(+)	–	+,d	1
D. hansenii	+,–	–	+	–,d	+,–	1
D. polymorphus	+,–	–	–	+	–	1
I. occidentalis	–	–	–	–	+	1
S. servazii	–	–	+	+	–	1
Y. lipolytica [4)]	–	–	–(+)	–	+,d	1

A = Assimilation; F = Fermentation; + = reaction positive; – = reaction negative; +,– = reaction variable; +(–) = reaction positive, seldom negative and vice versa; d = reaction delayed positive; [1)] = imperfect state of [1)]; * New nomenclature; ** from references in Tab. 1

Tab. 2:
Utilisation of carbon compounds by yeast species isolated from kefyr and kefyr grains [4, 67]

tion to the fermentation process, were the juice of fig tree, vinegar and milk clotting enzymes from the stomach of hare or kid. The first written references on cheese can be found in the bible, later Homer, Herodotus and others also referred to cheese [108].
The spread of cheese-making probably followed the pathways of bread. This geographical migration resulted, as expected, in new varieties of cheese. At present, literature reveals about 2'000 names applied to cheese.

3.2 The Yeast Flora of Cheese

Quite a large number of cheese varieties are characterised by the development of a specific surface microflora which is generally composed of moulds, yeasts, micrococci and coryneform bacteria. Yeasts, therefore, are frequently found within the microflora of many cheese types. Their occurrence is not unexpected because of their tolerance towards low pH and moisture, elevated salt concentration and low storage temperatures [35]. Also, they are widely dispersed in the dairy environment and appear as natural contaminants in the raw milk, the air, the dairy utensils, the brine and smear water [119 – 121]. The brine being one of the most important sources of contamination may be carrier of several yeasts species such as *Debaryomyces hansenii, Candida versatilis, Kluyveromyces marxianus, Saccharomyces cerevisiae, Torulaspora delbrückii, Trichosporon beigelii* and *Yarrowia lipolytica* [7, 109]. In the raw milk, following species were found: *D. hansenii, Clavsispora lusitaniae, Tr. beigelii, Rh. mucilaginosa* and *K. marxianus* [50]. The utilisation of lactic acid and the formation of alkaline metabolites by yeasts lead to an increase of the pH value which enables the growth of less acid tole-rant microorganisms such as the micrococci and coryneform bacteria [28].

In the first few days of the ripening period, the yeast count on the surface of the cheese increases very rapidly until it reaches a maximum after 10 days [19].The numbers can increase to 10^6 - 10^9 CFU/g [75, 102] or 10^7 - 10^8 CFU/cm^2 [28, 127]. In the following, the population remains at a nearly constant level and decreases only slightly to a final number of about 10^7 CFU/g [75]. In the interior of soft cheeses, there is an almost parallel development of the yeast population but at a 100 or even 10'000fold lower magnitude [20, 27, 74, 127]. In general, higher numbers are present in soft and blue-veined cheeses [13, 35].
Investigations of the yeast flora composition reveal a large diversity with more than 10 species among which *Kluyveromyces lactis, K. marxianus, Debaryomyces hansenii, Saccharomyces cerevisiae, Yarrowia lipolytica, Trichosporon cutaneum (beigelii), Rhodotorula mucilaginosa, Torulaspora delbrueckii* are the most frequent [13, 15, 19, 57 – 59, 74 – 76, 86, 100]. In the following tables Tab. 3 and Tab. 4, the yeast species isolated from different cheese types such as Cheddar, Gouda, Danablu, Roquefort, Tilsit, Tête de Moine, Gruyère, Münster, Brie, Camembert and many others are listed.

The composition of the yeast flora in young cheese seems to be rather heterogeneous and depends strongly on the cheese plant it has been produced [28]. In the cheese prior to brining, lactose positive species such as *K. lactis* and *K. marxianus* and *Torulaspora delbrückii* are predominant. These species are most probably also contributing to the characteristic open texture in blue-veined cheeses [35, 76] .
The technology of cheese-ripening also has an impact on the yeast flora composition. The typical yeast flora of mould-ripened cheeses seems to be mainly composed of *D. hansenii* and *G. candidum,* as well as of *K. marxianus* and *Y. lipolytica*, of smear-ripened cheeses mainly of *D. hansenii,* but also *Y. lipolytica* and *G. candidum* may be found, in blue-veined cheeses mainly of *D. hansenii* and *K. marxianus*, of acid-curd cheeses mainly of *D. hansenii, K. marxianus* and *G. candidum* and in fresh cheeses mainly of *K. marxianus* and *C. zeylanoides* [5, 27, 96, 113].

