1 Production of Bread, Cheese and Meat

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I. Introduction

Historic references to the fermentation of dough for baking and the fermentation of beer originate from the Sumerians and the Babylonians and, under the Pharaohs in ancient Egypt, the brewing of beer was a trade (Jørgensen 1948). At that time, the fermentation of bread was achieved by using a mixture of yeast and lactic acid bacteria maintained in a dough medium. After each fermentation, a portion of the dough was retained for starting the next batch or a close connection with beer brewing was established so that surplus yeast from breweries was used for production of bread. These same methods are still used in certain regions in Africa and probably other parts of the world, where ancient technologies have survived and can be experienced today. In the industrialised part of the world, these methods remained in use and did not change until late in the eighteenth century when yeast was first propagated for direct use in bread making in the Netherlands by the so-called Dutch method, which had a very low efficiency. As a result of the work of Louis Pasteur and the Danish botanists Emil Christian Hansen and Alfred Jørgensen and others in the late nineteenth century, the role of oxygen in yeast propagation was realised, the anaerobic condition of fermentation ("life without oxygen") was understood, *Saccharomyces cerevisiae* was described and the use of pure cultures was introduced. This was a very significant breakthrough for the industrialised production of baker's yeast. A similar process improvement followed in 1920, with the introduction of the "fed-batch" process. In this process, sugar is fed incrementally during yeast propagation, avoiding repressions and leading to increased biomass production. It forms the basis of commercial processes used today for manufacturing baker's yeast and has developed into a highly centralised industry offering a cheap bulk commodity. This is contrary to the historical development of other industrial yeast cultures like brewer's yeast (Jørgensen 1948). For reviews on the history of baker's yeast, see Rose and Vijayalakshmi (1993) and Jenson (1998).

In cheese, the role of yeast is not yet fully understood, but the yeast seems to take part in several microbial interactions important for the fermentation and maturation process of several cheeses (Jakobsen and Narvhus 1995). The species of particular interest are *Debaryomyces hansenii* (anamorph: *Candida famata*), *Yarrowia lipolytica* (anamorph: *Candida lypolytica*), *Geotrichum candidum* (teleomoprph: *Galactomyces candidus*) and *S. cerevisiae*. Furthermore, for the filametous fungi, the use of *Penicillium roqueforti* and *P. camemberti* has a long history in cheese production. According

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to early records, names of blue and white mould cheeses are dated to the year 879 for Gorgonzola, 1070 for Roquefort, 1785 for Stilton and 1791 for Camembert (Robinson 1995). These cultures are important starter cultures, which are commercially available and widely used by the dairy industry. Their technological properties have been studied over a number of years and, although not fully understood, useful information has been collected as reviewed by Gripon (1993).

Compared with bread and cheese, meat is the least developed area concerning the use of yeast and filamentous fungi as starter cultures. Apart from a few examples of using *P. nalgiovense* for surface ripening of sausages and *D. hansenii* for fermentation of sausages and other meat products, limited information is available. The role of micro-organisms is unclear, but bacteria rather than fungi appear to be responsible for flavour development in fermented meat (Montel et al. 1998). However, several important meat products like Parma and Serrano hams are still spontaneously fermented and the possible role of yeasts and mycelial fungi has not been studied in detail. An increasing interest is seen in research work leading to the understanding of the role of micro-organisms in traditional spontaneously fermented meat products including the significance of yeast and mycelial fungi. For a review on fungal ripening of meat, see Cook (1995), Lücke (1998) and Sunesen and Stahnke (2003).

II. Bread

A. Baker's Yeast

Yeast-fermented breads in Europe and the United States are mostly based on wheat, although rye is commonly used for some popular bread types in Scandinavia and other northern European countries. A large variety of bread is produced, and the European tradition for consumption of bread seems to spread over the world, including regions like South-East Asia and Africa, as a result of the strong impact of European eating habits. Traditional bread making in Africa does not seem to be widely known, but strong traditions exist for fermentation of cereals, with yeast playing a significant role, especially in co-cultures with lactic acid bacteria, as was also the case in the past in Europe. For Sudan, 11 types of sorghum and millet bread are described by Dirar (1993) and in other parts of Africa various cereal dough fermentations, like *kenkey* made from fermented maize dough in Ghana (Halm et al. 1993), play a substantial role in the daily food intake. A review on yeast in traditional African food is given by Jespersen (2003).

In most cases, the dominant yeast appears to be S. *cerevisiae* Meyen ex E.C. Hansen with the taxonomic delimitation given by Vaughan-Martini and Martini (1998). It is considered the principal species responsible for cereal fermentation and bread making as well as alcoholic fermentations, except for fermentation of lager beer.

Several methods based on molecular techniques have been reported for subspecies typing of *Saccharomyces* spp., one of the most popular methods has been determination of chromosome length polymorphism, e.g. by pulse field gel electrophoresis (PFGE) as done for S. *cerevisiae* isolates from spontaneously fermented maize dough in Ghana by Hayford and Jespersen (1999).

As chromosome length polymorphism is evident among the isolates, the technique clearly shows that several subspecies are involved in the fermentation process. Further, the observed chromosome profiles are quite similar to chromosome profiles observed for baker's yeast. According to general experience, the genomic stability of individual strains of baker's yeast appears to be high (Gasent-Ramirez et a1. 1999).

Other molecular methods which seem to be well accepted for species recognition and clarification of phylogenetic relationships are based on sequencing of rDNA, such as the ribosomal internal transcribed spacer (ITS) region (Montrocher et a1. 1998), or sequencing of the Dl/D2 domain of the large subunit (26S) rDNA (Kurtzman and Robnett 1998). Functional genomics and proteomics are also valuable tools in clarification of strain differentiation and elucidation of the relationship between geno- and phenotypes (Garrels et a1. 1997; Joubert et a1. 2000).

Mixed cultures of yeast species may occur in bakeries depending on the type of flour used and other conditions employed (Jenson 1998; Hammes et al. 2005). Other than *S. cerevisiae* species are of interest, because they may be better suited for applications where *S. cerevisiae* cannot meet desired technological properties, like resistance to osmotic stress, freezing and thawing (Jenson 1998; Hammes et al. 2005).

An alternative baker's yeast could be *Kazachstania exigua* (previously *Saccharomyces exiguus*) Rees ex E.C. Hansen or its anamorph form *Candida holmii* (Jørgensen), according to several investigations as reviewed by Jenson

(1998). Another candidate is *Torulaspora delbrueckii* (Lindner) and its anamorph form *Candida colliculosa* (Hartmann) because of its high osmotolerance and resistance to freezing (Oda and Tonomura 1993a, b). *Candida krusei* and its teleomorph form *Issatchenkia orientalis* are often seen in microbial successions with S. *cerevisiae* in dough fermentations where it appears to take over when the conditions become too inhibitory, probably too acid, to S. *cerevisiae* (Hayford and Jakobsen 1999; Hayford and Jespersen 1999). Molecular typing has indicated that the strains observed are different from pathogenic strains of *C. krusei* (Hayford and Jakobsen 1999), a point which should also be considered when selecting new strains, although the yeast is supposed to be killed during baking. Other indigenous fermented cereals in Africa and in smaller traditional bakeries in Europe which still rely upon spontaneous dough fermentations may be considered as sources for alternative cultures or for cultures to be applied in mixed cultures with *S. cerevisiae*. The cultures may not only be selected to ensure efficient bread leavening, but also for the purpose of adding aroma characteristics to the bread. Tomer et a1. (1992) specifically investigated the effect on volatile compounds in fermentation studies with S. *cerevisiae* and *Candida guilliermondii* without specifying which of the two species varieties (var. *guilliermondii* or var. *membranifaciens*) was used. They concluded that S. *cerevisiae* produced the highest number of aroma components and, in general, the yeast examined produced more flavour components than the lactic acid bacteria investigated.

It should be mentioned that lactic acid bacteria are often found in high levels during bread fermentations and the microbial interactions between baker's yeast and these bacteria can be very important, in particular in sourdough breads, as reviewed by Hammes and Ganzle (1998) and Hammes et al. (2005). Recent studies have made use of culture independent molecular methods for identification of the microbiota of bread fermentations (Zannini et al. 2009) and yeast population dynamics (Meroth et al. 2003).