3.3 The Role of Yeasts during Cheese Ripening

The major recognised action is the metabolism of lactic acid with consequent increase in pH values. This promotes the growth and action of cheese-ripening microorganisms sensitive to acid such as *Brevibacterium linens.* In addition, LAB show a higher survival rate which accelerates proteolysis, and consequently the ripening process [20, 35, 73].
As already mentioned above, the lactose fermenting species as for example *K. marxianus* are contributing to the open structure of mainly blue-veined cheeses. Their ability to ferment lactose results in the formation of carbon dioxide but also in flavouring compounds such as ethanol and acetaldehyde [20, 75]. However, it should be considered that there is a risk of to much openness and of a yeasty off-flavour if the count of yeasts exceeds a certain level. The utilisation of lactose also limits the acidification by LAB and, thus, affects the texture of the cheese [74].
Furthermore, yeasts contribute to the maturation of cheese by their lipolytic activity. Among the yeasts from cheese, *Y. lipolytica* is recognised as the species having the greatest lipolytic activity [15]. It was possible to accelerate ripening and to improve quality of cheese by the addition of this yeast [20, 75]. Esterase activity seems to be common for many yeast isolates from cheese [89].
Many yeasts are carrier of proteolytic enzymes. Species with a high proteolytic activity are *K. lactis, K. fragilis, C. pseudotropicalis* and *D. hansenii* [26, 43]. *Y. lipolytica, G. candidum* and *C. catenulata* are species with a strong extracellular proteolytic and/or peptidolytic activity [3, 102, 103, 113]. Intracellular proteinases were

Tab. 3:
Yeast species isolated from cheese

Yeast species	New nomenclature [67]	Reference
K. marxianus	*K. marxianus* var. *marxianus*	[13, 15, 20, 27, 28, 35, 59, 74, 75, 100, 102, 109, 119-121, 127]
K. lactis	*K. marxianus* var. *lactis*	[5, 13, 15, 35, 57, 59, 74-76, 86]
K. bulgaricus	*K. marxianus* var. *bulgaricus*	[15, 74]
K. fragilis	*K. marxianus* var. *marxianus*	[35, 76]
D. hansenii	*D. hansenii*	[5, 13, 15, 20, 28, 35, 36, 57, 59, 74-76, 86, 100, 102, 109, 119-121, 127]
G. candidum	*G. candidum*	[13, 20, 28, 59, 74-75, 91, 100, 121, 127]
G. capitatum	*G. capitatum*	[15, 127]
S. cerevisiae	*S. cerevisiae*	[13, 15, 20, 27, 35, 57, 74, 75, 86, 100, 102, 119]
S. unisporus	*S. unisporus*	[13]
S. italicus	*S. cerevisiae*	[74]
S. exiguus	*S. exiguus*	[57]
S. fragilis	*K. marxianus* var. *bulgaricus*	[35]
S. lactis	*K. marxianus* var. *lactis*	[35, 76]
C. catenulata	*C. catenulata*	[13, 27, 28, 102, 113, 119]
C. famata	*C. famata*	[5, 13, 35, 76, 113]
C. intermedia	*C. intermedia*	[13, 15, 27, 28, 59, 102, 109]
C. kefyr	*C. kefyr*	[13, 102]
C. krusei	*C. krusei*	[13]
C. lipolytica	*C. lipolytica*	[13, 35, 59, 102]
C. pseudotropicalis	*C. kefyr*	[35, 74]
C. robusta	*S. cerevisiae*	[13, 86]
C. rugosa	*C. rugosa*	[13, 109]
C. sake	*C. sake*	[15, 121]
C. sphaerica	*C. sphaerica*	[5, 13, 86]
C. tenuis	*C. tenuis*	[109]
C. utilis	*C. utilis*	[1, 15]
C. versatilis	*C. versatilis*	[20, 75, 109]
C. zeylanoides	*C. zeylanoides*	[96]
Cl. lusitaniae	*C. lusitaniae*	[13, 57, 127]
Cr. albidus	*Cr. albidus*	[102, 119, 120]
H. anomala	*P. anomala*	[13, 15]
I. orientalis	*I. orientalis*	[27, 100, 109]
P. fermentans	*P. fermentans*	[13, 15, 35]
P. jadinii	*P. jadinii*	[127]
P. kluyveri	*P. kluyveri*	[15]
P. membranaefaciens	*P. membranaefaciens*	[13, 15, 35, 100]
P. pseudocactophila	*P. pseudocactophila*	[127]
Rh. glutinis	*Rh. glutinis*	[119, 120]
Rh. minuta	*Rh. minuta*	[27, 28, 120]
Rh. rubra	*Rh. rubra/mucilaginosa*	[57, 58, 127]
Tor. sphaerica	*K. marxianus* var. *lactis*	[15, 35, 74]
Tor. mogii	*Zygos. rouxii*	[74]
Tor. versatilis	*C. versatilis*	[15, 74]
T. delbrueckii	*T. delbrueckii*	[13, 27, 28, 119-121]
Tr. cutaneum	*Tr. cutaneum*	[57, 127]
Tr. beigelii	*Tr. beigelii/cutaneum*	[27, 28, 109]
Tr. pullulans	*Tr. pullulans*	[100]
W. californica	*W. californica*	[127]
Y. lipolytica	*Y. lipolytica*	[13, 15, 27, 28, 35, 36, 75, 76, 119-121, 127]
Zygos. rouxii	*Zygos. rouxii*	[15, 20, 74]