B. Technological Properties of Baker's Yeast

Fermentation of Dough Carbohydrates. The main technological properties of baker's yeast can be summarised as the efficient fermentation of dough carbohydrates and formation of CO_2, influence on dough structure, shelf life, aroma formation, osmotolerance, acid tolerance and freeze–thaw resistance.

Baker's yeast does not ferment starch. The starch of the dough will be broken down by the action of α- and β-amylases from the dough, leading to the formation of mainly glucose, maltose and maltotriose, which can all be fermented by the yeast, and higher carbohydrates (dextrins) which S. *cerevisiae* cannot ferment. Maltose is present at the highest level and the faster the yeast can ferment maltose, the faster the fermentation will occur. Fast fermentation is one of the most desired properties of baker's yeast.

Of the three major sugars, glucose is preferentially utilised by *S. cerevisiae* (Stewart et a1. 1979), but efficient fermentation requires the rapid utilisation of both maltose and maltotriose. Gene dosage studies performed with laboratory strains of yeast have shown that the transport of maltose into the cell may be the rate-limiting step in the utilisation of this sugar (Goldenthal et a1. 1987). Information on the maltose and maltotriose transporter genes present in brewer's yeast is therefore of some value in selecting suitable strains and in predicting fermentation performance.

Maltose utilisation in *S. cerevisiae* is conferred by anyone of five *MAL* loci, *MALI* to *MAL4* and *MAL6* (Vanoni et al. 1989). Each locus consists of three genes: gene 1 encodes a maltose transporter, gene 2 encodes a maltase (α-glucosidase) and gene 3 encodes a transcriptional activator of the other two genes. Thus, for example, the maltose transporter gene at the *MAL6* locus is designated *MAL61*. The five *MAL* loci each map to a different yeast chromosome, as follows: *MAL1*, chromosome VII; *MAL2*, chromosome III; *MAL3*, chromosome II; *MAL4*, chromosome XI; *MAL6*, chromosome VIII. The *MAL* loci exhibit a very high degree of homology and are telomere linked, suggesting that they evolved by translocation from telomeric regions of different chromosomes (Michels et al. 1992). Since a fully functional or partial allele of the *MAL1* locus is found in all strains of *S. cerevisiae*, this locus has been proposed as the progenitor of the other *MAL* loci (Chow et al. 1983; Jespersen et al. 1999).

Based on information about the regulation of maltose utilisation, recombinant DNA technology has been used for constructing strains which are not repressed by glucose and are constitutive in their maltose uptake (Osinga et al. 1989). Such recombinant yeast show rapid gas production from maltose in plain dough. Recent studies (Higgins et al. 1999) suggest that varying constitutive maltase and maltose permease levels in strains of *S. cerevisiae* are important targets for selection of strains with improved maltose utilisation.

If the amylase activity of the dough is not sufficient to provide enough fermentable carbohydrates, external enzymes or fermentable sugars, e.g. glucose, fructose or sucrose, can be added. Baker's yeast normally has invertase in excess to

hydrolyse the extra sucrose added. Wheat flour contains about 2% (w/w) glucose, fructose, sucrose and maltose. The wheat amylases or added external enzymes can increase the amount of maltose to about 3% (w/w).

The optimum fermentation temperature is about 40°C. With regard to bread quality, a temperature of 25°C and a fermentation time of several hours should be applied. To decrease production time, higher temperatures, e.g. 35°C, are often used. During the initial phases of baking, fermentation continues, but it stops at around 50°C when *S. cerevisiae* and some relevant enzymes are inactivated.

Gas Production and Influence on Dough Structure. The main influence of fermentation on dough structure appears to be explained by mechanical stretching and modification of the dough protein (gluten) caused by the CO_2 evolution (Reed and Nagodawithana 1991). This is obviously linked directly to the fermentation capacity of the yeast as well as the amount of yeast present and hence the amount of CO_2 formed.