B = Brettanomyces; C = Candida; Cl = Clavispora; Cr = Cryptococcus; D = Debaryomyces; G = Geotrichum; I = Issatchenkia; K = Kluyveromyces; P = Pichia; Rh = Rhodotorula; S = Saccharomyces; T = Torulaspora; Tor = Torulopsis; Tr = Trichosporon; W = Williopsis; Y = Yarrowia; Zygos = Zygosaccharomyces

Tab. 4:
Utilisation of carbon compounds by yeast spezies isolated from cheese [4, 67]

Yeast species *	Utilisation of carbon sources						Frequency of mentioning in literature **
	Lactose		Galactose		Glucose	Lactic acid	
	A	F	A	F	F	A	
D. hansenii [1)]	–	–	+	–	+,–	+,–	15
K. marxianus var. *lactis*	+	+	+	+	+	+	15
K. marxianus var. *marxianus* [4)]	+	+,–	+	+	+	+	13
S. cerevisiae	–	–	+,–	+,–	+	+,–	13
G. candidum	–	–	+	–	–,w	+,–	10
C. intermedia	–	–	+	+,d	+	–	6
C. kefyr [4)]	+	+,–	+	+	+	+	6
Y. lipolytica [2)]	–	–	–,d	–	–	+,d	6
C. versatilis	d	d	+	+,d	+	+,–	5
C. famata [1)]	–	–	+	–	+,–	+,–	4
C. lipolytica [2)]	–	–	–,d	–	–	+,d	4
P. membranaefaciens [5)]	–	–	–	–	–,d	+,–	4
Zygos. rouxii	–	–	+,–	–	+	–	4
C. catenulata	–	–	+	–,d	+,–	+	3
K. marxianus var. *bulgaricus*	–	–	+,–	+,–	+		3
P. fermentans	–	–	–	–	+	+	3
Tr. beigelii/cutaneum	+	–	+	–	–	+	3
C. rugosa	–	–	+,d	–	–	+,d	2
C. utilis	–	–	–	–	+	+	2
I. orientalis [3)]	–	–	–	–	+	+	2
P. anomala	–	–	+,d	–,d	+	+	2
Rh. rubra	–	–	+,–	–	–	+,–	2
T. delbrueckii	–	–	+,–	+,–	+	+,–	2
C. krusei [3)]	–	–	+	+	+	–	1
C. valida [5)]	–	–	–	–	–,d	+,–	1

A = Assimilation; F = Fermentation; + = reaction positive; – = reaction negative; +,– = reaction variable; +(–) = reaction positive, seldom negative and vice versa; d = reaction delayed positive; w = reaction weak positive; [1)] = imperfect state of [1)]; * New nomenclature; ** from references in Tab. 3