Yeast excretions may also affect the structure of the dough. It has been reported that excreted glutathione and cysteine could affect protein disulphide bonds in gluten (Stear 1990). Yeast quality and hydrolysis of yeast with release of proteolytic enzymes may also influence dough structure. However, regardless of the possible influence of the yeast, the mechanical treatment of the dough, in particular in modern bakeries, has a major, and more likely dominant, effect.

Influence on Bread Flavour. In comparison with the role of the yeast strain used for the fermentation and formation of flavour compounds in beer and wine, the influence of baker's yeast on bread flavour appears to be limited (Rose and Vijayalakshmi 1993; Jenson 1998). Few studies on the flavour characteristics of baker's yeast have been published, and the search for strains with special flavour properties does not seem to be given much attention.

The flavour components produced by the yeast in bread making include organic acids, aldehydes, ethanol, higher alcohols, esters and ketones. In addition, the less well defined yeasty flavour is often noticeable. This may occur in particular in the case of active dry yeast where a relatively higher concentration of yeast dry matter may be applied. Trials have been reported showing that *S. cerevisiae* and *C. guilliermondii* produced larger amounts of volatile flavour components than the lactic acid bacteria investigated, *Lactobacillus brevis* and *Lactobacillus plantarum* (Tomer et al. 1992). The effects of stress exposure and interactions between yeast and lactobacilli on generation of aroma compounds in sourdough has been reported (Guerzoni et al. 2007)

C. Manufacture of Baker's Yeast

Baker's yeast is the oldest and still one of the most important products within biotechnology. Globally, the yearly production amounts to more than 2×10^6 t. The process has been thoroughly investigated and well described (White 1954; Reed and Nagodawithana 1991). It has also been reviewed in several publications, e.g. by Rose and Vijayalakshimi (1993) and Jenson (1998).

Traditionally, yeast biomass production occurs using a molasses-based heat-treated substrate, added nutrients, like urea or ammonium sulphate, vitamins and minerals, in fermenters equipped with aeration and agitation. Growth conditions are important for producing the maximum amount of yeast and for obtaining the desired yeast quality including a rapid dough fermentation and a high resistance to oxidative stress, drying, freezing and other forms of stress (Attfield 1997).

The optimal growth conditions during fermentation are primarily defined by nutrient feed rates, aeration, temperature, pH, ethanol level and the respiratory quotient (CO_2: O_2), For control purposes, fermenters are equipped with sensors for measurement of ethanol, CO_2 and O_2 in the exit gas. High aeration rates and fed-batch fermentation ensure that the carbohydrate level is kept low, allowing the yeast to produce biomass using respiratory metabolism. At the end of the fermentation, nutrient feed is stopped, but aeration is continued for 30–60 min, during which time a significant synthesis of trehalose takes place. High aeration, removal of nitrogen and carbohydrate derepression favour the gluconeogenic pathway and synthesis of trehalose. Trehalose is the typical storage carbohydrate in yeast. It plays an important protective role for the yeast cell when exposed to stresses like drying and freezing. The protective role of trehalose has also been demonstrated by Shima et al. (1999), by constructing trehalase mutants derived from *S. cerevisiae*. During fermentation, degradation of intracellular trehalose was inhibited in the trehalase mutants, which all showed improved freezing tolerance which may make the strains useful in frozen dough.

After fermentation, the yeast is recovered by filtration or centrifugation and processed into a commercial product. It can be in the form of cream yeast, compressed yeast or viable dry yeast.

Cream yeast is a liquid yeast obtained after concentration, washing and, if desired, stabilisation of the yeast at the end of fermentation (Cees 1991; Jenson 1998). The solids level is about 16–20% and the shelf life is approximately 14 days at 4°C. The cream yeast offers better opportunities for control over yeast activity and ease the use in large plant bakeries that can pump the yeast to several points and dose it accurately. For viable dry yeast, the yeast slurry is mixed with additives, e.g. sorbitan esters, carboxy methylcellulose and antioxidants. These additives protect the yeast against drying and reconstitution at use. Following mixing with additives, the yeast is concentrated by filtration then extruded mildly through a screen to form threads which are cut up and dried in a fluidised-bed dryer at room temperature. To maintain viability, the dry yeast needs to be protected against oxygen and is packaged accordingly (Jenson 1998). Compressed yeast has a solids level of about 30–35% and a shelf life of approximately 4 weeks at 4°C.