detected in yeasts of the genera *Trichosporon* and *Debaryomyces*. The activity of these proteinases (pH_{opt} = 5.5 – 6; T_{opt} = 60 °C) is specific on caseins. Exopeptidases i.e. aminopeptidases and carboxypeptidases seem to play a major role in the proteolysis of milk proteins. The aminopeptidases with a pH optimum of 7.5 – 8 are present in nearly all the yeast species. The carboxy-peptidases have a pH optimum of 4. Most of them are located inside the cell [15, 19, 74, 75].
All intracellular enzymes would be much more significant to the cheese ripening process if released by cell lysis [34, 102, 129].
The enzymatic activity of yeasts may also play an important role in the breakdown of bitter peptides which are usually a result of an unbalanced activity of both proteinases and peptidases. By releasing smaller peptides and amino acids, the aminopeptidases and carboxypeptidases contribute essentially to the breakdown of bitter peptides [72, 75]. Especially *G. candidum* is known to show such an activity [3,19, 20].
Furthermore, synergistic effects of yeasts with LAB were observed with the result of a stronger proteolysis by *D. hansenii* [18, 34].
From the mentioned it can be concluded that yeasts are of importance in the maturation of cheese. However, only little is known about their specific proteolytic and lipolytic activity on milk proteins and fat. Further investigations on their physiological biochemical characteristics are needed in order to select relevant strains for starter cultures [13, 35, 75, 100].
In the following, four important species used for cheese production are described. The names in brackets indicate the imperfect form of the species.

3.3.1 Debaryomyces hansenii (Candida famata)

D. hansenii is one of the most prevalent yeast species in dairy products, especially on cheese surfaces. In Roquefort cheese, as an example, it is largely responsible for the formation of a slime on the surface. At equivalent a_w, it tolerates salt better than glucose [17]. Therefore, its high tolerance to salt is not surprising [104]. It shows a maximal growth rate between 25 and 30 °C but is also able to grow at 5 and up to 32 – 37 °C [4, 35].
Intracellular proteinases (pH_{opt} 5.8) which hydrolyse preferably caseins, and extracellular proteinases, as well as leucinaminopeptidases and carboxypeptidases could be detected [15, 19]. It has been demonstrated that the proteolytic activity of *D. hansenii* cultured in skim milk together with LAB was greater than the sum of their activities when cultured separately. *D. hansenii* also prolonged the survival of LAB in cheese [128]. However, it is not able to hydrolyse casein at ripening temperatures of 10 °C [114].
This yeast utilises aerobically and anaerobically lactic acid, preferably the L(+) isomer, as well as acetic acid. Thus, its role in de-acidifying the surface of cheese is apparent. The anaerobic pathway is much slower but there is still evidence that the reduction of the lactic and acetic acid concentration in cheese may inhibit the growth of *Clostridium tyrobutyricum* [33]. Furthermore, good growth reactions on citrate even in the presence of salt were observed [36, 104, 114].
Also, a higher amount of free fatty acids in cheese inoculated with *D. hansenii* could be detected [18], even though only little release of free fatty acids from butterfat at 10 °C was observed in another work [114]. In general, it was found that this yeast led to a more rapid proteolysis as well as overall ripening [18]. In cheese curd slurries, *D. hansenii* increased pH significantly and was proteolytic. It generated an alcoholic, acidic, fruity and also cheesy aroma [80, 126]. The species is a very heterogeneous yeast species which consists of several phenons [109, 127].

3.3.2 Yarrowia lipolytica (Candida lipolytica)

Interest in *Y. lipolytica* arose from its rather uncommon physiological characteristics. Strains were more often isolated from lipid- and protein-containing than from sugar-containing substrates, because it has a strong extracellular lipolytic and proteolytic activity [114]. Thus, they occur often in dairy products such as cheese, yoghurts, or salads containing meat or shrimps [123], as well as in spoiled food [17].
Y. lipolytica is strictly aerobe, it utilises lactic and citric acid [36,103]. A concentration of 1 % citric or lactic acid (pH 4.5) did not inhibit growth, whereas 1 % acetic acid was lethal [17]. It is able to release high amounts of formic acid [36]. This might be the reason, why in cheese curd slurries it did not affect pH at all [126]. Growth was observed at 5 – 10 °C, but the optimal temperature lays between 25 – 30 °C and the maximal temperature in the range of 33 – 37 °C [102].
Due to its strong extracellular enzymatic activity, *Y. lipolytica* is proposed for the production of a cheeseflavoured basis [11]. In cheese model systems, it exhibited a putrid, cabbage and strongly cheesy (Parmesan, Sbrinz, Munster), but not fruity aroma [80, 126]. In fact, as a consequence of using *Y. lipolytica* as an adjunct culture, it proved to affect flavour of cheese positively [47, 124]. It is thought to produce itself volatile flavour compounds such as methanthiol, dimethylsulfide and to enhance synthesis of aroma compounds by bacteria [80].

3.3.3 Pichia jadinii (Candida utilis)

This species is known for its strong fermentative ability (facultatively fermentative). Thus, the response of a glucose grown culture to oxygen limitation is alcoholic fermentation after a lag phase of about 1 h, during which glycerol, pyruvate

and D-lactate as the main fermentation products are formed [56]. It utilises lactate but not lactose. In contrary to the three other yeast species, growth is possible up to 44 °C [17]. Concerning its role in cheese production, it is added to mesophilic starters to enhance flavour development and improve texture [111]. Nevertheless, *C. utilis* was also found to cause blowing in young cheese [1]. Since it possesses high extra- and intracellular lipolytic activity, the yeast is utilised in the manufacture of raw-dried sausages to improve flavour development [83].

3.3.4 Galactomyces geotrichum (Geotrichum candidum)

G. candidum is usually mentioned separately when listing the yeast flora of cheese because its position in classification for a long time remained unclear. It was considered either as a yeast or a yeast-like fungus depending on the morphology of the colonies [20]. Two main biotypes may, therefore, be distinguished. One is characterised by clearly white strains, more or less felting, by a rapid growth and an optimal temperature of 25 – 30 °C, a strong proteolytic activity, the formation of a true mycelium and an alkalising action. The other type forms cream-coloured colonies and has a yeast-like appearance, only weak growth and proteolytic activity, an optimal temperature between 22 – 25 °C and an acidifying action [44, 45, 74]. However, now it is considered as a yeast.
Growth can be observed in the range of 5 – 38 °C with an optimum at around 25 °C and at pH 5 – 5.5. *G. candidum* is sensitive to salt and growth is limited at concentrations above 1 % [91]. The yeast produces extracellular lipases and proteinases, and two endopeptidases with a pH optimum of 5.5 – 6 [3, 75]. In the production of Camembert cheese, it has been shown that *G. candidum* decreases considerably bitterness by the breakdown of bitter peptides through aminopeptidase activity [84], as well as by the inhibition of *Penicillium* growth [115].

G. candidum strains are also able to deaminate glutamic and aspartic acid [42] as well as tryptophan, leucine, methionine and phenylalanine [46]. The catabolism of amino acids by *G. candidum* strains can produce alcohols and volatile sulphur compounds such as dimethyldisulfide, methanethiol and various S-methyl thioesters which are important for flavour development [55]. In cheese model systems, *G. candidum* yielded in fact cheesy, sulphur-like and alcoholic odours [80, 126].

4 Yeasts as Spoilage Organisms in Dairy Products

Characteristics such as the ability to grow at low pH, temperatures and water activities, at high salt concentrations and such as certain enzymatic activities make yeasts not only desirable for dairy products as we have seen it in the cases of cheese and kefyr. Yeasts may also cause spoilage in dairy products because of these very characteristics. The most common defects are gas production, thus blowing of packages, yeasty and other off-flavours, discolorations and changes of texture [54].
Yeasts and moulds are considered to be the most common spoilage organisms in fermented milks (yoghurt, quark) causing blowing of the packages and off-flavours [99]. Not only the lactose fermenting species *Kluyveromyces marxianus* is usually found, but also *Hansenula* sp. and *Saccharomyes cerevisiae*, *Pichia membranaefaciens*, *Candida guilliermondii* and *Geotrichum candidum*, some of them capable to ferment galactose [32]. Major contamination sources are the fruit bases used for yoghurt production. In this case, following yeast species may be isolated: *Candida magnoliae, C. parapsilosis, C. silvicola, C. valida, Zygosaccharomyces bailii, Metschnikowia pulcherrima* and *Issatchenkia orientalis* [81].
Similarly, yeasts are also encountered in the spoilage of soft and fresh cheese causing gassy and flavour defects. *Torulaspora delbrueckii, Candida parapsilosis,*

C. sake, Cryptococcus sp., *Debaryomyces hansenii, Kluyveromyces marxianus, Pichia fermentans, P. guilliermondii, P. membranaefaciens, P. norvegensis, Rhodotorula* sp and *Yarrowia lipolytica* were most commonly isolated [121, 122]. Yeast occurrence is usually due to recontamination from the production and packaging area.
In other cheese types with longer ripening periods, yeasts may be responsible for browning defects due to the activity of tyrosinase, an enzyme mainly produced by *Yarrowia lipolytica* [14, 106]. Even though the overall flavour and texture quality of the cheeses are not affected, the appearance, however, will not be appreciated by consumers.

5 Short Conclusion

As a conclusion it can be said, that the very same yeasts can play an important beneficial role such as ripening agents as well as a rather negative role in the spoilage of dairy products by means of their me-tabolic pathways. Even though in recent years, the interest in dairy yeasts has grown a lot, there is still only little knowledge especially on their beneficial contribution to the quality of dairy products as for example of cheese.

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