Evaluation of thermally dried *Kluyveromyces marxianus* as baker's yeast has recently been published. In comparison with commercial baker's yeast, no significant differences in the volatile aroma compounds and overall quality were observed (Dimitrellou et al. 2009).

III. Cheese

A. Yeasts

Yeasts are found in all sorts of cheeses. *Debaryomyces hansenii* (anamorph form: *C. famata*) is the predorminant yeast species in semi-soft cheeses, whereas soft cheese are characterised by the additional presence of *G. geotrichum* (teleomorph form: *Galactomyces candidus*). Other predorminant yeast species include *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Kluyvermyces lactis* and *K. marxianus*. The number of yeasts can be in the range 10^6 colony-forming units (cfu)/g or even higher. The development of yeasts in cheeses occurs spontaneously, while the controlled use of yeasts as starter cultures in cheeses primarily is used for the production of some kinds of mould-ripened cheeses and smear-ripened cheeses.

The positive role of yeasts in the maturation and aroma formation in Camembert has been suggested in several investigations (Anderson and Day 1966; Schmidt and Lenoir 1978, 1980a, b; Schmidt and Daudin 1983; Rousseau 1984; Siewert 1986; Baroiller and Schmidt 1990; Gripon 1993). Baroiller and Schmidt (1990) concluded that the following five yeasts are predominant in white mould cheeses: *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *D. hansenii*, *S. cerevisiae* and *Zygosaccharomyces rouxii*. A positive role for yeasts has been proposed for blue mould cheeses, and the yeasts in the interior of the cheese reach significantly higher numbers than observed for white mould cheeses (Hartley and Jezeski 1954; Galzin et al. 1970; Nunez et al. 1981; Kaminarides and Anifantakis 1989; Besancon et al. 1992; Hostin and Palo 1992; van den Tempel and Jakobsen 1998). According to the investigations mentioned, the predominant yeast populations are rather similar for the white and blue mould cheeses.It is characteristic of the ecosystem of the cheese, the cheese brine and the environmental conditions prevailing in the dairy that they select towards a uniform and well defined yeast population (Baroiller and Schmidt 1990; van den Tempel and Jakobsen 1998). Thus, a situation may exist which is very similar to a deliberate use of yeasts as starter cultures, which is still the exception rather than the rule in the dairy industry.

1. *Debaryomyces hansenii*

Originally two species were described: *Debaryomyces hansenii* (Zopf) Lodder et Kreger van Rij and *Debaryomyces fabryi* Ota. It was proposed to subdivide the species *D. hansenii* (Zopf) Lodder et Kreger van Rij into varieties: *D. hansenii* var. *hansenii* and *D. hansenii* var. *fabryi* and their respective anamorphs, *Candida famata* var. *famata* and *C. famata* var. *flareri* (Nakase and Suzuki 1985). Physiologically, the two varieties can be distinguished by maximum growth temperatures and the enzyme glucose-6-phosphate dehydrogenase. The maximum growth temperature of the variety *hansenii* is 31–35°C, whereas that of the variety *fabryi* is 36–39°C.

However, based on molecular techniques, several authors have proposed to reinstate the two original species (Prillinger et al. 1999; Corredor et al. 2003; Romero et al. 2005; Quiros et al. 2006; Jacques et al. 2009; Nguyen et al. 2009). Futhermore, a number of strains within the variety *fabryi* have been proposed to form a new species *Debaryomyces subglobosus* (= *Candida flareri*). Finally, growth at 37°C may no longer be used to differentiate *D. hansenii* from *D. fabryi*. In contrast, riboflavin production seems more specific for *D. fabryi* and *D. subglobosus* strains compared to *D. hansenii* strains. In cheese *D. hansenii* appears to be the dominant form (Petersen et al. 2001). *D. hansenii* occurs in high numbers (10^6–10^8 cfu/g) in surface-ripened cheeses as well as in blue and white mould cheeses.

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